Induction of Progesterone Serum Levels Mediated the Behavioral Responses to Cocaine Through Activation of Progesterone Receptors

by

Hui-Bing (Katie) Wu

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_____	_____
Date	Chair of Examining Committee Vanya Quinones-Jenab
_____	_____
Date	Executive Officer Joseph Glick
Supervisory Committee:	Vanya Quinones-Jenab, Ph.D.
	Shirzad Jenab, Ph.D.
	Victoria Luine, Ph.D.
	Yan Zhou, Ph.D.
	Yuan Shan Zhu, Ph.D.
	Jim Gordon, Ph.D.

THE CITY UNIVERSITY OF NEW YORK

Abstract

The Mechanisms of Action of Progesterone Receptors in Cocaine-induced Psychomotor Responses

by

Hui-Bing (Katie) Wu

Adviser: Professor Vanya Quinones-Jenab

Although accumulating evidences suggest cocaine-induced behavioral sex differences, the mechanism of action remains unclear. The aim of this proposal is to delineate the underlying mechanism of actions at different levels.

At the behavioral level, we extended previous studies by demonstrating that sex differences occurred in certain aspects of cocaine-induced behavioral activation, development, and sensitization. Furthermore, we demonstrated that this sensitization effect maybe gonadal hormone mediated as cocaine-treated GDX female rats had similar ambulatory counts as cocaine-treated intact male rats.

At the endocrine level, female rats have an exaggerated HPA axis response compared with male rats. We extended this finding by demonstrating that chronic cocaine administration did not alter corticosterone plasma levels, indicating the possible development of tolerance of HPA activity.

RU 486, a progesterone receptor (PR) antagonist, significantly attenuated cocaine-induced ambulatory and rearing behaviors in males, while tamoxifen, an estrogen receptor (ER) antagonist, spared all aspects of locomotor measurements in both male and female rats. Taken together, our results suggest that ER may play a limited role, while

PR activation is a necessary step in the cascades of events that may account for the behavioral and neuroendocrinological responses to acute cocaine administration.

At the receptor level, we found that PR protein levels and PR-DNA complex formation were differentially affected dose-dependently by cocaine. 15mg/kg of cocaine upregulated nuclear PR-A protein levels and PR-DNA complex, while 30 mg/kg of cocaine upregulated nuclear PR-A protein levels, but downregulated PR-DNA complex at a later time point. The results suggest that different doses of cocaine may activate different pathways, which in turn, may mediate the short- and long-term effects of progesterone. In addition, regardless of drug treatment or time course, whole cell extracts had no effect on PR protein or PR-DNA complex levels, suggesting that the receptor activation is internalized into the nucleus while little or no activity occurs in the cytoplasm.

Taken together, the effects of cocaine on circulating hormone levels, protein levels, and functional DNA complexes may play a crucial role in cocaine-induced behavioral sex differences.

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CHAPTER 1: General Introduction

History of Cocaine

Cocaine is an active alkaloid found in the leaves of Erythroxylon coca, a tree indigenous to Peru and Bolivia. The drug has been used as a psychostimulant among the people of Colombia for 2,000-5,000 years (Platt, 1997). In the United States, however, its use remained relatively limited until the late 1800s (Platt, 1997). Around this time cocaine began to see heightened popularity when a young physician by the name of Sigmund Freud advocated its use as a treatment for a multitude of personality disorders and even morphine addiction (McKim, 1996). It wasn't long before reports of cocaine addiction threatened its new founded popularity and by 1894, American Medical Association was beginning to question its use (Platt, 1997). Finally, in 1914 the use of cocaine was banned by the Harrison Narcotic Act (Platt, 1997).

Since the ban of this substance as a narcotic and its potential to be addictive, its use has fluctuated affecting all classes and races of people. Today, there is a lack of understanding about the unequal distribution of cocaine use between the sexes. In 2000, the national Household Survey on Drug Abuse reported that 33% of the 1.2 million Americans that currently used cocaine were woman (Substance Abuse and Mental Health Services Administration, 2003). Even though males are more likely than females to have an initial opportunity to use drugs, there seems to be no difference in the progression to intense drug use following the initial use (Van Etten and Anthony, 1999; Van Etten et al., 1999). Thus, sex differences in the pattern of drug abuse may be circumstantial, thereby providing males with greater opportunities to progress from initial to habitual use (Van Etten and Anthony, 1999; Van Etten et al., 1999).

Accumulating evidences from recent studies have suggested sex differences to cocaine-induced behavioral and neurochemical alterations in humans and animals (Becker and Ramirez, 1981; see chapter 2; 3; Lukas et al., 1996; Quinones-Jenab et al., 2001; Robinson et al., 1982a). Although progress has been made in understanding the clinical basis of cocaine abuse, more extensive studies are needed at the neurochemical, molecular, and behavioral levels to address these sex differences.

Effects of Cocaine on the Monoamine System

Cocaine acts by binding non-selectively to monoamine transporters to prevent monoamine re-uptake, thereby, increasing the concentration of neurotransmitters at the synapse (Heikkila et al., 1975). The mesolimbic dopamine system has been postulated to have a primary role in mediating the reinforcing properties of many drugs of abuse including cocaine, amphetamine and opioids (Koob, 1992). The mesolimbic system includes dopamine cell bodies in the ventral tegmental area projecting to the nucleus accumbens, which appears to be necessary for cocaine reward (Dahlstrom and Fuxe, 1964; Fallon and Moore, 1978; Roberts et al., 1980). The ventral tegmental area and nucleus accumbens has been postulated to be involved in the locomotor and reinforcing properties of cocaine (Koob, 1992; Wise and Bozarth, 1987). Other areas, such as, medial prefrontal cortex, ventral pallidum, and olfactory tubercle have been implicated in cocaine reinforcement (Goeders and Smith, 1993; Hubner and Koob, 1990; Koob, 1992; Kornetsky et al., 1991; Roberts et al., 1980).

Cocaine enhancement of locomotor and stereotypic behaviors are thought to be mediated primarily by modulation of dopaminergic neurotransmission in the mesolimbic

dopamine system (Collins et al., 2001; Kelley and Iversen, 1975; Kelly and Iversen, 1976; Post et al., 1988; White and Cooper, 2001). Repeated, intermittent cocaine augments the motor response and parallels the cocaine-induced dopamine efflux in the nucleus accumbens, and in the striatum of sensitized rats, thereby contributing to a long-lasting effect (Akimoto et al., 1989; Fujiwara et al., 2000; Kalivas and Duffy, 1990; Kalivas et al., 1991; Pettit et al., 1990; Post and Rose, 1976; Robinson and Becker, 1986; Robinson et al., 1988; Vezina, 1993; White and Cooper, 2001; White and Kalivas, 1998). Lesions of dopaminergic terminals in the nucleus accumbens with 6-OHDA (6-hydroxydopamine) inhibited cocaine-induced motor response (Kelley and Iversen, 1975).

Cocaine-Induced Intracellular Signal Transduction

Acute and chronic administration of cocaine increases extracellular concentration of dopamine, which binds postsynaptically to D1 and D2 family of receptors (Fitzgerald and Nestler, 1995). Activation of Dl receptors coupled to Gs proteins lead to stimulation of adenylyl cyclase activity, thereby, converting ATP (adenosine triphosphate) to cAMP (cyclic adenosine monophosphate) (Fitzgerald and Nestler 1995; Nestler and Aghajanian, 1997). cAMP formation in turn enhances PKA (protein kinases A) activity, which phosphorylates proteins, including CREB (cAMP response element binding protein), a transcription factor (Fitzgerald and Nestler, 1995; Nestler and Aghajanian, 1997). Unlike D1 receptors, activation of D2 receptors inhibits adenylyl cyclase activity by coupling to Gi proteins and activates inward rectifying K^+ channels (Fitzgerald and Nestler, 1995; Figure 1).

Previous studies have indicated the involvement of D1 and D2 receptors in

modulating the behavioral and neurochemical responses to psychostimulants (Cabib et al., 1991; Ushijima et al., 1995), while alterations in CREB modulates the effect of cocaine reward (Carlezon et al., 1998). Enhancement of the cAMP second messenger system increases the locomotor activity and self-administration to cocaine. However, reduction or suppression of CREB enhances cocaine reward (Carlezon et al., 1998).

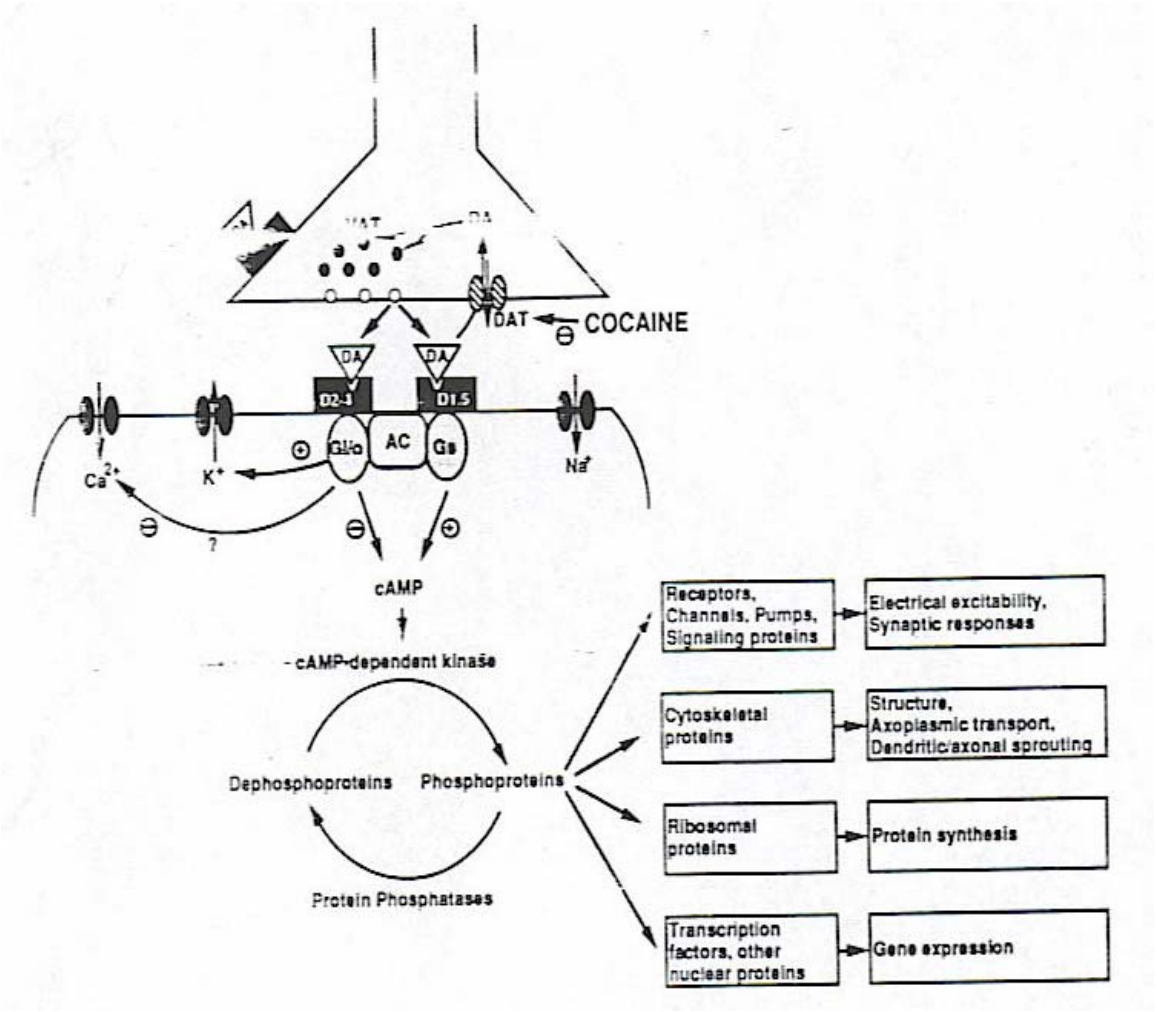


Figure 1. Schematic illustration of cocaine's effect on signal transduction pathways. Acute administration of cocaine blocks DAT, thereby, increasing synaptic DA levels, in turn, binds to DA receptors to regulate adenylyl cyclase activity to alter cAMP levels. This in turn leads to modifications of cAMP-dependent protein kinase, which regulates phosphorylation of multiple protein substrates that influence neuronal functioning. Adopted by Fitzgerald and Nestler 1995 (Fitzgerald and Nestler, 1995).

Evidence for Alternative Mechanism of Action of Cocaine:

The persistence of cocaine self-administration from recent studies with DATKO (dopamine transporter knockout) mice model challenged the notion that dopamine is predominantly responsible for the reinforcing properties of drugs of abuse (Koob et al., 1987; Wise and Bozarth, 1984). Carboni et al. (2001) found that cocaine increased dialysate dopamine in the nucleus accumbens of DATKO mice and proposed that the increase may be due to blockade of norepinephrine. Blockade of dopamine transporter or serotonin transporter via GBR 12909 or fluoxetine, respectively, did not alter the dialysate dopamine in nucleus accumbens, but blockade of norepinephrine via reboxetine increased dialysate dopamine in nucleus accumbens but not in the striatum where NET containing terminals are absent (Carboni et al., 2001). This finding is not consistent with studies that report a decrease of extracellular dopamine levels in the nigrostriatal and mesolimbic pathways of animals subject to lesion in the locus coeruleus, main source of norepinephrine. This result suggests that other mechanisms may be modulating the monoamine release, which affects cocaine-induced reinforcement.

Sex differences in drug abuse

Women may be more sensitive to the addictive properties of cocaine than men. In addition, women reported more drug cravings induced by cocaine cues than men (Robbins et al., 1999). Furthermore, women are also admitted to the emergency room more frequently than men following crack use (Dudish et al., 1996). Many factors may contribute to this sex difference (Blume, 1986; Blume, 1990; Reed and Mowbray, 1999; Van Etten et al., 1999).

Sex differences in men and women reports of subjective effects, cocaine plasma levels, and menstrual cycle differences in response to cocaine are controversial (Evans et al., 1999; Lukas et al., 1996; Sofuoglu et al., 2001; Sofuoglu et al., 1999). The discrepancy may be due to differential route of administration (i.v., intranasal or smoked) or subject pool (addicts or occasional users) (Lukas et al., 1996; Mendelson et al., 1999b; Volkow et al., 2000). Smoked cocaine significantly increased reports of "high" than intranasal cocaine (Volkow et al., 2000), which has a greater effect than i.v. cocaine (Foltin and Fischman, 1991). Women who experienced smoked cocaine reported lower paranoid/suspicious and heart racing/pounding than men and women in luteal phase reported a decreased in euphoric effect compared to women in follicular phase and men (Sofuoglu et al., 1999). Evans et al. (1999) reported higher plasma cocaine levels in women who repeated smoked cocaine than men. Despite reported sex differences in response to cocaine, Mendelson et al. (1999) did not report any gender or menstrual cycle differences in cocaine plasma level, subjective effects, or heart rate in addicts treated with i.v. cocaine.

In rat studies, accumulating evidence suggest sex differences in cocaine-induced behavioral response (Bowman et al., 1999a; see chapter 2; 3; Festa et al., 2003; Sell et al., 2000; Van Haaren and Meyer, 1991). Overall, female rats are more sensitive to cocaine-induced behavioral response and behavioral sensitization than male rats (see Table I; see chapter 2; 3; Glick et al., 1983a; Sell et al., 2000; Van Haaren and Meyer, 1991; Walker et al., 2001c). Female rats also acquire cocaine discrimination at a faster rate and display a greater motivation to self-administer cocaine than male rats (Craft and Stratmann, 1996; Roberts et al., 1989). Moreover, cocaine reinstatement is greater in female rats and

female rats also exhibit an augmented or exaggerated response to cocaine-induced alterations after the first administration compared to male rats (Berul and Harclerode, 1989; Caihol and Morméde, 1999; see chapter 3; Lynch and Carroll, 2000; Sircar and Kim, 1999; Van Etten and Anthony, 1999; Walker et al., 1997). Cycling female rats after acute 'binge' pattern cocaine displayed similar but higher locomotor activity than male rats treated with chronic 'binge' pattern cocaine (Quinones-Jenab et al., 2000). Moreover, ovariectomy (OVX) eliminated behavioral sex differences in response to cocaine, while castration has no effect on behavioral response (see Table II). This suggest that ovarian hormones and not testicular hormones may play a pivotal role in mediating some aspects of cocaine-induced behavioral sensitization possibly through learning and memory impairment because estrogen has been reported to influence learning acquisition and memory processes (Luine, 1997).

Hormonal fluctuation throughout the estrous cycle has been implicated to affect the behavioral response to cocaine. After acute cocaine administration, cocaine-induced motor activity is lowest during diestrus when estrogen levels are beginning to rise and progesterone levels are low (see Table III). On the other hand, chronic cocaine administration disrupts or has no effect on estrous cyclicity (see Table III).

Estrogen replacement paradigms have been studied extensively and have demonstrated that a steady state of estrogen via Silastic capsules can potentiate cocaine-induced behavioral effects and sensitization, while a pulsatory increase of estrogen levels via a subcutaneous injection has no effect on the response to an acute injection of cocaine. On the other hand, implants of either progesterone or testosterone both attenuated hyperactivity and sensitization in response to an acute cocaine injection (see

Table IV). Taken together, estrogen may play a pivotal role while progesterone and testosterone may play a limited role in the acute effect of cocaine (see Table V).

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*

↑ Represents an increase in behavioral activity.

Δ Represents an effect observed only in female rats. Adapted by Festa et al., 2004

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

		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. Adapted by Festa et al., 2004

Table III. 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. Adapted by Festa et al., 2004

Table IV. Effects of gonadal hormones on cocaine-induced activity following acute 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*

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

↑↓ Represents changes in behavioral activity across "binge" injections of cocaine. Adapted by Festa et al., 2004.

Table V. Effects of gonadal hormones on cocaine-induced activity following chronic cocaine administration

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

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

↑↓ Represents changes in behavioral activity across "binge" injections of cocaine.

Possible Mechanisms of Action Underlying Behavioral Sex Differences

HPG Axis

Activation of HPG is responsible for the regulation of female and male reproductive cycle. Secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus is regulated by a plethora of neurotransmitters and neuropeptides, such as, serotonin, dopamine, opioid peptides, GABA (gamma-aminobutyric acid), CRH (corticotrophin-releasing hormone). GnRH stimulates the pituitary to release follicular stimulating hormone (FSH) and leutinizing hormone (LH). FSH and LH are released into the bloodstream and target the gonads to release estrogen and progesterone, respectively (Pinel, 2000).

Cocaine Activation of HPG Axis

Some studies reported differences in HPG activation that may be due to differential route of administration, while other reported no effect of cocaine-dependence on activation. Overall, i.v. injections of cocaine to male addicts significantly increased LH levels above baseline (Mendelson et al., 2003; Mendelson et al., 2001; Mendelson et al., 1992). LH levels in menstrual female at follicular and luteal phase were elevated after i.v. cocaine but LH levels remained elevated longer in men than in women (Mendelson et al., 2001). LH and to a lesser extend FSH levels were significantly induced after intranasal cocaine administration to male volunteers without drug abuse history (Heesh et al., 1996). The induction of LH levels parallel the increase of cocaine plasma levels, suggesting that LH may contribute to the abuse-related effects of cocaine (Mendelson et al., 2003). Cocaine may modulate GnRH release directly or indirectly via

modulation of neurotransmitter transmission that may in turn act on GnRH release in the hypothalamus to influence LH release from the pituitary (Canez et al., 1992).

Accumulating evidences from estrous cycle studies in rats suggest that sex differences in cocaine-induced psychomotor responses are mediated by gonadal hormones. Female rats in estrus phase exhibit higher locomotor activity and stereotypic behaviors than any other stages of the cycle and males. Animals during estrus stage also exhibit greater motivation to self-administer cocaine compared to other stages of the cycle (Roberts et al., 1989).

Estrogen treatment enhance cocaine-induced locomotor behaviors compared to OVX treatment group (Castner et al., 1993; Peris et al., 1991; Perrotti et al., 2001c; Sell et al., 2000). Removal of estrogen via chemical blockade (tamoxifen) or lesion (OVX) both reduced acquisition of cocaine self-administration from initial use to regular use, while estrogen replacement increased behavioral sensitization and restored cocaine self-administration. Estrogen plus progesterone treatment group augments the locomotor activity, while progesterone slightly attenuating estrogen effect (Perrotti et al., 2003; Perrotti et al., 2001c; Sell et al., 2000). On the other hand, progesterone alone decreased cocaine-induced locomotor behaviors compared to OVX treatment group (Sell et al., 2000).

Sex Difference in Cocaine-Induced Steroid Plasma Levels

In women who are cocaine dependent, progesterone and estrogen plasma levels were significantly higher during luteal phase than during follicular phase after 0.2 and 0.4 mg/kg i.v. cocaine administration (Mendelson et al., 2001). Mello et al. (2000) reported

an induction of estrogen plasma level as early as 15 minutes after i.v. infusion of .8 mg/kg cocaine, but no change in progesterone plasma levels across all time points. Despite the discrepancy of reported changes in ovarian hormones that can be due to route of administration, testosterone plasma levels were not significantly altered after different routes of administration (i.v. and intransal) in men with or without drug abuse history, respectively (Heesh et al., 1996; Mendelson et al., 2003).

Cocaine increased blood levels of progesterone were observed in male and cycling female rats as well as pregnant dams (Quinones-Jenab et al., 1997; Quiñones-Jenab et al., 2000b). Progesterone induction was greatest in proestrous, where estradiol peaks, than during diestrus, where estradiol is lower (Quiñones-Jenab et al., 2000a; Quiñones-Jenab et al., 2000b; Walker et al., 2001d). In male rats, both single and "binge" pattern cocaine administration increased progesterone plasma levels (Quinones-Jenab et al., 2000b; Walker et al., 2001d), while only single acute administration of cocaine increased progesterone plasma levels in female rats (Festa et al., 2003). Although progesterone has been implicated in drug addiction, little is known about molecular interactions between progesterone and cocaine. Currently, interactions between cocaine and estrogen plasma level is limited in literature, especially in animal models.

Steroid Receptors

Steroid hormones are lipophilic allowing simple diffusion into target cells and blood-brain-barrier to exert their primary effects on steroid receptors (Allera and Wildt, 1992; Pardridge and Mietus, 1979). Steroid receptors are part of the nuclear/intracellular

receptor superfamily of ligand-dependent transcription factors (Bamberger et al., 1996; Conneely et al., 2002; Evans, 1988; Leonhardt et al., 2003; Levine et al., 2001; Xu et al., 1996). Transcription factors are mediators in genome activation. Due to the scope of the overviews, only progesterone receptor (PR) and estrogen receptor (ER) will be discussed.

Classical steroid receptor comprised of a variable N-terminal, a highly conserved DBD (DNA binding domain), and a LBD (ligand binding domain) on the C-terminal. The N-terminal contains two transcriptional activation domains [AF 1 and AF 3 (Sartorius et al., 1994; Wen et al., 1994) that have been implicated for transcriptional activation (Beato, 1989; Beato and Klug, 2000; Evans, 1988; Fuller, 1991; Leonhardt et al., 2003). The DBD contains two zinc fingers that recognizes and binds to the DNA consensus sequence called the HRE (hormone response element) (Chandler et al., 1983; Evans, 1988; Klein-Hitpass et al., 1986). LBD in the C-terminal is responsible for hormone binding, receptor dimerization, nuclear translocation, and contains a transcriptional activation domain (AF 2) interacts with N-terminal transcriptional activation domains to facilitate interaction with steroid receptor cofactors (Fawell et al., 1990; Kumar et al., 1987; Muller et al., 2000) (Figure 2).

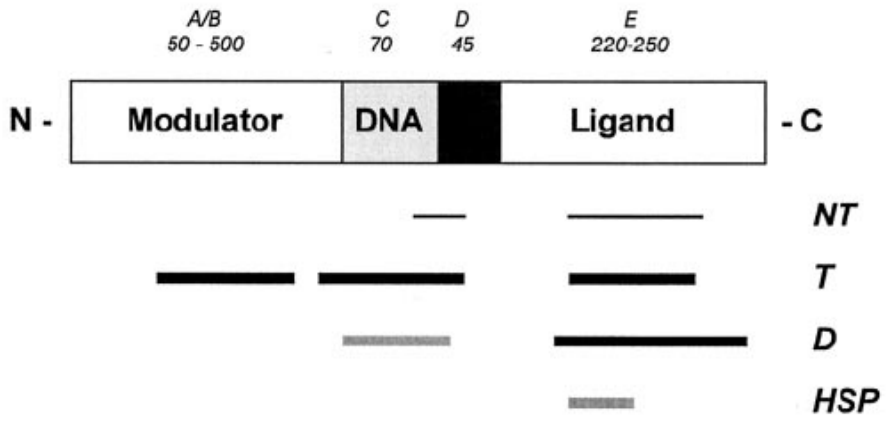


Figure 2. General structure and functional organization of steroid receptors.

N= N terminal, which contains regions A/B with variable lengths
DNA= DNA binding domain that contains regions C and D with two zinc fingers.
Ligand= Ligand binding domain that contain region E for ligand binding and nuclear translocation signaling.
C= C terminal that contains ligand binding domain.
NT= nuclear transcription; T= transactivation; D= dimerization; HSP= heat shock protein binding.
Adopted by Falkentstein et al., 2000.

Human and rat progesterone receptor (PR) is expressed in two distinct isoforms, PR-A and PR-B; both are derived from the same gene but produced by different mRNAs (Conneely et al., 1989; Kastner et al., 1990). Recent studies have discovered another isoform, PR-C that is about 60 kDa (Wei and Miner, 1994). However, PR-C is transcriptionally inactive and is not extensively studied (Wei and Miner, 1994). As a result, we will focus only on PR-A and PR-B. PR-B has 933 amino acids resulting in a higher molecular weight of 120 kDa compared to PR-A, which lacks the first 164 amino acids, resulting in 769 amino acids and lower molecular weight of 94 kDa (Sartorius et al., 1994).

PR mRNA is predominantly found in the specific regions of the hypothalamus, such as, the arcuate nucleus, medial preoptic nucleus and ventrolateral part, which are involved in sexual behavior (Hagihara et al., 1992; Kato et al., 1994). Other areas of the hypothalamus are either not detected or detected at a moderate amount, such as, the supraoptic nucleus or anterior periventricular nucleus, respectively (Kato et al., 1994). Moderate amounts are also found in the isocortex, hippocampus, amygdala (Hagihara et al., 1992; Kato et al., 1994), and septum (Kato et al., 1994). PR mRNA levels are weak or absent in all the regions of the thalamus (Kato et al., 1994).

ER α was first discovered in the 1960s and cloned in the mid 1980s (Green et al., 1986; Jensen and DeSombre, 1972; Jensen and Jacobsen, 1962; Walter et al., 1985). ER β was only discovered recently and cloned (Kuiper et al., 1996; Mosselman et al., 1996). The molecular weight for classical ER α is 67 kDa and the molecular weight for the more recent discovery of ER β is 54 kDa (Kuiper et al., 1996; Mosselman et al., 1996; Ogawa et al., 1998). The isoforms can exist as homodimers or heterodimers, suggesting a functional interaction between ER α and ER β (Moore et al., 1998; Ogawa et al., 1998; Pace et al., 1997; Pettersson et al., 1997; Shaun et al., 1997).

Both ER isoforms are predominantly found in areas, such as, amygdala, hypothalamus, and septum that are involved in emotional processing, cognition, and procreation (Blurton-Jones et al., 1999; Donahue et al., 2000; Keefer and Stumpf, 1975; Osterlund and Hurd, 2001; Osterlund et al., 2000a; Osterlund et al., 2000b; Pfaff and Keiner, 1973; Shughrue et al., 1997). Both ER α and ER β mRNA levels are expressed low-to-moderate in the cerebral cortex, which may play a role in regulating cognition and memory (Suzuki, 1996). The isoforms are absent in the human basal ganglia regions,

such as, caudate putamen, nucleus accumbens, and globus pallidus (Osterlund and Hurd, 2001). However, ER α and ER β mRNA levels are differentially expressed in rodents (Laflamme et al., 1998; Osterlund et al., 1998; Shughrue et al., 1997) and in primates (Gundlah et al., 2000; Osterlund et al., 2000a; Osterlund et al., 2000b).

Mechanism of Action of Genomic or Classical Pathway

Steroid hormones, such as, progesterone, estrogen, and androgen exert their effects on target cells via genomic or classical pathways and nongenomic pathways. A pathway is termed "genomic" when the effect is long lasting and is sensitive to inhibitors of transcription and translation (Falkenstein et al., 2000), while "nongenomic" is termed when the effect is rapid (within seconds to minutes) and reproducible in presence of inhibitors of transcription and protein synthesis. Interactions between genomic and nongenomic pathways have been reported.

The classical pathway involves binding of steroid hormones to cognate intracellular receptors to regulate gene transcription for long-lasting effects (Beato et al., 1996; Beato and Klug, 2000). Unbound steroid receptors are associated with heat shock proteins (HSPs), such as, hsp 70 and 90 and immunophilins (Falkenstein et al., 2000; Schumacher et al., 1999) that acts to keep the steroid receptor functional (Godowski and Picard, 1989) and to repress DNA binding in the absence of the hormone (Baulieu et al., 1990; DeMarzo et al., 1991; Pratt, 1992). Ligand binding induces a cascade of events that leads to transcription of target genes.

Upon ligand binding to the cavity on LBD, HSPs are dissociated (Pratt and Toft, 1997) and a cascade of events that occurred simultaneously. Phosphorylation of steroid

receptors leads to conformational changes, in turn, activating the receptor to recruit steroid receptor coactivators (SRC) and corepressors (Beato and Klug, 2000) to the hydrophobic binding pocket of LBD (Brzozowski et al., 1997). Phosphorylation has been implicated as a crucial determinant in transactivation (Takimoto et al., 1992). Most steroid hormone receptors are phosphorylated on the serine residue (Orti et al., 1992), while ER is the only member of the steroid receptor family that is phosphorylated on both the tyrosine and serine residues (Kuiper and Brinkmann, 1994; Orti et al., 1992). Upon activation, ligand-bound receptors are homo/heterodimerized and nuclear localization sequence (NLS) in the LBD region is activated to signal nuclear/cytoplasmic shuttling (Guiochon-Mantel et al., 1991).

After nuclear translocation, the ligand-receptor complex needs to interact with the hormone response element (HRE) on the DNA, which is wrapped around histones (van Holde et al., 1992). The coactivators on the steroid hormone receptors have been reported to reduce the affinity of histones to DNA through histone-acteyltransferase, thereby, exposing the DNA consensus sequence to the complex (Beato and Klug, 2000). Coactivators, such as, SRC-1 (steroid receptor-coactivator 1) and GRIP1 (glucocorticoid receptor interacting protein 1) facilitate transactivation through remodeling of chromatin, while corepressors, such as, SMRT (silencing mediator of retinoic and thyroid receptors) hinder transactivation (Giangrande et al., 2000; Giangrande and McDonnell, 1999). Two zinc fingers on the DBD region recognizes and binds to the DNA nucleotide sequence on the promoter region to regulate gene expression by interacting with transcription machinery (Beato, 1989). Turning off and on a gene is determined by the ratio of coactivator to corepressor, thereby, upregulating or downregulating gene expression.

Mechanism of Action of Nongenomic Pathways:

In 1942, Hans Selye was the first to discover the rapid effects of progesterone (Selye, 1942). Modulation of Na^+ exchange in the erythrocytes that lack a nucleus (Spach and Streeten, 1964) and rapid effects of estrogen on Ca^{2+} flux in endometrial cells (Pietras and Szego, 1975) all points to a nongenomic mechanism of steroid action. All steroid hormones exhibit nongenomic actions through different mechanisms. It could be mediated through intracellular receptor in a hormone-independent manner, second messenger system, active neurosteroids, and more recently, membrane-bound receptors (Figure 3).

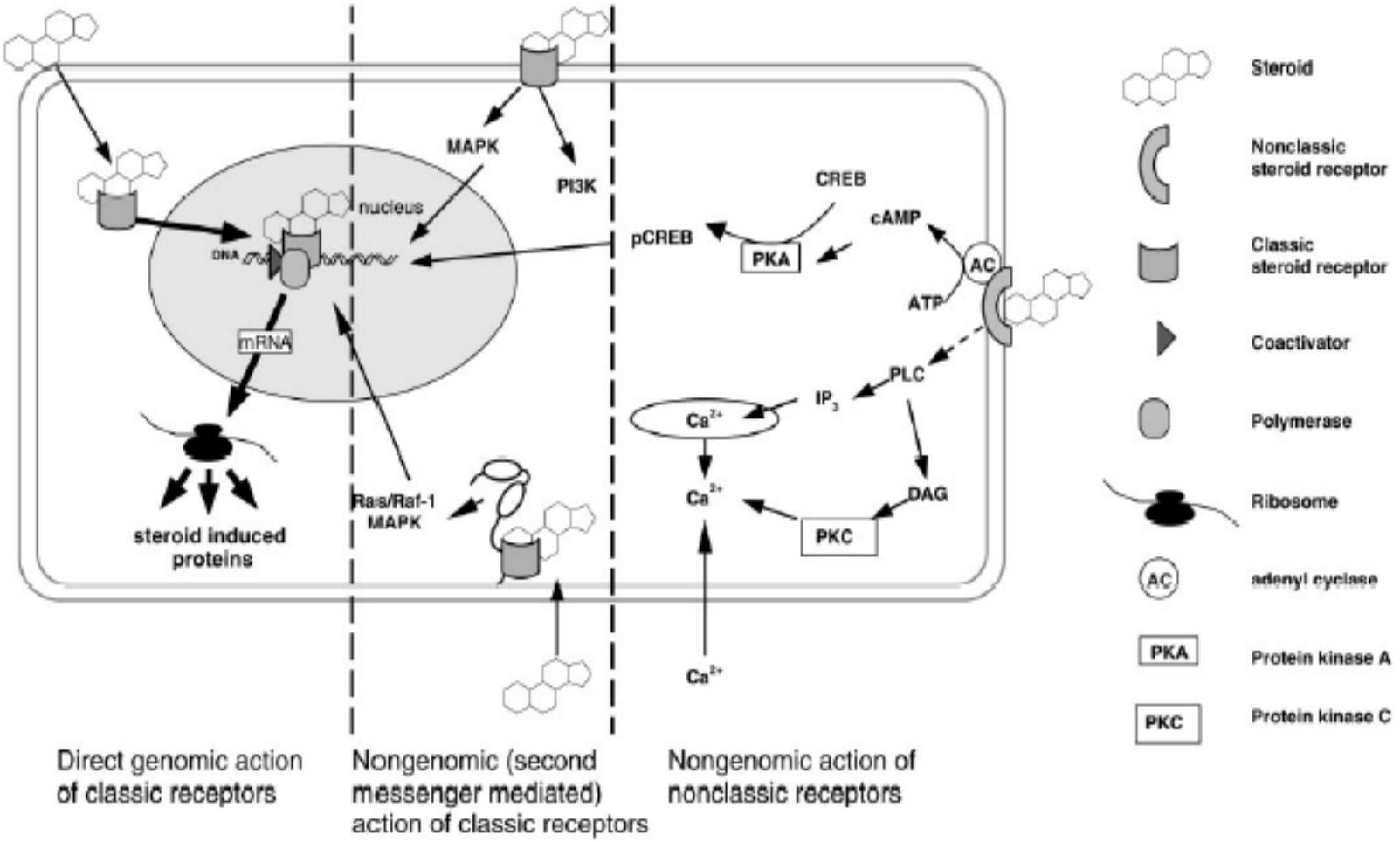


Figure 3. Crosstalk between genomic and nongenomic mechanisms that may involve unidentified membrane receptors. Adopted from Losel et al., 2003 (Losel et al., 2003).

Nongenomic Mechanism of Action of Steroid Receptors

Intracellular PR (iPR) can mediate nongenomic effects of progesterone. One of the nongenomic effects mediated by iPR is rapid induction of intracellular Ca2+ levels in spermatozoa that is without a functional nucleus (Blackmore et al., 1990; Blackmore et al., 1991).

Membrane bound PR have been reported to mediate the rapid effects of progesterone (Blackmore and Lattanzio, 1991; Meizel and Turner, 1991; Tesarik et al., 1992; Towle and Sze, 1983). Within seconds to minutes, plasma membrane PR can rapidly modulate Ca2+ levels in oocyte and spermatozoa, which both lack a nucleus and the action is not blocked by antiprogestin RU486, suggesting nongenomic mechanisms mediated by progesterone in sperm cells (Baldi et al., 1991; Baldi et al., 1998; Blackmore, 1998; Herrero et al., 1997; Morrill and Kostellow, 1999; Purohit et al., 1998; Turner et al., 1994). An induction of Ca2+ was followed by a decrease in cAMP leading to a reduction of PKA activity and a transient increase in cGMP (Finidori-Lepicard et al., 1981; Kostellow and Morrill, 1980; Kostellow et al., 1980; Maller and Krebs, 1977; Morrill and Kostellow, 1999; Sadler and Maller, 1981). Modulation of PKA in progesterone-induced sperm acrosomal reaction has also been reported (Harrison et al., 2000). Concurrently, progesterone actively stimulates phosphatidylinositol 4,5-biphosphate hydrolysis, leading to formation of DAG and IP_3, which further augments intracellular Ca2+ levels (Thomas and Meizel, 1989). The first membrane-bound PR has been cloned and displayed no significant similarity to iPR or other known functional proteins (Falkenstein et al., 1996; Gerdes et al., 1998; Schmidt et al., 2000).

Another rapid effect of progesterone is activation of a member of the MAPK family called ERK-2 with R 5020 treatment (Losel et al., 2003). This effect was inhibited by antiprogestin RU486, suggesting the involvement of iPR (Losel et al., 2003). Cells that lack PR cannot stimulate ERK-2 activity, further indicates the requirement of iPR (Migliaccio et al., 1998).

Rapid effects of estrogen have been shown to be mediated by intracellular ER (iER) and membrane bound ER in both an estrogen-dependent and estrogen-independent manner (Anuradha et al., 1994; Caulin-Glaser et al., 1997; Chiaia et al., 1983; Garcia-Segura et al., 1987; Lantin-Hermoso et al., 1997; Migliaccio et al., 1993; Pappas et al., 1995; Pietras and Szego, 1977; Razandi et al., 1999; Watson et al., 1995). Within seconds to minutes, both iER and plasma membrane ER can rapidly increase levels of Ca^{2+}, stimulate adenylate cyclase activity, activate PLC (phospholipase C), and activate members of the MAPK family, such as, ERK 1/2 (extracellular regulated kinase) (Aronica et al., 1994; de Jager et al., 2001; Duan et al., 2001; Improta-Brears et al., 1999; Le Mellay et al., 1997; Migliaccio et al., 1996). Estrogen induced activation of ERK leads to nuclear translocation to interact with nuclear transcription factors [CREB, AP-1 (activator protein 1)] and immediate early genes, such as, cFos and cJun (Marshall, 1995). 17β-estradiol-induced endothelial NO (nitric oxide) synthase (Chen et al., 1999b; Lantin-Hermoso et al., 1997) was inhibited by classical estrogen receptor antagonists, tamoxifen and ICI 182780 (Caulin-Glaser et al., 1997; Lantin-Hermoso et al., 1997) or by Ca^{2+} inhibitors, tyrosine kinases, or MAPK (Shaul, 1999), suggesting the involvement of iER in the rapid response to estrogen treatment. Moreover, overexpression of ER α further enhanced 17β-estradiol-induced NO (Shaul et al., 1997). Estrogen-independent

activation acts through growth factor signaling pathways (Cabral et al., 1994; Chen et al., 1999a). Growth factor can activate ER α through MAPK phosphorylation of the serine residue (Bunone et al., 1996).

Hormone Independent Activation of Steroid Receptors

A third mechanism of action by which steroid modulates behavioral activity is through hormone independent activation of steroid receptors. Steroid receptors are phosphoproteins that are hyperphosphorylated in the presence of hormone (Kuiper and Brinkmann, 1994; Toran-Allerand et al., 1996). Mechanisms that phosphorylate steroid receptors in the absence of hormone may act as a nongenomic mechanism of steroid action to elicit rapid response. This is called hormone-independent activation. Denner et al. (1990), was the first to report that PKA can activate chicken PR (cPR)-mediated transcription in the absence of steroid hormone. Cell treated with kinase activators or D1 receptor agonist can mimic and enhanced gene transcription, likewise, treatment with 8-bromo-cAMP can invert the antiprogestin action of RU486 to activation of gene transcription (Beck et al., 1993; de Ruiter et al., 1995; Katzenellenbogen et al., 1995; Nordeen et al., 1993; Power et al., 1991).

Nongenomic Action Through Non-Steroidal Receptors

Finally, steroids can also affect behavioral responses through non-genomic action of non-steroidal receptors. According to *Paul and Purdy* (1992), neurosteroids, steroids synthesized in the brain, can rapidly alter excitability of neurons by binding to membrane-bound receptors, such as, GABA receptors. GABA is major inhibitory

neurotransmitter in the brain. GABA activates two types of membrane-bound receptors, $GABA_A$, a chloride channel-gating receptor, and $GABA_B$, a G-protein coupled receptor (Olsen and Tobin, 1990). Progesterone metabolite, allopregnenolone, and deoxycorticosterone metabolite, 3α, 5α-TH Prog (3α, 5 α-tetrahydrodeoxycorticosterone) are potent allosteric modulators of $GABA_A$ receptors and can mimic and enhance the effects of GABA (Harrison and Simmonds, 1984; Majewska et al., 1986; McCarthy, 1995). This may explain the anesthetic, hypnotic, anxiolytic and anti-epileptic effects in humans (Majewska, 1992; Paul and Purdy, 1992; Selye, 1923). However, the underlying mechanism is not well understood as there are multiple isoforms of $GABA_A$ receptors, nonselective to binding to GABA subunits, and direct binding of steroids to $GABA_A$ receptors that require further speculation.

Significance

As previously described, accumulating evidences indicate that gonadal hormones may be the basis of the sex differences in the development and maintenance of cocaine-induced behavioral sensitization, which may highlight differences in the pattern of cocaine abuse or relapse between men and women. Of key importance is the female addict, where interactions between endogenous gonadal hormones and cocaine may ultimately affect the behavioral and subjective responses to cocaine. In particular, the development of sensitization or tolerance to cocaine may be affected according to which steroid-based contraceptive a woman is using or whether she is in her reproductive cycle.

This study is conducted to extend our understanding of cocaine's effects on circulating steroid hormones, receptor distribution and functional protein levels in the

male rat brain that may play a crucial role in the cocaine-induced behavioral response. Figure 4 is a model of the progesterone/estrogen activation of PR/ER and PR-DNA/ER-DNA complex in the absence of cocaine treatment. It is well documented that progesterone/estrogen crosses the plasma membrane and binds to the PR/ER in either the cytoplasm or nucleus to affect transcription of target genes that contains PRE/ERE at its promoter region, thereby, producing long-term biological responses. However, little is known on cocaine's influence on this activation pathway.

We hypothesized that induction of progesterone or estrogen serum levels may mediate the behavioral responses to cocaine through activation of steroid receptors (see Fig. 5A). After cocaine administration, that surge of progesterone/estrogen serum levels at the periphery may translate to activation of PR/ER inside the cell nucleus to affect transcription of target genes, thereby, altering the behavioral response to cocaine. If this hypothesis is confirmed then obstruction of progesterone or estrogen receptors with steroid antagonists (RU 486 or tamoxifen, respectively) would attenuate the behavioral effects of cocaine (see Fig. 5B). Steroid receptor antagonists can block receptor activation throughout cascades of events, but they act primarily by stabilizing the cytoplasmic heat shock proteins. In addition, the antagonist can translocate to the nucleus and heterodimerize with an active steroid receptor complex, thereby, rendering its function to activate the transcription machinery, in turn, affecting the behavioral response.

Specific Aim I: To test the hypothesis that cocaine-induce behavioral responses (locomotor and stereotypic activities), develop and maintain sensitization in male and female Fischer rats.

Specific Aim II: To test the hypothesis that genomic activation of steroid receptor is needed to produce cocaine-induced behavioral response. To this end, RU-486 and tamoxifen, steroid antagonists that block the formation of active steroid receptor DNA-binding complex, will be co-administered with cocaine and behavioral activation will be analyzed.

Specific Aim III: To test the hypothesis that activation of steroid receptor and response element are a pivotal pathway after cocaine administration. A) Because the presence of progesterone receptor (PR) protein levels in the striatum (region that regulates locomotor behavior) is limited in the literature, distribution of PR protein levels was assessed via Western blotting. B) To determine the optimal acute cocaine dose that mediates steroid receptor and DNA-binding activation. C) Through analysis of whole cell and nuclear extracts, we will test the hypothesis that steroid receptors are internalized after acute cocaine administration. Western blotting analysis and electro-mobility shift assay (EMSA) will be conducted to assess if steroid receptor levels and DNA-binding response element are altered after cocaine administration, respectively.

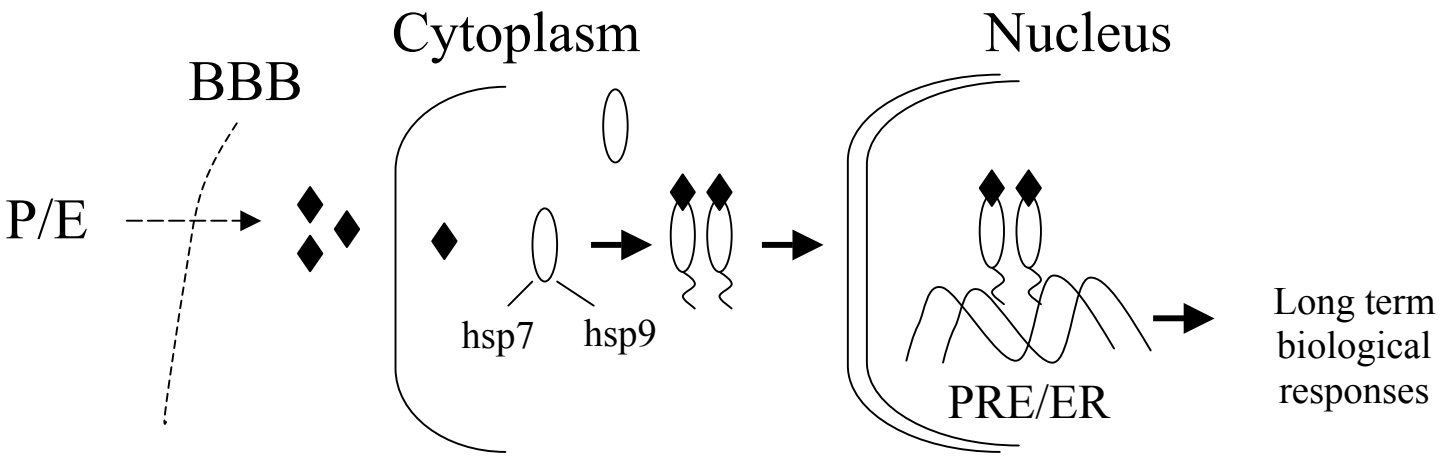


Figure 4. A model of progesterone/estrogen induced activation of PR/ER and PR-DNA/ER-DNA complex in the absence of cocaine treatment. It is well documented that progesterone/estrogen crosses the plasma membrane and binds to the PR/ER in either the cytoplasm or nucleus to affect transcription of target genes that contains PRE/ERE at its promoter region, thereby, producing long-term biological responses.

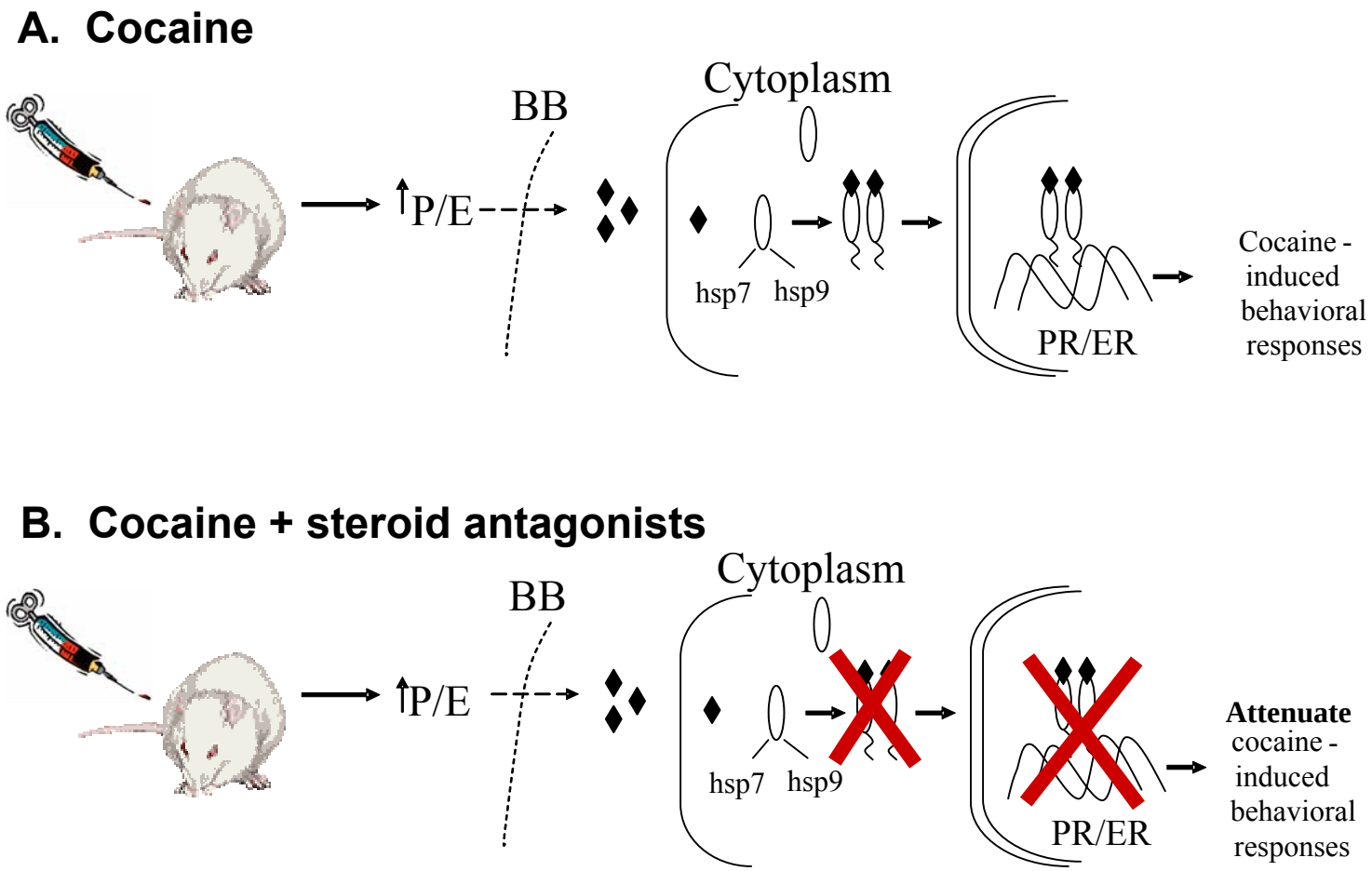


Figure 5. Proposed model that activation of steroid receptors are the key mediator in the behavioral response to cocaine. (A) After cocaine administration, progesterone/estrogen serum levels maybe increase and internalize into the cell nucleus to activate PR/ER complex formation, thereby, initiating the transcription machinery to affect cocaine-induced behavioral alterations. **(B)** After co-administration of cocaine and steroid antagonist, steroid serum levels, receptor levels, and DNA formation will be altered by RU 486 to render an attenuation of cocaine-induced behavioral responses.

CHAPTER 2: Sex differences in cocaine-induced behavioral sensitization

Although recent progress has been made towards understanding the clinical basis of cocaine abuse, more extensive studies are needed at the molecular and behavioral levels in females, where the understanding of the effects of drug addiction in the central nervous system is extremely limited. A plethora of evidence suggests sex differences in the cocaine-induced alterations in both human and animal models. For example, after acute cocaine administration, Kuhn and Francis (1997) (Kuhn and Francis, 1997), reported sex differences in cocaine-induced HPA-axis activation in rats; where female rats had exaggerated hypothalamic-pituitary-axis (HPA) responses to cocaine when compared to male rats. Female rats, when compared to male rats, also displayed greater hyperactivity, less toxicity to cocaine, as well as, more intense cocaine-induced locomotor activity and markedly enhanced stereotypic behaviors (Craft and Stratmann, 1996; Glick et al., 1983b; Kalivas et al., 1991; Post et al., 1981; Van Haaren and Meyer, 1991).

Behavioral sensitization to cocaine has been defined as a progressive increase in motor stimulation after repeated cocaine administration (Kalivas et al., 1991). Two different sensitization paradigms have been used in rats: a continued-chronic administration or a challenge dose of cocaine given following a period of withdrawal from chronic cocaine administration. In male rats, both protocols have been shown to produce sensitization (Kalivas et al., 1991; Robinson et al., 1982b). Although, female rats demonstrated higher levels of sensitization to repeated cocaine administration and were sensitized with a lower dose of cocaine than male rats, it is not clear if there are sex differences in the development and maintenance of psychomotor sensitization to cocaine

(Glick et al., 1983b; Post et al., 1981). Sex differences in development or maintenance of cocaine-induced behavioral sensitization may have important consequences on different patterns of drug abuse and relapses between the two sexes. To extend our understanding of sex differences in cocaine-induced alterations, this study was designed to determine how sex influences different components of locomotor activity (rearing, ambulations, total locomotion, and stereotypic behaviors) after acute, sub-chronic, chronic, or challenge dose administration of cocaine.

Methods

Animals

Eight-week-old intact female and male Fischer rats (Charles River Laboratories, North Carolina, USA) were individually housed in standard cages with free access to food and water, and maintained on a 12-hour light/dark cycle with lights on at 10:30 A.M. EST. One week after arrival, the rats were randomly assigned to either cocaine- or saline-treatment groups, and then, further subdivided into one of three sub-treatment conditions, acute (1 day), chronic (14 days), or challenge cocaine administration. Thirty minutes after acute, chronic or challenge drug treatments, rats were decapitated following a brief exposure (20 seconds) to CO_2 and trunk blood was collected. All NIH guidelines for the care and use of laboratory animals were followed.

Cocaine administration

Rats received daily i.p. injections of cocaine (15 mg/kg; dissolved in 0.9% saline) or saline for 1 day (acute; n=6) or 14 days (chronic; n=12). A fifth and six groups

received 14 days of chronic cocaine administration, 6 days of withdrawal, followed by a single challenge dose of cocaine or saline on day 7 (n=6/group). All injections were administered in each rat's home cage.

Behavioral assays

All assays were performed in home cages for 30 minutes after cocaine administration. Both stereotypic and locomotor activities were analyzed for each animal.

Locomotor activity

Spontaneous locomotor activity during the following 30 minutes after cocaine or saline treatment was monitored with a Photobeam Activity System from San Diego Instruments (CA) which record vertical and horizontal activity, as previously described (Perrotti, et al., 2000). Total locomotor activity, all behavioral activity, represents the sum of all counts in the horizontal frame. Ambulatory activity is the number of counts produced by two consecutive photobeams interruption in the horizontal frame. Rearing activity represents total counts of vertical motion.

Stereotypic activity

Rats were videotaped for 1 minute at 15 and 30 minutes post-injection. The videotapes were later analyzed for qualitative behavioral stereotypy activity by three trained observers blind to each animal's treatment group. The rating for cocaine-induced stereotypic behaviors was based on the Creese and Iversen (1974) scale (Creese and Iversen, 1974). This scale (summarized in Table 1) consists of 10 scores, ranging from a score of 1 (given to an animal that was asleep or inactive) to 10 (given to an animal that

exhibited splayed hind limbs). A score of 10 was never observed during the course of this experiment.

Table VI: ***Rating Scale from Daunais and McGinty (1995)***

Score	Behavior
1	Asleep, inactive
2	Alert, actively grooming
3	Increased sniffing in one location
4	Intermittent rearing and sniffing
5	Increased locomotion and sniffing
6	Intense sniffing in one location
7	Continuous pivoting and sniffing
8	Continuous rearing and sniffing
9	Maintained rearing and sniffing for >25 seconds
10	Splayed hid limbs

Plasma levels of cocaine metabolite and corticosterone

Trunk blood was allowed to clot and then centrifuged 3,000 RPM for 15 minutes at 4□C. Plasma was collected and stored at -80□C until used. Samples were analyzed for benzoylecognine and corticosterone with Coat-A-Count Radioimmunoassay kits (Diagnostic Product Corporation, CA). Intra-assay coefficient of variance averaged less than 10%. Results were determined using a log-logit computer program.

Data analysis

Locomotor activities

To examine the responses after cocaine or saline administration between male and female rats, three-way RM ANOVAs were used: CONDITION (saline vs. cocaine) X SEX (male vs. female) X LENGTH OF TREATMENT [1, 7, 14 or challenge]. Significant differences between groups (male vs. females: 1, 7, 14, and challenge) were examined using separate one-way ANOVAs. This method was chosen because there is no valid post hoc test to make between versus within groups comparisons (Winer et al., 1991).

Stereotypic behaviors

Friedman RM ANOVAs were used to locate differences between sex and drug treatment groups on each treatment day, followed by Kruskal Wallis H tests, when appropriate.

Plasma levels of cocaine metabolite and corticosterone

Two way ANOVAs were used to examine the effects of acute (1 day), chronic (14 days), and challenge dose administration on plasma levels of benzoylecognine and corticosterone.

Results

Cocaine-induced total locomotor activity

There was significantly greater total locomotor activity in cocaine-treated rats than saline-treated control groups ([$F(1,54) = 88.380$, $p<0.001$]; Figure 4A). Furthermore, cocaine-treated female rats displayed higher total locomotor activity than male rats [$F(1,54) = 9.362$, $p<0.005$]. Across the length of drug treatment, there was a significant interaction between drug and day of cocaine administration [$F(3,162) = 2.775$, $p<0.05$]; cocaine-treated male rats displayed higher counts of total locomotor activity on Days 14 and 21 (after the cocaine challenge) when compared to Days 1 and 7 [Day 14: $p<0.05$; $p<0.005$; Day 21: $p<0.05$; $p<0.02$; respectively]. However, cocaine-treated female rats had significantly higher total locomotor activity only after cocaine challenge when compared to Days 1, 7 and 14 [$p<0.02$; $p<0.05$; $p<0.05$; respectively]. No differences were observed in the total locomotor activity of female or male saline-treated rats [$F(3,87) = 1.33$, $p>0.1$], [$F(3,87) = 1.85$, $p>0.1$] respectively.

Ambulatory Activity

Overall, cocaine induced increases in ambulatory activity when compared to saline-treated controls ([$F(1,54) = 44.421$, $p<0.001$]; Figure 4B). Similar to total locomotor activity, cocaine-treated female rats had significantly higher levels of ambulatory activity than cocaine treated male rats [$F(1,54) = 10.514$, $p<0.005$]. Cocaine-treated male rats had significantly higher ambulatory counts only on Day 14 when compared to Day 1 ($p<0.05$). On the other hand, female rats had significantly higher ambulatory counts after both Day 14 ($p<0.02$) and the challenge dose of cocaine ($p<0.002$) when compared to Day 1.

Rearing Activity

Overall, cocaine-treated rats displayed more rearing activity than saline-treated rats ([$F(1,106)=66.251$, $p<0.001$]; Figure 4C). Although cocaine-treated female rats reared more than male rats [$F(1,106)=4.327$, $p<0.05$], there were no statistically significant changes in rearing activity over time in both male and female rats [$F(3,318)=1.988$, $p>0.1$].

Cocaine induction of stereotypic behaviors:

On Day 1, saline-treated control female rats had statistically significant differences between 15 and 30 minutes scores of stereotypic behavior [Friedman ANOVA: ($df1=5.444$; $p<0.02$]. This could be due to stress-induced stereotypic activity after the first time of handling. Since no statistically significant changes were observed in any of the other treatment groups or any of the other treatment days, the sum of scores (cumulative stereotypic activity) after 15 and 30 minutes was used for all groups.

Cocaine-treated rats had higher scores of stereotypic behavior than saline-treated controls [Day 1: $H(1, N=48)=25.979$, $p<0.001$; Day 7: $H(1, N=48)= 24.968$, $p<0.001$; Day 14: $H(1, N=48)=24.913$, $p<0.001$; Challenge dose: $H(1, N=24)= 14.175$, $p<0.001$; Figure 4D]. When compared to male rats, cocaine-treated female rats had significantly higher stereotypic scores after 1, 7 and 14 days of cocaine administration [Day 1: $H(1, N=24)=6.857$, $p<0.01$; Day 7: $H(1, N=24)=4.133$, $p<0.05$; Day 14: $H(1, N=24)=8.767$, $p<0.005$]. Furthermore, across the length of cocaine administration, no differences in the stereotypic response to cocaine were observed within either female or male rat groups [Females: ANOVA ($N=6$, $df=3$)$=0.666$, $p>0.1$; Males: ANOVA ($N=6$, $df=3$)$= 3.631$, $p>0.1$].

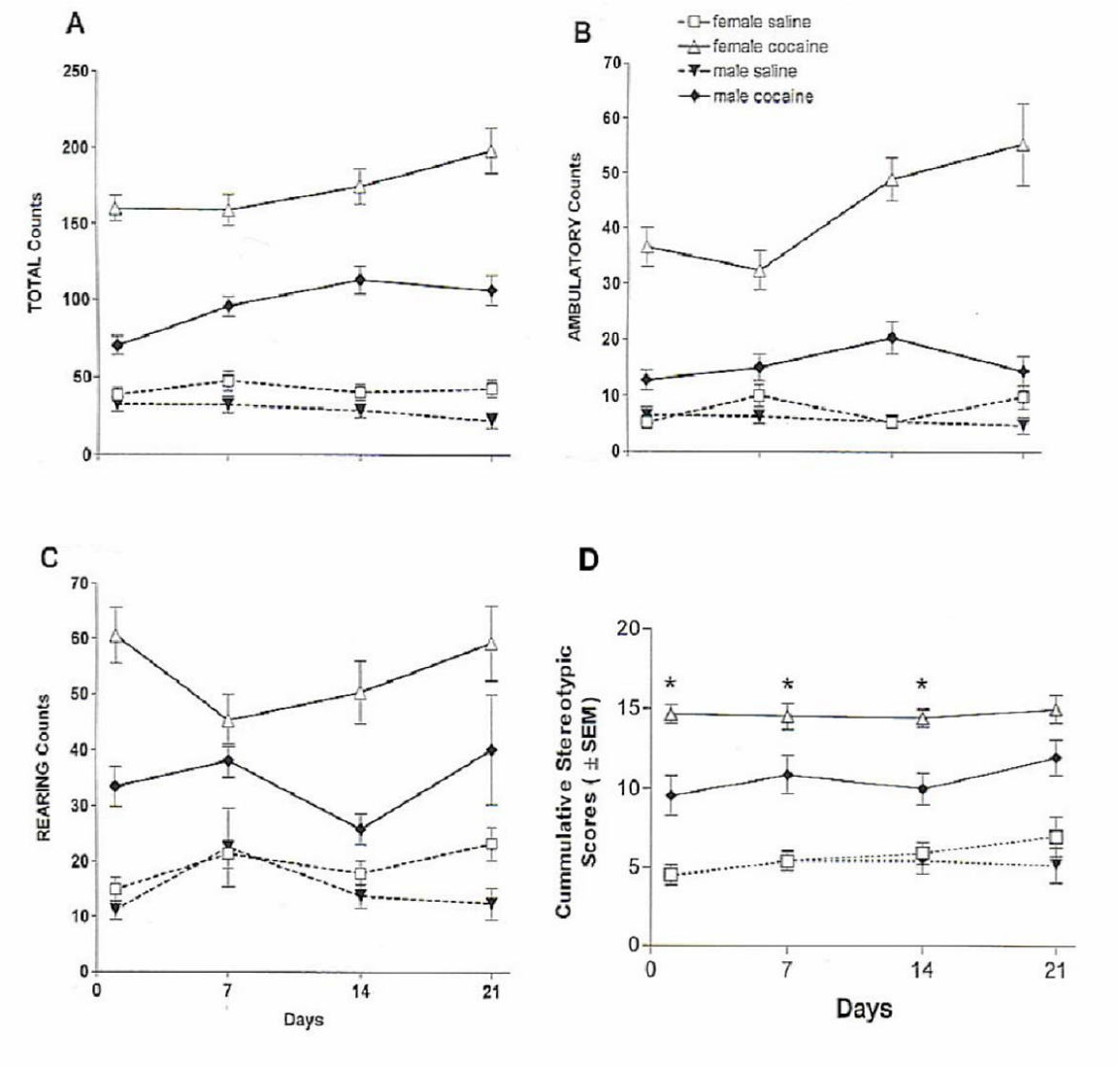


Figure 6. (A) Total locomotive, (B) Ambulatory, and (C) Rearing activity of male and female rats after 1, 7, 14 and 21 days of cocaine administration. All data represent a cumulative Mean ± SEM of 30 minute bins. (D) Stereotypic scores of male and female rats after 21 days of cocaine administration. All data represent a cumulative of 15 and 30 minute scores ± SEM (*$p<0.05$). This data was adapted from Chin et al., 2001 and was analyzed by two-way ANOVA.

Sex differences in benzoylecognine and corticosterone plasma levels

When compared to cocaine-treated male rats, female rats had higher levels of benzoylecognine after acute cocaine administration ($t=2.66$, $p<0.02$), but not after chronic and challenge cocaine administration ($p>0.2$, respectively; Figure 5 A,B,C).

Overall, female rats had higher plasma levels of corticosterone than male rats ([$F(1,17)$ 6.689, $p<0.001$], Figure 5 A,C). Cocaine-treated female rats had higher corticosterone plasma levels after acute and challenge cocaine treatments when compared to their respective saline-treated controls [Day 1: $p<0.05$ and Challenge dose: $p<0.02$]. No significant differences in corticosterone plasma levels were observed in male rats throughout the different cocaine administration paradigms ($p<0.1$).

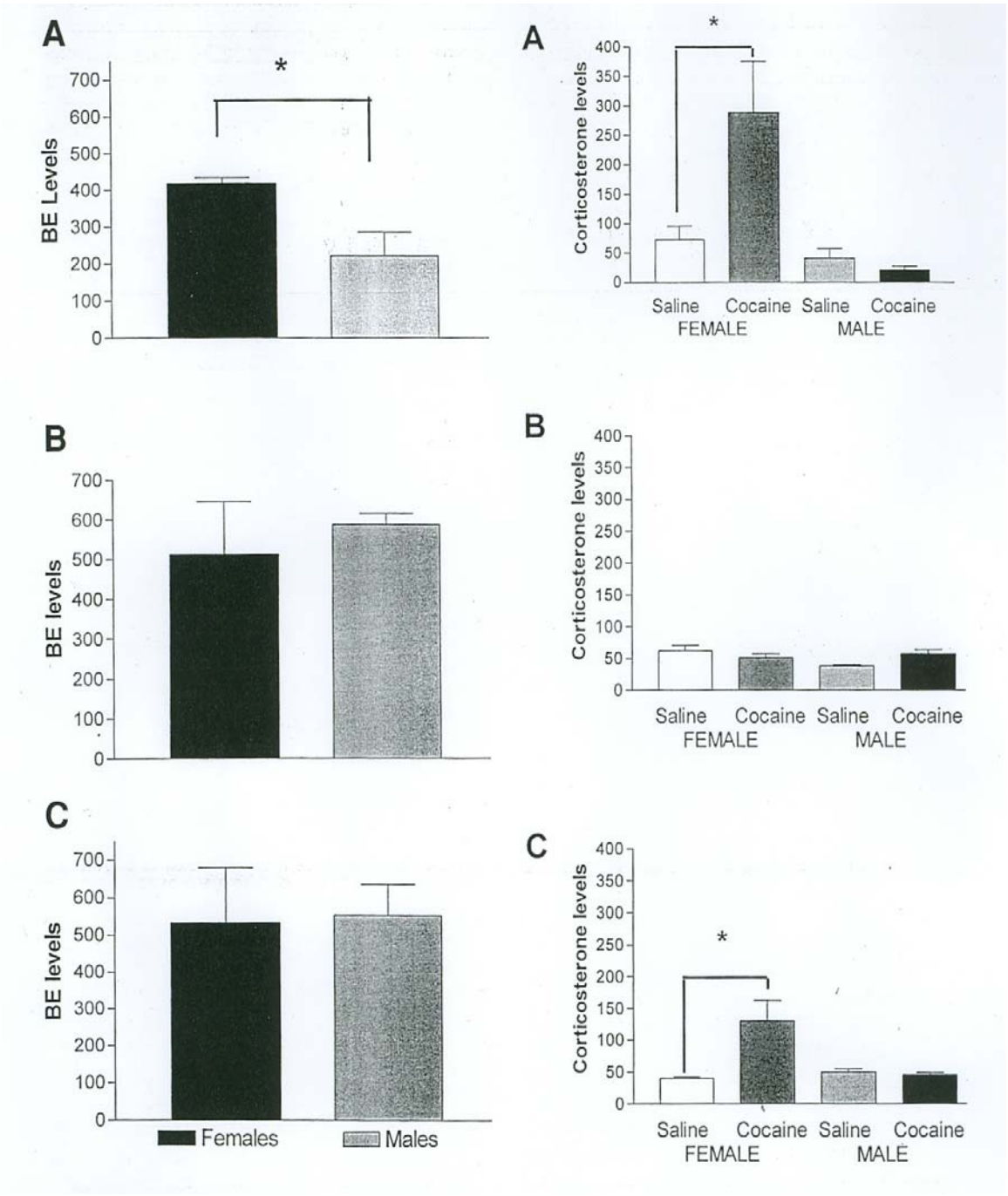


Figure 7. Levels of benzoylecognine (BE) or corticosterone measured in ng/ml of cocaine-treated female and male rats after (A) acute administration, (B) chronic administration, or (C) a challenge dose of cocaine. All data represent Mean ± SEM (*$p<0.05$). This data was adapted from Chin et al., 2001 and was analyzed by two-way ANOVA.

Discussion

Similar to previous observations, male and female rats had different psychomotor responses to acute cocaine. Female rats had significantly higher ambulatory and total counts after acute cocaine administration than male rats (Craft and Stratmann, 1996; Glick et al., 1983b; Kuhn and Francis, 1997; Kuhn et al., 1999; Van Haaren and Meyer, 1991). Female rats also demonstrated higher rearing activity after acute cocaine administration than male rats. Thus, overall, after acute cocaine administration, female rats demonstrated hyperactivity in all the elements of the locomotor activity measured when compared to cocaine-treated males.

Similar to previous studies, after chronic cocaine administration in both male and female rats, we observed the development of sensitization to cocaine-induced ambulatory and total locomotor activities. Moreover, this report expands previous observations by determining that there are sex differences in the maintenance of cocaine-induced sensitization. Female rats demonstrated cocaine-induced behavioral sensitization in ambulatory and total locomotor activity after the challenge with cocaine, while male rats maintained their behavioral sensitization only in total locomotor activity. Furthermore, although overall rearing activity was not affected by repeated injections or a challenge dose of cocaine administration in both male and female rats, female rats reared more during the first 5 minutes after the challenge dose of cocaine on day 21 (data not shown). Taken together, this study suggests that there are sex differences in cocaine-induced behavioral sensitization on these different psychomotor responses both in the development and maintenance of sensitization. This may highlight differences in the pattern of chronic cocaine abuse between males and females.

Similar to Walker et al., (1997) (Walker et al., 1997), we observed that female rats have markedly enhanced stereotypic behaviors in response to the first dose of cocaine than males. This study extends these observations by demonstrating that although, female rats' stereotypic response to acute, chronic and sub-chronic cocaine administration was higher than male rats, no sensitization to cocaine-induced stereotypic activity was observed in either male or female rats. Thus, sensitization to cocaine occurs in only certain aspects of cocaine-induced behavioral activity, i.e. locomotor behavior but not stereotypic activity.

The hypothalamo-pituitary-adrenal (HPA) axis activation has been postulated to be essential for the control of behavioral and neurochemical alterations by cocaine. For example, the manipulation of corticosterone levels influences locomotor responses to cocaine as well as the development of sensitized behavioral responses following cocaine administration (Marinelli et al., 1994; Rough-Pont et al., 1995). Similar to previous observations, female rats exhibited greater HPA activation than male rats after acute cocaine administration (Kuhn and Francis, 1997). We extend these results by demonstrating that chronic cocaine administration did not alter corticosterone plasma levels in female rats, indicating the possible development of tolerance of HPA activity. However, a challenge dose of cocaine in behaviorally sensitized female rats caused an increase in corticosterone levels. Thus, suggesting a desensitization of HPA activity after withdrawal of cocaine or a return to a hypersensitive HPA activity. Although, augmented responses in corticosterone plasma levels have been previously shown after challenge with cocaine in sensitized male rats (Marinelli et al., 2000), we did not observe alterations in corticosterone levels in male rats over time. Discrepancies between both

reports may reside in the strain of rat or cocaine administration paradigm used. This remains to be elucidated. It is provocative to postulate that differences in HPA activity might underlie some mechanisms in which sex differences to cocaine- induced psychomotor activity may occur.

Major metabolites of cocaine are benzoylecgonine (BE) and ecgonine methyl ester. It has been postulated that cocaine metabolism may be influenced by an individual's hormonal status, which in turn, may underlie the differences in vulnerability to cocaine's effects between males and females (Mendelson et al., 1999b; Van Haaren and Meyer, 1991). We have previously demonstrated that there are estrous cycle differences and ovarian hormone effects on BE levels after "binge" but not acute cocaine administration (Perrotti et al., 2001b; Quinones-Jenab et al., 2000a; Quiñones-Jenab et al., 1999). Van Haaren and Meyer (1991) (Van Haaren and Meyer, 1991) reported no sex differences in BE serum levels after acute cocaine administration (10 mg/kg i.p.). However, Bowman et al., (1999b) reported that BE levels were 2-fold lower in plasma and brains of female as compared to male rats after acute cocaine administration using a similar dose and route of administration. Although we found sex differences in BE levels, contrast to Bowman et al., (1999) (Bowman et al., 1999b) female rats had approximately 2-fold higher BE plasma levels than male rats after acute cocaine administration. However, after chronic and challenge cocaine administration, no sex differences in BE plasma levels were observed. This is contradictory to previous results by Van Haaren and Meyer (1991) (Van Haaren and Meyer, 1991) who reported that after chronic cocaine administration, female rats had lower BE serum levels than male rats. However, Van Haaren and Meyer (1991) (Van Haaren and Meyer, 1991) used a different dose and

length of cocaine administration (22 days continuous, i.p., 10 mg/kg). Overall, sex differences in BE do not completely explain the exaggerated locomotor and stereotypic responses after the different cocaine administration paradigms, nor the difference in sensitization to the behavioral effects of cocaine of female rats. This may support the idea that differences in the endocrinological profiles of females versus males or HPA activity are more likely to underlie sex differences to cocaine-induced alterations than cocaine metabolism.

It is provocative to postulate that sex differences in cocaine-induced behavioral activity and sensitization may involve differential regulation by gonadal hormones (i.e., estrogen, progesterone, and testosterone) in CNS activity. Moreover, these steroids have been postulated to be important in the control and development of different components of CNS activity associated with learning and memory (Luine, 1997). Due to the possible overlap of CNS mechanisms of cocaine-induced behavioral sensitization and learning and memory, the development and maintenance of behavioral sensitization may be similarly regulated by gonadal hormones. Of key importance are the health disparities that may relay in sex differences on the development and maintenance of cocaine behavioral and endocrinological sensitization. These sex differences in cocaine-induced behavioral sensitization, HPA activity, and/or cocaine metabolism may lead to overdose or other clinical complications. This important clinical issue needs further investigation.

CHAPTER 3: Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration

Previous studies in rats have demonstrated sex differences in cocaine-induced behavioral and neurochemical alterations. For example, toxic effects of cocaine are sexually dimorphic: male rats developed a cardiovascular toxic reaction to cocaine at lower plasma concentrations of the drug than females (Morishima et al., 1993). Female rats do acquire cocaine discrimination at a faster rate than males (Craft and Stratmann, 1996) and display an increased motivation to self-administer cocaine than males (Roberts et al., 1989). Additionally, reinstatement of extinguished cocaine-reinforced responding is greater in female rats (Lynch and Carroll, 2000) and, overall, females are usually more hyperactive after the first cocaine administration (demonstrating either augmented or exaggerated responses to most of the cocaine-induced behavioral alterations) (Berul, C. I. and Harclerode, J. E.89; Caihol and Morméde, 1999; see chapter 2; Sircar and Kim, 1999; Van Etten and Anthony, 1999; Walker et al., 1997). Sex differences on the development of cocaine-induced behavioral sensitization after repeated or intermittent administration of cocaine (see chapter 2; Grimm and See, 1997; King et al., 1990; Quinones-Jenab et al., 2000a) have been demonstrated where female rats have higher levels of sensitization to repeated cocaine administration (Glick et al., 1983b), were sensitized with a lower dose of cocaine than male rats (Post et al., 1981), and demonstrate longer-lasting behavioral sensitization to cocaine (see chapter 2).

Gonadal hormones have been postulated to be important determinants of cocaine effects by influencing neuronal activity and plasticity of the brain (Cunningham, K. A., 95; Hruska and Pitman, 1982; Morishima et al., 1993; Perrotti et al., 2001a; Perrotti et al.,

2000; Quinones-Jenab et al., 2001; Sell et al., 2000; Sell et al., 1998; Van Etten and Anthony, 1999). For example, estrogen regulates cocaine-induced behavioral responses after acute and chronic cocaine administration. Progesterone has also been reported to modulate different aspects of cocaine alterations (Castner et al., 1993; Hruska and Pitman, 1982; Sell et al., 2000). In turn, cocaine modulates progesterone plasma levels in intact (Roberts et al., 1989) and pregnant female (Quiñones-Jenab et al., 2000a) as well as male rats (Kuhn et al., 1999; Quinones-Jenab et al., 2000c; Walker et al., 2001c). On the other hand, in male rats, testosterone levels are also affected by cocaine administration whereas "binge" (Quinones-Jenab et al., 2000c) or single dose (Berul, C. I. and Harclerode, J. E.89; Quinones-Jenab et al., 2000c) cocaine administration significantly decreases testosterone plasma levels.

Little is known about the contribution of endogenous gonadal hormones on cocaine-induced behavioral responses and the development of sensitization. Therefore in this study, we conducted experiments to address the question of how endogenous gonadal hormones affect cocaine-induced locomotor and stereotypic activity as well as known cocaine-induced neuroendocrinological alterations in some components of the hypothalamic-pituitary-adrenal (HPA) and/or hypothalamic-pituitary-gonadal (HPG) axis. To address these questions, the present study investigates the effects of acute (1 day), sub-chronic (7 days), chronic (14 days), or withdrawal/challenge (on day 21) cocaine on behavioral (locomotor and stereotypic) and neurochemical (corticosterone, progesterone, testosterone, and benzoylecgonine plasma levels) responses in intact and gonadectomized male and female rats.

Methods

Animals and cocaine administration

Two cohorts (24 animals each) of eight-week-old intact and gonadectomized (GDX) female and male Fischer rats (Taconic Laboratory) were individually housed in standard cages with free access to food and water. Rats were maintained on a 12-hour light/dark cycle with lights on at 10:30 A.M. EST. One week after arrival, the rats were randomly assigned to either cocaine- or saline-treatment groups and one of the following experimental conditions: intact/female, gonadectomized (GDX)/female, intact/male, or GDX/male (n=6/groups).

Rats received daily i.p. injections of cocaine (15 mg/kg; dissolved in 0.9% saline) or saline for 14 days followed by 6 days of withdrawal and on day 21, according to the animal's respective experimental group, a single cocaine or saline injection. All injections were administered in each rat's home cage. Thirty minutes after the last drug treatment, rats were briefly (30 seconds) exposed to CO_2. Following decapitation, trunk blood was collected. NIH guidelines for the care and use of laboratory animals were strictly followed.

Behavioral assays

All behavioral assays were performed in home cages for 30 minutes after cocaine administration. Both stereotypic and locomotive activities were analyzed for each animal on days 1, 7, 14, or after the challenge dose of cocaine or saline administration.

Locomotor activity

The spontaneous locomotor activity of each animal was monitored with a Photobeam Activity System from San Diego Instrument, which records vertical and horizontal activity as previously described [6, 24]. 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.

Stereotypic activity

Rats were videotaped for 40 seconds at 15 and 30 minutes post- injection. 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 was based on the Creese and Iversen scale [8]. This scale consists of 10 scores, ranging from 1 (given to an animal that was asleep or inactive) to 10 (given to an animal that exhibited splayed hind limbs; see Table 1.

Plasma levels of cocaine metabolite and steroid hormones

Trunk blood was allowed to clot and then centrifuged 3,000 RPM for 15 minutes at 4° C. Plasma was collected and stored at - 70° C until used. Samples were analyzed with Coat-A-Count radioimmunoassays (Diagnostic Product Corporation (CA)) for benzoylecgonine, testosterone, progesterone, or corticosterone. The intra-assay coefficient of variance averaged less than 12%. Results for assays were determined using a log-logit computer program.

Data analysis

Locomotor activity

To examine the response to cocaine administration between male and female rats, a four-way analysis of variance (ANOVA) was used: condition (saline vs. cocaine) X Sex (male vs. female) X GDX (intact vs. gonadectomized) X Length of drug treatment (1, 7, 14, or challenge). When significant, differences between groups were then examined using separate one-way ANOVAs or respective *post hoc* tests. This method was chosen since there is no valid *post hoc* test to make within group comparisons [43].

Stereotypic behaviors

To examine the effects of gonadectomy on cocaine-induced stereotypy, a Kruskal-Wallis analysis of variance (ANOVA) was used, followed by Mann-Whitney U tests with Bonferroni adjustment for multiple comparisons.

Plasma levels of cocaine metabolite, progesterone, testosterone, and corticosterone

To examine the effects of a challenge dose of cocaine administration on plasma levels of benzoylecgonine, gonadal hormones, and corticosterone, ANOVAs were used, followed by Newman-Keuls *post hoc* tests or t-tests when appropriate.

Results

Ambulatory Activity

Overall, cocaine significantly increased ambulatory activity when compared to saline-treated controls [Day 1: $F(1,232)=2.786$, $p<0.005$; Day 7: $F(1,192)=9.86$, $p<0.002$; Day 14: $F(1, 223)=3.43$, $p<0.02$; Day 21: $F(1, 222)=25.42$, $p<0.001$], Figure 6. A significant interaction between sex, drug and day of administration was obtained [F

(9,555)=2.786, $p<0.005$] where sex differences were observed after Day 7 and 14 and also after cocaine challenge [Day 7: $F(3,192)=3.392$, $p<0.02$; Day 14: $F(3,223)=3.423$, $p<0.02$; Day 21: $F(3,222)=6.293$, $p<0.001$], but not after acute cocaine administration [$p>0.05$]. Across time, cocaine-treated female rats had significantly higher levels of ambulatory activity than GDX or intact male and GDX female rats [$F(1,54)= 10.514$, $p<0.0005$], Figure 6 A,B.

Intact female rats had significantly higher ambulatory counts after 7 and 14 days or cocaine challenge when compared to acute cocaine administration ($p<0.05$ for all comparisons). Similar to total locomotor activity, ambulatory activity was progressively higher through time, where ambulatory activity after 7 days of cocaine administration was significantly lower when compared to activity after 14 days of cocaine administration. In turn, ambulatory activity after 14 days of cocaine administration was significantly lower than after cocaine challenge administration ($p<0.005$). Although acute cocaine administration induced ambulatory activity in GDX female rats ($p<0.02$), after 7 and 14 days of drug administration, no statistically significant differences were observed between saline- and cocaine-treated GDX female groups. Furthermore, unlike intact female rats, GDX females had significantly higher ambulatory counts only after the challenge dose of cocaine when compared to activity after 1 and 7 days of cocaine administration ($p<0.05$ for all comparisons).

In saline-treated intact female rats, ambulatory activity also increased through time, where ambulatory activity after acute saline administration was significantly lower than activity after day 7 to day 21 of saline-challenge ($p<0.05$ for all comparisons). No

differences in ambulatory activity through time were observed in saline-treated GDX females group ($p>0.5$).

As in intact female rats, through time, ambulatory activity in intact male rats gradually increased after cocaine administration, whereas after 7 and 14 days or a cocaine challenge, ambulatory counts were higher than acute cocaine administration [$p<0.05$], Figure 6B. In addition, ambulatory activity after chronic- and challenge- cocaine administration was higher than after 7 days. This gradual increase in ambulatory activity was also observed in saline-treated intact males where ambulatory counts were significantly lower on day 1 when compared to day 7 and day 21 (challenge administration, $p<0.05$).

After 14 days of cocaine administration, GDX male cocaine rats had significantly higher ambulatory activity when compared to day 1, 7, or cocaine challenge [$p<0.01$; $p<0.002$; $p<0.02$, respectively]. Saline-treated GDX male rats also had higher ambulatory activity after 14 days of saline administration when compared to 1, 7, or saline-challenge on day 21 [$p<0.001$; $p<0.005$; $p<0.005$, respectively].

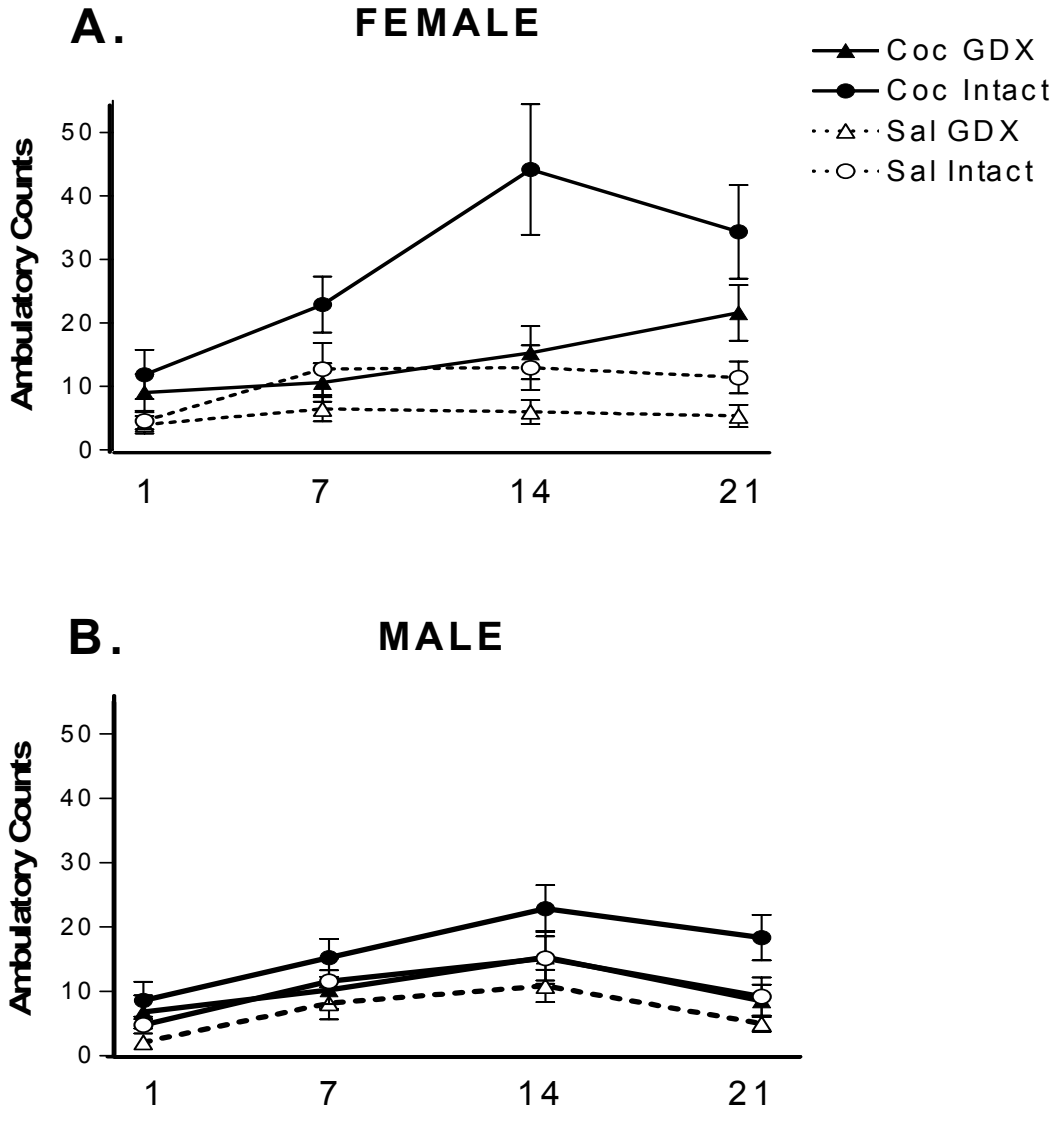


Figure 8. ***Total ambulatory activity after acute, sub-acute, chronic, and withdrawal/challenge cocaine or saline administration.*** Total ambulatory activity is represented as cumulative mean ± SEM after cocaine (solid line) or saline (dashed line) administration in female (A) or male (B) rats in one of the following groups: GDX saline (open triangle), GDX cocaine (solid triangles), Intact saline (open circles), or Intact cocaine (solid circles). N=6 per group. This data was adapted from Chin et al., 2002 and was analyzed by two-way ANOVA.

Rearing Activity

Overall, cocaine-treated rats displayed more rearing activity than saline-treated rats [Day 1: $F(1,224)=31.66$, $p<0.001$; Day 7: $F(1, 192)=32.70$, $p<0.001$; Day 14: $F(1,223)= 31.20$; $p<0.001$; Day 21: $F(1,222)=41.99$, $p<0.05$], Figure 7. Furthermore, a significant interaction between sex, drug, and the length of cocaine administration was observed [$F(9,555)=2.70$, $p<0.005$] where intact and GDX female rats had higher rearing activity than GDX and intact male rats [$F(3,222)=2.69$, $p<0.05$]. Intact female rats had increase rearing activity on 7, 14, or after the challenge when compared to acute treatments [$p<0.005$], Figure 7A. Although rearing activity was higher after cocaine challenge when compared to groups treated with one day of cocaine, it failed to reach statistical significance ($p=0.056$).

After acute cocaine administration, no statistically significant differences were observed between cocaine- or saline-treated intact ($p=0.26$) or GDX ($p=0.054$) male rats, Figure 7B. Both intact and GDX male rats exhibited significantly higher rearing behaviors after 14 days of cocaine administration than after acute cocaine administration [$p<0.05$, for all comparisons].

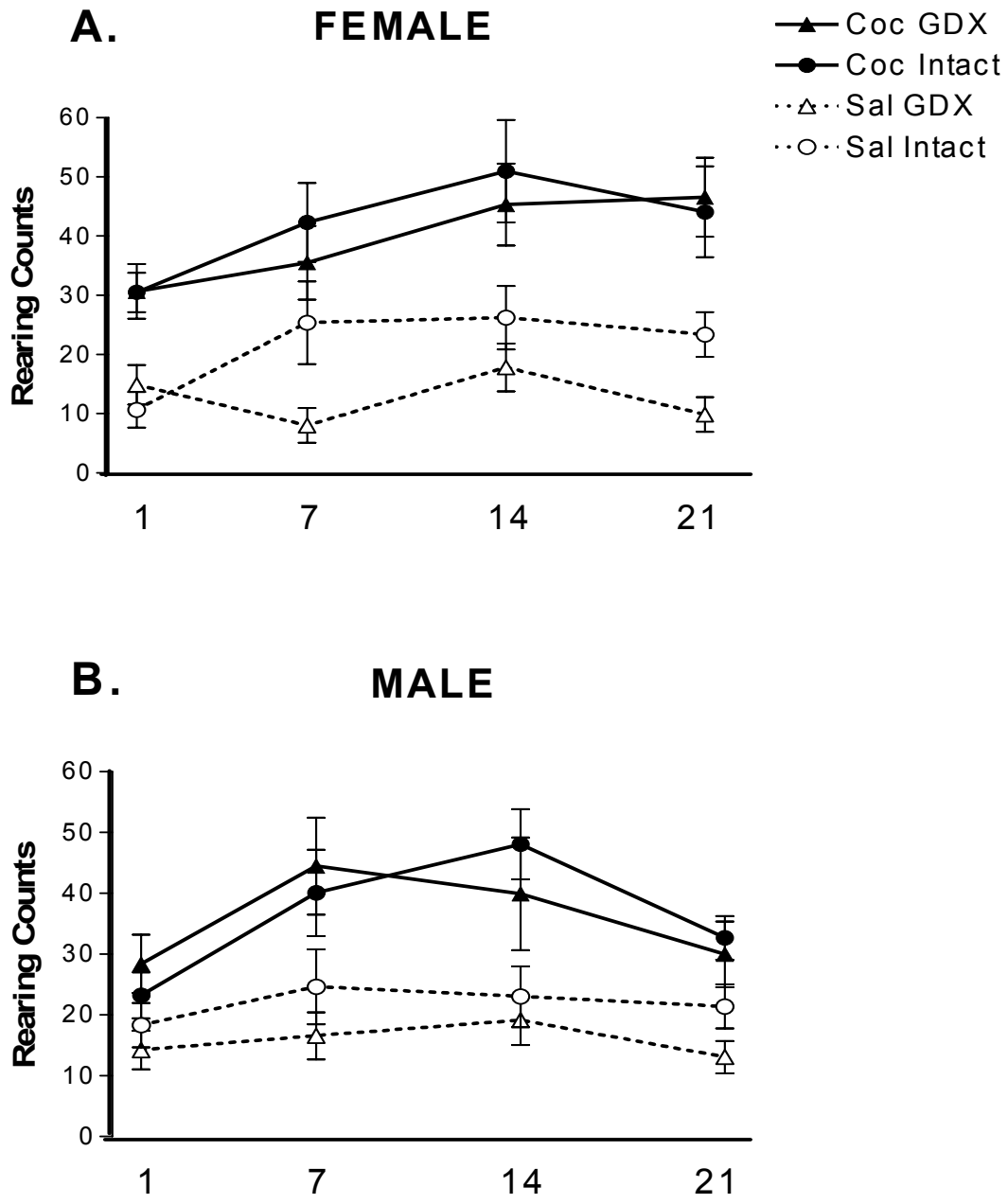


Figure 9. ***Total rearing activity after acute, sub-acute, chronic, and withdrawal/challenge cocaine or saline administration.*** Total rearing activity is represented as cumulative mean ± SEM after cocaine (solid line) or saline (dashed line) administration in female (A) or male (B) rats in one of the following groups: GDX saline (open triangle), GDX cocaine (solid triangles), Intact saline (open circles), or Intact cocaine (solid circles). N=6 per group. This data was adapted from Chin et al., 2002 and was analyzed by two-way ANOVA.

Cocaine induction of stereotypic behaviors

No statistically significant differences were observed on stereotypic scores between 15 and 30 minutes after cocaine administration; thus, cumulative scores are represented on Figure 8. Overall, cocaine-treated rats had higher scores of stereotypic behaviors than saline-treated controls [$p<0.05$], Figure 8. In saline controls, no significant differences between intact or GDX male and female rats were observed through time in stereotypic activity [$p>0.05$]. However, across the length of cocaine administration, stereotypic response to cocaine was higher on days 7 and 14 for intact male rats when compared to acute cocaine administration [$p<0.05$]. GDX male rats demonstrated significantly higher cocaine-induced stereotypic activity after the challenge when compared to acute cocaine administration [$p<0.05$]. No statistically significant differences were observed in any of the cocaine-treated intact or GDX female rats.

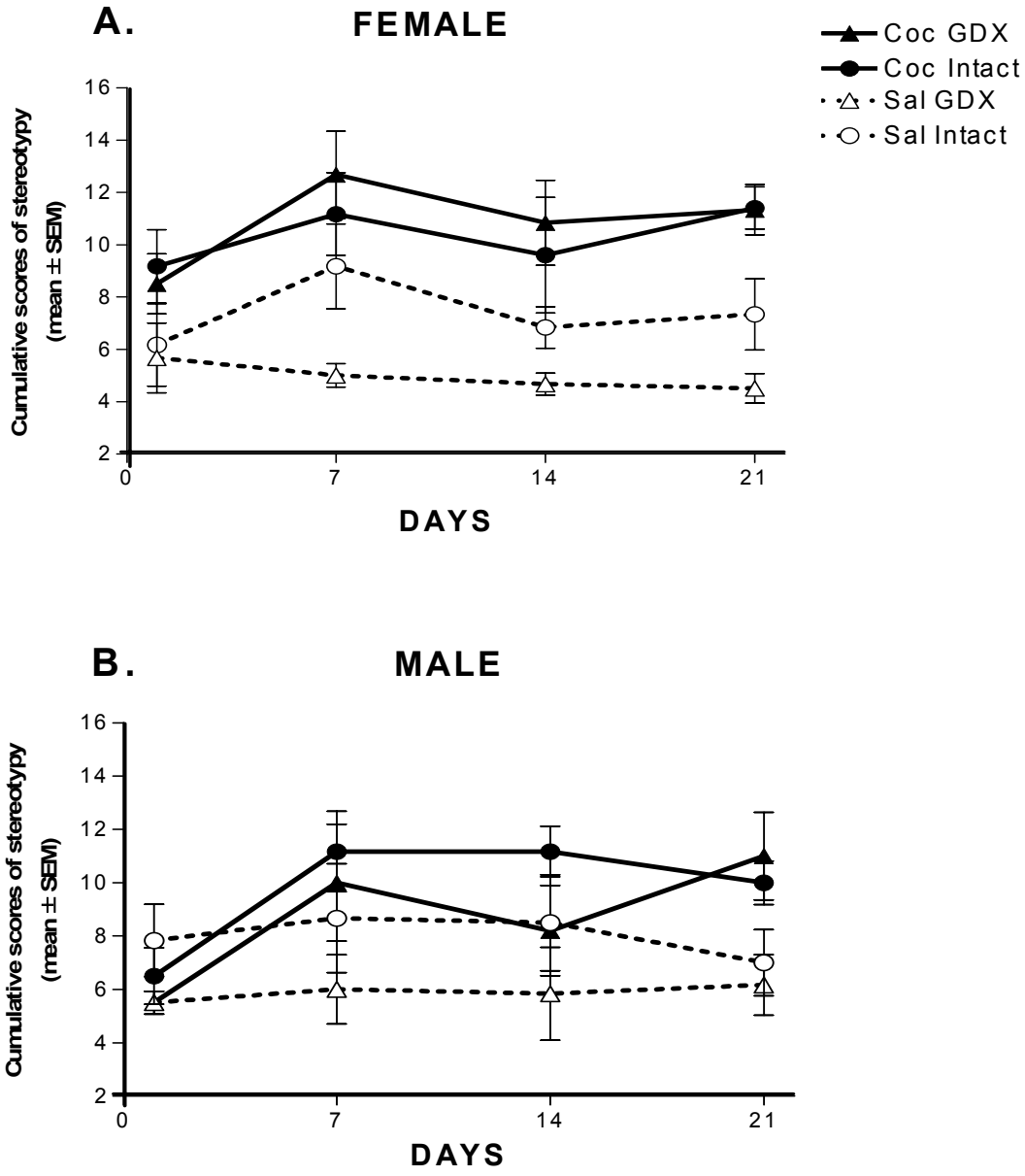


Figure 10. ***Stereotypic behaviors after acute, sub-acute, chronic, and withdrawal/challenge cocaine or saline administration.*** Stereotypic activity is represented as cumulative mean ± SEM after cocaine (solid line) or saline (dashed line) administration in female (A) or male (B) rats in one of the following groups: GDX saline (open triangle), GDX cocaine (solid triangles), Intact saline (open circles), or Intact cocaine (solid circles). N=6 per group. This data was adapted from Chin et al., 2001 and was analyzed by two-way ANOVA.

Sex differences in benzoylecgonine, testosterone, progesterone, and corticosterone plasma levels

After the cocaine challenge, no significant differences were observed in levels of the cocaine metabolite benzoylecgonine (BE) in any of the experimental groups [$p>0.5$], Table 2. Testosterone levels were significantly lower in cocaine-treated intact male rats when compared to saline groups [$p<0.05$], Table 2. No statistically significant differences were observed in testosterone plasma levels of saline- vs. cocaine- treated intact/GDX females or GDX males. On the other hand, cocaine [$F(1,35)=15.77$, $p<0.005$] and sex [$F(3,35)=28.07$, $p<0.00001$] effects were observed on progesterone plasma levels where progesterone plasma levels were significantly higher on cocaine-treated intact female rats when compared to saline controls [$p<0.0001$]; Table 2. Although cocaine-treated GDX male rats had higher plasma levels of progesterone when compared to saline-treated controls, it failed to reach statistical significance ($p=0.052$).

Overall, cocaine induced corticosterone levels [$F(3,37)=8.19$, $p<0.0002$]. Intact females and GDX female and male rats had significantly higher corticosterone plasma levels after cocaine challenge when compared to their respective saline controls, Table 2. No significant differences in corticosterone plasma levels were observed in intact male rats ($p<0.1$). Although there were apparent sex differences on cocaine-induced alterations in corticosterone plasma levels, it failed to reach significance [$F(1,37)=3.61$, $p=0.055$].

	Intact Female		*GDX Female*		*Intact Male*		*GDX Male*	
	Saline	***Cocaine***	***Saline***	***Cocaine***	***Saline***	***Cocaine***	***Saline***	**Cocaine**
Benzoylecgonine	NA	4.41±1.20	NA	3.97±0.82	NA	2.82±0.92	NA	3.59±1.01
Testosterone	2.54±0.26	2.41±0.05	2.70±0.13	4.95±0.87	**251.62±21.40**	**158±18.00***	1.86±0.07	1.98±0.09
Progesterone	**4.08±1.58**	**18.37±4.70***	0.29±0.08	0.68±0.25	0.46±0.06	0.44±0.16	0.29±0.06	1.07±0.43
Corticosterone	**50.04±13.40**	**221.08±49.70***	**33.24±84.48**	**334.32±74.46***	77.35±22.14	155.14±70.77	**54.90±9.33**	**188.63±47.40***

Table VII: Benzoylecgonine, testosterone, progesterone and corticosterone plasma levels for all experimental groups after withdrawal. Mean ± SEM plasma levels of progesterone, corticosterone and testosterone are expressed as ng/ml. Benzoylecgonine levels are expressed as mg/ml. N=6/group. * and bold numbers represent significant ($p<0.05$) differences between saline- and cocaine-treated rats analyzed by a two-way ANOVA. NA= to non-detectable levels. This data was adapted from Chin et al., 2002.

Discussion

Consistent with previous studies, female rats demonstrated exaggerated ambulatory responses to acute and chronic cocaine administration when compared to male rats (see chapter 2; Glick et al., 1983b; Kuhn and Francis, 1997; Quinones-Jenab et al., 2001; Van Haaren and Meyer, 1991). However, in terms of other components of psychomotor activation, such as stereotypic and rearing activity, no sex effects were observed after different cocaine administration paradigms. These results suggest that there are sex differences in only certain aspects of the psychomotor response to cocaine, i.e., ambulatory activity. Overall, gonadectomy did not affect the behavioral responses to acute cocaine administration. Thus, this suggests that endogenous gonadal hormones have little or no effect on acute cocaine-induced behavioral activity.

Sex differences on the development of cocaine-induced behavioral sensitization have also been reported (female rats demonstrated higher levels of sensitization to repeated cocaine administration (see chapter 2; Glick et al., 1983b) and were sensitized with a lower dose of cocaine than male rats (Post et al., 1981). Using the same manner and doses of cocaine administration as this study, we have recently reported sex differences in the development and maintenance of sensitization to different components of the cocaine-induced behavioral activities (see chapter 2; Craft and Stratmann, 1996). We have extended our previous published results by demonstrating a role of endogenous gonadal hormones in certain aspects of the development and maintenance of cocaine-induced behavioral sensitization. For example, although both intact male and female rats were able to develop a gradual behavioral sensitization to ambulatory activity after sub-chronic cocaine administration and maintained higher behavioral activities after

withdrawal and challenge with cocaine, after castration, rats had only statistically higher levels of behavioral activity after chronic cocaine administration. On the other hand, ovariectomy delayed the development of sensitization to after challenge with cocaine. Thus, this suggests a possible role of endogenous gonadal hormone in the mediation of some aspects of cocaine-induced ambulatory behavioral sensitization. Since gonadal hormones play a pivotal role in the development and maintenance of learning and memory (reviewed in (Quinones-Jenab et al., 2001)), it is possible that gonadectomy (and thus the removal of estrogen, progesterone, and testosterone) may ultimately affect the development of sensitization via learning and memory impairments. This remains to be elucidated. Interestingly, some discrepancies between this and previous results from our laboratory may reside in vendor differences (Taconic vs. Charles River) (Perrotti et al., 2001a).

While no significant differences were observed in rearing activity between GDX and intact male rats, overall, intact female rats rear more than GDX female rats. Furthermore, intact rats demonstrated behavioral sensitization from sub-chronic to challenge administration while GDX rats did not demonstrate sensitization in rearing activity. Interestingly, GDX and intact male groups both were sensitized after 14 days and had equivalent levels of rearing activity. Thus, suggesting that endogenous gonadal hormones may affect rearing activity in female rats but not in male rats.

Similar to Walker et al., (1997) and Chin et al., (2001) overall, female rats had significantly higher stereotypy than male rats after acute, chronic, or a challenge dose after withdrawal from chronic cocaine administration. In intact and GDX male rats, a progressive response of stereotypic activity was observed, suggesting the development of

behavioral sensitization. However, no behavioral sensitization in stereotypic activities was observed in intact or GDX female rats. It is possible that the lack of development of stereotypic behavioral sensitization may be due to a ceiling effect in stereotypic activity. Further, gonadectomy did not affect the stereotypic responses to cocaine in both females and males. These results suggest little effect of endogenous gonadal hormones in cocaine-induced stereotypic responses.

Uslaner et al., (Uslaner et al., 2001) have recently shown that there are different patterns of immediate early gene expression, which are associated with testing in a different environment vs. the home cage. In all behavioral assays, cocaine was administered in the subject home cage. Interactions between the environmental cues and subjective effects of the drug may have occurred. Thus, it is possible that endogenous hormones may modulate cocaine-induced behavioral effects in a non-context situation differently. The issue of context vs. non-context behavioral and the role of the endocrine milieu needs further investigation.

Taken together, both castration and ovariectomy affect the behavioral responses to cocaine as well as the development and maintenance of sensitization to some behavioral components of cocaine-induced effects, thus suggesting a contribution of endogenous gonadal hormones in some cocaine-induced behavioral alterations. Moreover, gonadal regulation of cocaine behavioral sensitization was observed in only some behavioral components; i.e., ambulatory and not stereotypic activity, suggesting that these hormones do not have a ubiquitous effect on all cocaine-induced behavioral activity. We postulate that gonadal hormone effects on the development and maintenance of cocaine-induced behavioral sensitization may highlight differences in the

pattern of cocaine abuse or relapse between males and females. Of key importance is the female addict, where interactions between endogenous gonadal hormones and cocaine may ultimately affect the behavioral and subjective responses to cocaine. In particular, the development of sensitization or tolerance to cocaine may be affected according to which steroid-based contraceptive a women is using or where she is in her reproductive cycle. This important clinical issue in females needs further investigation.

Cocaine-induced behavioral and neurochemical alterations may be mediated through hypothalamic-pituitary-adrenal axis (HPA) activation. For example, manipulation of corticosterone levels influenced locomotor responses to cocaine as well as the development of sensitized responses following cocaine administration (Marinelli et al., 2000; Marinelli et al., 1994; Rough-Pont et al., 1995). Kuhn and Francis, (1997) and Chin et al., (2001) have previously demonstrated that female rats exhibit greater activation of the HPA axis than male rats after acute cocaine administration. The observations reported herein are consistent with previous reports from our groups, which demonstrated that in sensitized intact female rats a challenge dose of cocaine caused greater activation of the HPA axis than saline control in male rats. Gonadectomy did not affect this neuroendocrine response in female rats, thus suggesting little effect of estrogen/progesterone in cocaine-induced corticosterone activation. On the other hand, although no effects on corticosterone levels were observed after challenge with cocaine in intact male rats, castration affected the corticosterone levels, suggesting a role of testosterone on HPA modulation. It is provocative to postulate that differences in HPA activity might was underlie at least one site in which behavioral sex differences may

occur. Moreover, at least in male rats, HPG axis interactions with HPA axis may be critical components in the cascade of events which modulate corticosterone secretion.

A number of studies have demonstrated cocaine modulation of the HPG axis (reviewed in (Quinones-Jenab et al., 2001)). At the gonads, after acute cocaine administration, cocaine modulates progesterone plasma levels in intact (Quiñones-Jenab et al., 2000b) and pregnant females (Quiñones-Jenab et al., 2000a). The stage of the estrous cycle affects cocaine-induced alterations in progesterone plasma levels, whereas animals in proestrus showed significantly higher cocaine-induced progesterone plasma levels than those in other stages of the cycle (Quiñones-Jenab et al., 2000b). In male rats "binge" pattern and single cocaine administration also increased progesterone plasma levels (Kuhn et al., 1999; Quinones-Jenab et al., 2000c; Walker et al., 2001c). In addition, testosterone levels are affected by cocaine administration, where in male rats, "binge" (Quinones-Jenab et al., 2000c) or single (Berul, C. I. and Harclerode, J. E.89; Quinones-Jenab et al., 2000c) cocaine administration significantly decreases testosterone plasma levels. Consistent with this observation, we reported an induction of progesterone plasma levels in the female rat as well as a reduction of testosterone plasma levels in the male rat after withdrawal and challenge of cocaine administration.

Cocaine affects different components of the reproductive cycle, including the duration and phases of the menstrual cycle (King et al., 1990; Mello et al., 1997). However, chronic cocaine administration does not interfere with normal estrous cyclicity (Booze et al., 1999). It has been postulated that the disruptive effects of cocaine on the reproductive cycle are transient and/or may be completely reversible; thus, tolerance in HPG alterations may occur after continuous and repetitive cocaine use (Quinones-Jenab

et al., 2001). However, based on observations presented in this study, both intact male and female rats maintain the dis-regulation of HPG activity (higher progesterone plasma levels and lower testosterone plasma levels), suggesting that cocaine effects in gonadal hormones are not transient. This issue of disruption of reproductive cycle activity (and thus, HPG axis) is in need of much further investigation, as cocaine-abusing human females can and do become pregnant (thus, have the ability to ovulate).

One of the major metabolites of cocaine is benzoylecgonine (BE). It has been postulated that metabolisms of cocaine may be influenced by a subject's hormonal status and that these differences could have implications for understanding differences between males and females in vulnerability to cocaine's effects (Mendelson et al., 1999a; Mendelson et al., 1999b; Morishima et al., 1993). We have previously demonstrated that there are estrous cycle differences and ovarian hormone effects on BE levels after acute or "binge" cocaine administration (Quinones-Jenab et al., 2000 a,b). Van Haaren et al., (1991), reported no sex differences in BE serum levels after acute cocaine administration (10 mg/kg i.p.), while Chin et al., (2001), and Bowman et al., (1999a) reported that BE levels were 2-fold higher in plasma of female as compared with male rats after acute cocaine administration. Consistent with these previous reports, after a challenge cocaine administration, no sex differences in BE plasma levels were observed after cocaine challenge (see chapter 2). Our results extend these reports by demonstrating that in male and female rats, gonadectomy had no effect on BE levels, suggesting little effect of endogenous gonadal hormones on cocaine metabolism. Overall, these results suggest that BE plasma levels do not explain differences in either behavioral sensitization or effects of cocaine in either HPA or HPG axis. This may support the idea that the

endocrinological profile of females and males is more likely to underlie sex differences to cocaine-induced alterations than cocaine metabolisms. Sex differences and gonadal hormone effects in cocaine-induced behavioral sensitization as well as in HPG and HPA axis may contribute to current health disparities in drug effects. Understanding how gonadal hormone-cocaine interaction affects sex differences in the regulation of CNS plasticity is essential to develop effective pharmacotherapy for both men and women.

CHAPTER 4: Effects of RU 486 and tamoxifen on cocaine-induced behavioral and endocrine activations in male and female Fischer rats

Accumulating evidence suggests sex differences in response to cocaine administration. For example, women report more drug cravings, have more severe drug use at intake, and are admitted to the emergency room more frequently than men (Dudish et al., 1996; Kosten et al., 1993; Robbins et al., 1999). Similarly, female rats acquire cocaine discrimination at a faster rate, display a greater motivation to self-administer cocaine, and exhibit more augmented behavioral responses to cocaine than do male rats (see chapter 3; Festa et al., 2003; Festa and Quinones-Jenab V, 2004; Lynch and Carroll, 1999; Roberts et al., 1989; Russo et al., 2003b; Sircar and Kim, 1999; Walker et al., 2001a; Walker et al., 1997). Female rats also develop conditioned place preference to cocaine with lower doses and fewer conditioning days than male rats require (Russo et al., 2003a). These results suggest that females are more sensitive than males to the addictive properties of cocaine.

Through different experimental approaches, it has been shown that gonadal hormones may contribute in part to sex differences in behavioral and endocrinological responses to cocaine. For example, rodent studies have shown that hormonal fluctuations during the female reproductive cycle modulate cocaine-induced behavioral responses; higher behavioral activities have been shown during estrous and proestrus than diestrus (Quiñones-Jenab et al., 1999; Sell et al., 2000; Walker et al., 2002). Gonadectomy (GDX) of female rats decreased overall cocaine-induced behavioral responses, but in male rats the effects of GDX were inconsistent (see chapter 3; Hu and Becker, 2003; Van Haaren and Meyer, 1991; Walker et al., 2001a). In female rats, gonadal hormone

replacement affects cocaine-induced behavioral response; i.e., estrogen increased, whereas progesterone did not affect or attenuate, acute cocaine-induced activity (Peris et al., 1991; Perrotti et al., 2003; Perrotti et al., 2001b; Sell et al., 2000). However, the mechanisms by which estrogen and progesterone influence cocaine-stimulated responses are not well understood.

RU 486 (mifepristone), a progesterone receptor antagonist, and tamoxifen, a selective estrogen receptor modulator (SERM), are commonly used to study the role of steroid receptors in the modulation of behavioral activities; i.e., both compounds inhibit lordosis behaviors (Brown et al., 1987; Etgen, 1979; Etgen and Shamamian, 1986; Patisaul et al., 2004). In this study, these pharmacological agents were used to test the hypothesis that activation of progesterone and/or estrogen receptors is a necessary step in mediating some behavioral effects of gonadal hormones on cocaine-induced activity in both male and female rats.

Methods

Animals

Eight-week-old intact male and female Fischer rats purchased from Charles River (Raleigh, NC) were individually housed in standard cages with free access to standard lab chow and water *ad libitum*. Rats were maintained on a 12-hour light/dark cycle (lights on at 9 a.m.) and handled for 1 week prior to experimental manipulations. Studies were run in three separate cohorts with a total of 7 to 12 animals per group. Because vaginal lavages have been shown to cause behavioral and neurochemical changes that may account for the differences when female rats are compared with male rats, females were

randomly assigned to experimental groups regardless of their estrous cycles (Walker et al., 2002). All NIH guidelines for the care and use of laboratory animals were strictly followed and were approved by the Institutional Animal Care and Use Committee of Hunter College (IACUC).

Drugs

Rats were pretreated for 1 hour with RU 486 (0, 3, or 25 mg/kg) or tamoxifen (0, 1, or 3 mg/kg) followed by intraperitoneal injections of saline or cocaine (15 mg/kg dissolved in 0.9% saline). Two different control groups were run to offset for differences in vehicle-treatment and manner of administration (DMSO and injected intraperitoneally for RU 486; sesame oil and injected subcutaneously for tamoxifen). This cocaine dose has previously been reported to effectively induce behavioral and hormonal responses in both male and female rats (see chapter 2; 3). These RU 486 and tamoxifen doses were chosen because they have been previously shown to decrease lordosis and nociceptive behavioral responses in female rats (Apostolakis et al., 1996; Brown et al., 1987; Etgen, 1979).

Behavioral activity

All behavioral activities were recorded for 1 hour after drug treatment in the rat's home cage, as previously described (see chapter 3). Spontaneous locomotor activity was monitored with a Photobeam Activity System from San Diego Instrument (San Diego, CA). 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.

Serum levels of progesterone and corticosterone

Following decapitation of the animals, serum was collected after centrifuging the trunk blood at 3,000 rpm for 30 minutes. Samples were analyzed using Coat-A-Count radioimmunoassay kits for progesterone and corticosterone from Diagnostic Products (Los Angeles, CA). Intra-assay coefficients of variation were less than 10.0% ± 1.0%. Hormone levels were determined by a log-logit analysis using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Serum levels of progesterone and corticosterone were expressed in ng/mL.

Statistical analysis

Data on locomotor and hormone levels were presented as the average of each treatment group ± standard error of the mean (SEM). For each behavioral measurement, two-way analyses of variance (ANOVA) were conducted to determine within the sexes the effects of cocaine and the antagonists' pretreatments {drug (cocaine or saline) x antagonist doses] or to determine between the sexes the effects of cocaine and behavioral responses {drug (cocaine or saline) x sex (male vs. female)]. When appropriate, Fisher LSD post-hoc tests were used. A p-value of <0.05 was considered significant in all statistical analyses.

Results

Cocaine-induced behavioral sex differences in male and female rats

In both male and female rats, cocaine increased all behavioral activities [**Males:**

Ambulation: $F(1,74) = 31.00$, $p<0.05$; Rearing: $F(1,74) = 30.91$, $p<0.05$; **Females:** Ambulation: $F(1,79) = 50.35$, $p<0.05$; Rearing: $F(1,79) = 86.74$, $p<0.05$; Figure. 9 A,B]. A main effect of drug and sex was observed in rearing counts [$F(1,72) = 49.10$, $p<0.05$; $F(1,72) = 7.42$, $p=0.01$, respectively; Figure. 9B]; cocaine-treated female rats reared significantly more than cocaine-treated male rats ($p=0.01$).

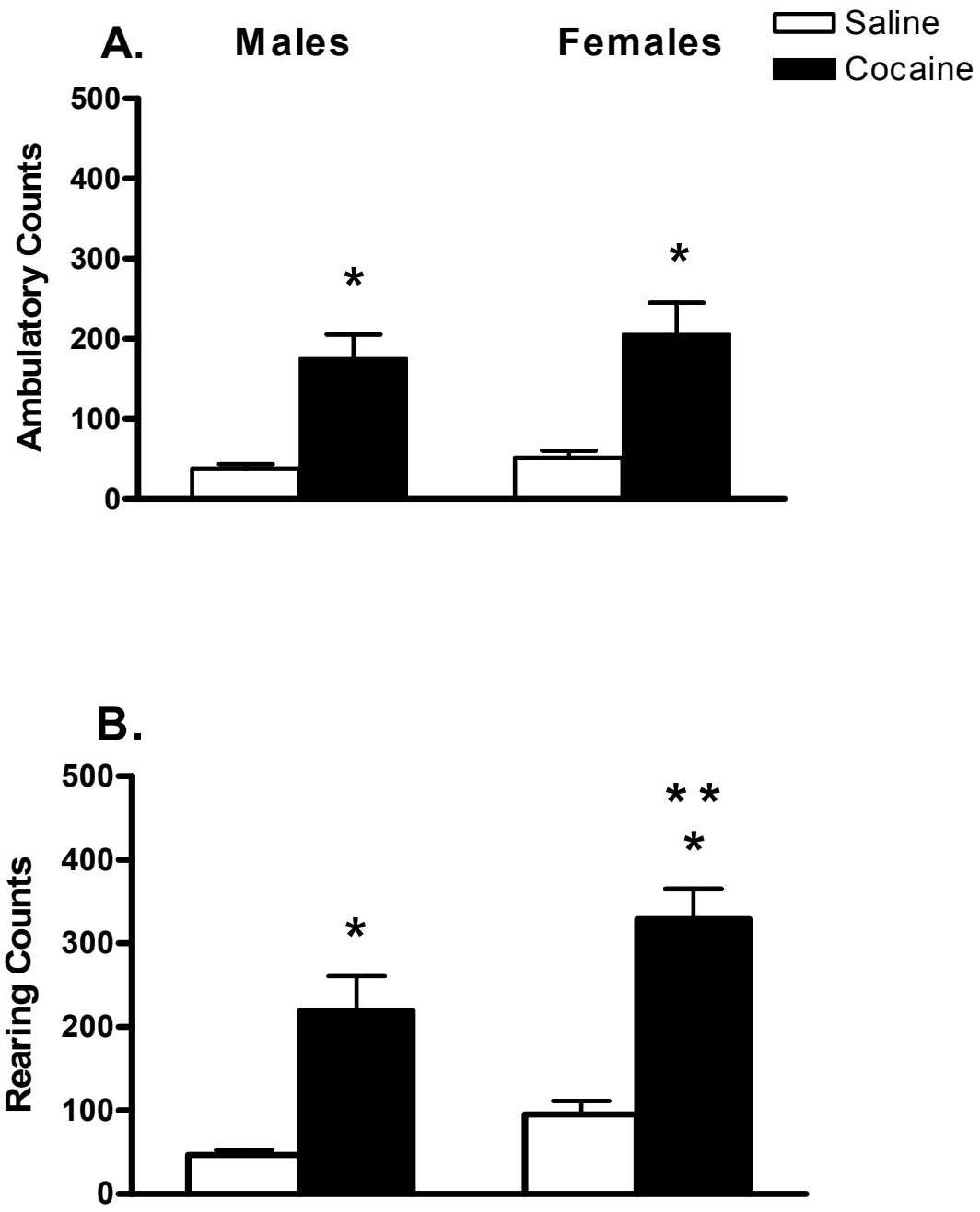


Figure 11. ***Effects of cocaine on ambulatory (A) and rearing counts (B) in male and female rats.*** Data are represented as cumulative ambulatory and rearing counts for the 30 minutes of behavioral testing. * Denotes significant differences saline- and cocaine-treated rats. ** Represents statistical differences between male and female treatment groups ($p<0.05$). The data was analyzed with a two-way ANOVA.

Effects of RU 486 on cocaine-induced behavioral activation in male and female rats

In males, significant interactions between drug and RU 486 treatments were observed for both ambulatory and rearing activities [Ambulation: $F(2,74) = 4.22$, $p=0.02$; Rearing: $F(2,74) = 3.42$, $p=0.04$; Figure 10 A,B]; 3 mg/kg of RU 486 significantly attenuated ambulatory and rearing activities in cocaine-treated rats as compared with vehicle- or 25 mg/kg-treated groups (Ambulation: $p<0.05$, $p<0.05$; Rearing: $p<0.05$, $p<0.05$, respectively). In female rats, no significant differences in cocaine-induced ambulatory or rearing counts were observed between vehicle and any of the RU 486 pretreatments.

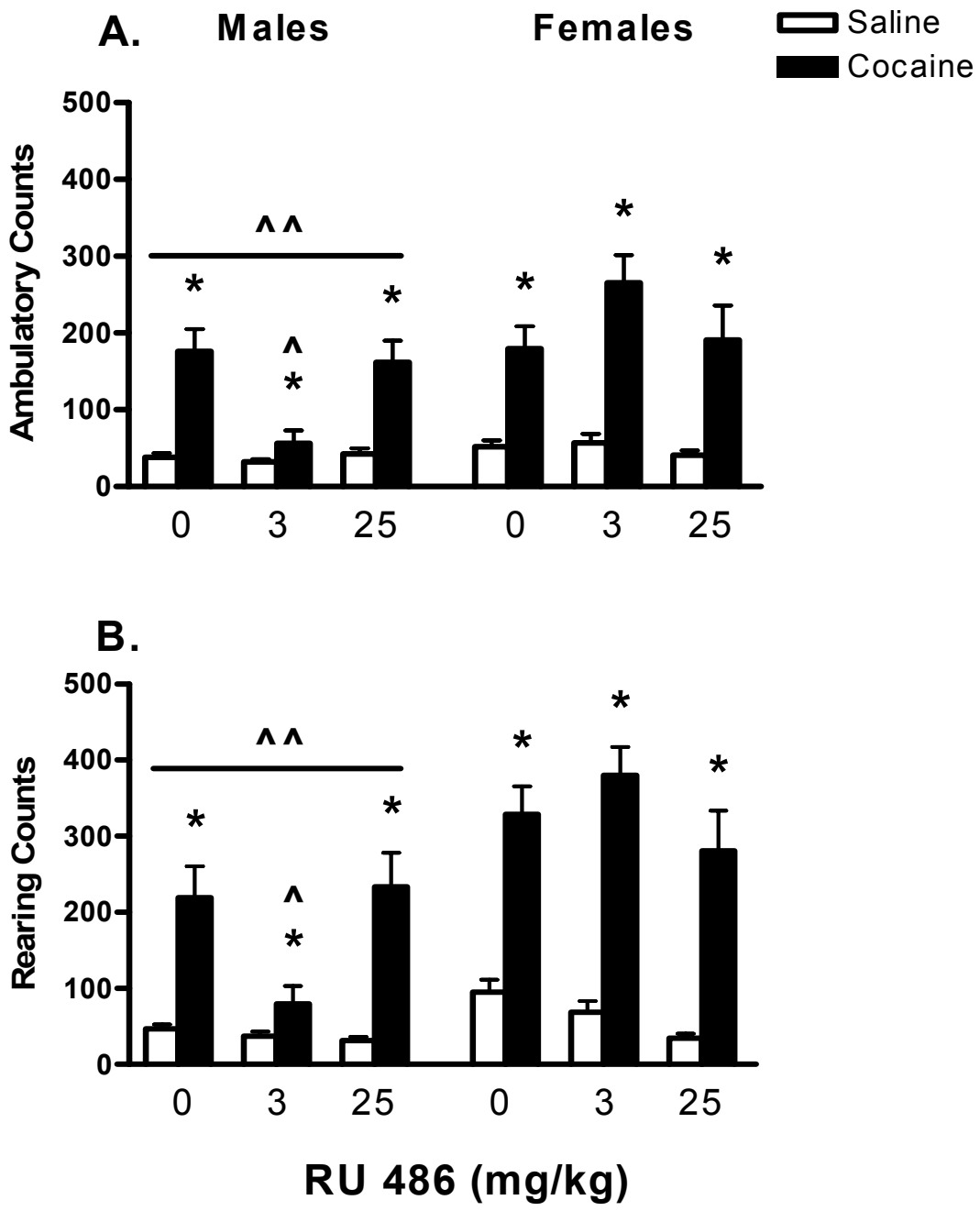


Figure 12. ***Dose effect of RU486 on cocaine-induced ambulatory (A) and rearing counts (B) in male and female rats.*** Data are represented as cumulative ambulatory and rearing counts for the behavioral testing. * denotes significant differences between saline- and cocaine-treated rats. ^ denotes a significant main effect of RU 486 dose. ^^ denotes a significant interaction effect of cocaine and RU 486 dose ($p < 0.05$). The data was analyzed with a two-way ANOVA.

Effects of RU 486 on cocaine-induced changes in serum levels of progesterone and corticosterone

In female rats, RU 486 administration raised serum levels of corticosterone from baseline in saline-treated controls [$F(2,28) = 10.89$, $p<0.05$; Table 3]; saline-treated rats receiving either 3 mg/kg or 25 mg/kg of RU 486 had higher serum levels of corticosterone than vehicle-treated saline rats ($p<0.05$, $p<0.05$, respectively). To account for these baseline effects, progesterone and corticosterone data from all studies were normalized to percentage of saline. As shown in Table 4, after cocaine administration, in males only, RU 486 altered progesterone serum levels [$F(2,23) = 8.47$, $p<0.05$]; 3 mg/kg of RU 486 significantly increased progesterone serum levels in response to cocaine as compared with vehicle- or 25 mg/kg-treated groups ($p<0.05$, $p<0.05$, respectively).

In both male and female rats, significant interactions between serum levels of corticosterone and RU 486 treatment were obtained [$F(2,31) = 7.95$, $p<0.05$; $F(2,32) = 10.06$, $p<0.05$, respectively; Figure 11B]. However, in male rats, 3 mg/kg of RU 486 significantly increased cocaine-induced corticosterone serum levels ($p=0.04$), but in female rats, both 3 mg/kg and 25 mg/kg of RU 486 significantly attenuated corticosterone serum levels as compared with their respective vehicle controls ($p<0.05$; Figure 11B).

Table VIII. Mean ± SEM of cocaine-induced percentage of corticosterone serum levels (ng/mL) after RU 486 administration

RU 486 mg/kg	Drug	Male	Female
0	Sal Coc	417.4 ± 70.5 536.4 ± 54.2	185.8 ± 33.7& 528.5 ± 97.4
3	Sal Coc	407.6 ± 68.1 644.8 ± 65.1	737.2 ± 129.7&^ 868.7 ± 83.8^
25	Sal Coc	558.8 ± 88.0 510.2 ± 97.1	848.0 ± 126.2&^ 830.2 ± 104.1^

& denotes a significant main effect of saline-treated controls.

^ denotes a significant main effect of RU 486 doses from vehicle.

Sal = saline; Coc = cocaine.

The data was analyzed with a two-way ANOVA.

Table IX. Mean ± SEM of cocaine-induced percentage of progesterone serum levels (ng/mL) after RU 486 administration

RU 486 mg/kg	Drug	Male	Female
0	Sal Coc	4.3 ± 1.7 8.6 ± 2.3	14.9 ± 3.4 21.3 ± 4.8
3	Sal Coc	**2.8 ± 1.1^^** **16.7 ± 4.2*^^**	21.8 ± 7.0^ 36.3 ± 3.2^
25	Sal Coc	10.9 ± 3.7 5.1 ± 2.4	32.7 ± 3.6^ 25.5 ± 5.5^

* denotes significant differences between saline- and cocaine-treated rats.

^ denotes a significant main effect of RU 486 doses from vehicle.

^^ denotes a significant interaction effect of cocaine and RU 486 doses from vehicle.

Sal = saline; Coc = cocaine.

The data was analyzed with a two-way ANOVA.

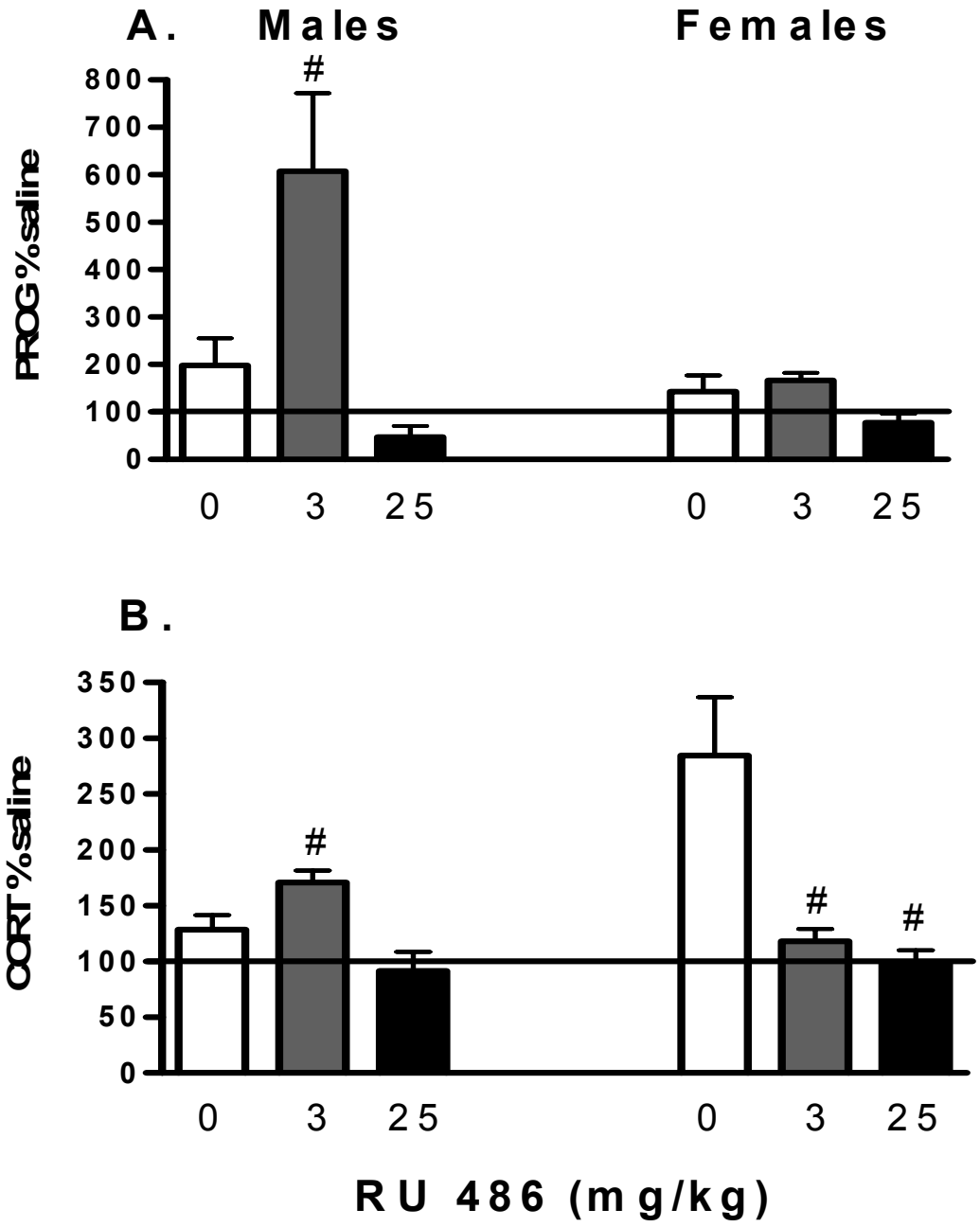


Figure 13. ***Dose effect of RU486 on cocaine-induced progesterone (A) and corticosterone (B) serum levels in male and female rats.*** Progesterone (PROG) and corticosterone (CORT) serum levels were normalized to saline to account for baseline effects for cocaine-treated (white bars), 3mg/kg of RU 486-treated (gray bars) or 25mg/kg of RU 486-treated (black bars) rats. # Represents statistical difference between vehicle and antagonist-treated group ($p < 0.05$). The data was analyzed with a one-way ANOVA.

Effects of tamoxifen on cocaine-induced behavioral response and serum levels of progesterone and corticosterone

Overall, a main effect of drug was observed in that cocaine-treated males and females had higher ambulatory and rearing counts than did saline-treated groups [**Males:** Ambulatory counts: $F(1,61) = 14.53$, $p<0.05$; Rearing counts: $F(1,62) = 22.18$, $p<0.05$; **Females:** Ambulatory counts: $F(1,66) = 48.60$, $p<0.05$; Rearing counts: $F(1,68) = 68.76$, $p<0.05$; Figure 12]. However, tamoxifen did not alter cocaine-induced behavioral responses in either sex nor did tamoxifen have significant impact on cocaine-induced effects on serum levels of either progesterone or corticosterone (Figure 12; Tables 5 and 6; Figure 13).

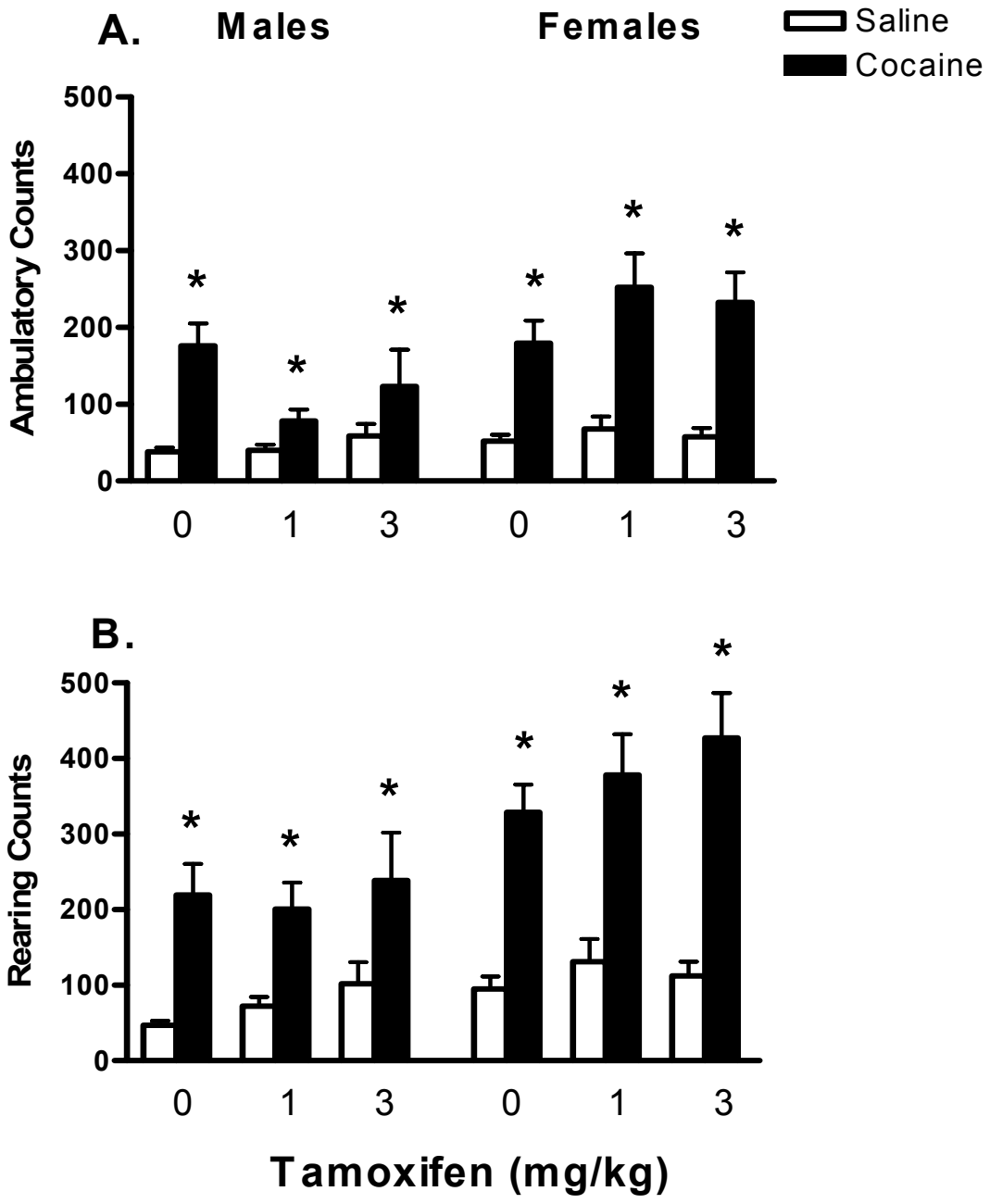


Figure 14. ***Dose effect of tamoxifen on cocaine-induced ambulatory (A) and rearing counts (B) in male and female rats.*** Rats were pre-treated for one hour with tamoxifen (1 or 3mg/kg) followed by 30 minutes with saline (white bars) or cocaine (solid bars) administration. * denotes significant differences between saline- and cocaine-treated rats. The data was analyzed with a two-way ANOVA.

Table X. Mean ± SEM of cocaine-induced percentage of progesterone serum levels (ng/mL) after tamoxifen administration

Tamoxifen mg/kg	Drug	Male	Female
0	Sal Coc	9.9 ± 5.0 7.6 ± 3.6	12.5 ± 3.2 15.3 ± 3.3
1	Sal Coc	0.3 ± 0.1^ 0.2 ± 0.1^	8.3 ± 1.9 11.2 ± 2.3
3	Sal Coc	0.3 ± 0.1^ 0.3 ± 0.1^	7.5 ± 1.8 12.7 ± 1.7

^ denotes a significant main effect of tamoxifen doses from vehicle.

Sal = saline; Coc = cocaine.

The data was analyzed with a two-way ANOVA.

Table XI. Mean ± SEM of cocaine-induced percentage of corticosterone serum levels (ng/mL) after tamoxifen administration

Tamoxifen mg/kg	Drug	Male	Female
0	Sal	117.7 ± 33.5	74.4 ± 13.2
	Coc	241.2 ± 88.9*	227.4 ± 46.5*
1	Sal	124.4 ± 51.5	68.5 ± 22.6
	Coc	173.5 ± 53.1*	139.9 ± 28.3*
3	Sal	32.2 ± 8.5	156.7 ± 38.1
	Coc	103.9 ± 37.3*	634.0 ± 103.4*^(^^)

* denotes significant differences between saline- and cocaine-treated rats.

^ denotes a significant main effect of tamoxifen 3 mg/kg dose from vehicle and 1mg/kg dose.

^^ denotes a significant interaction effect of cocaine and tamoxifen 3 mg/kg dose from vehicle and 1 mg/kg dose.

Sal = saline; Coc = cocaine.

The data was analyzed with a two-way ANOVA.

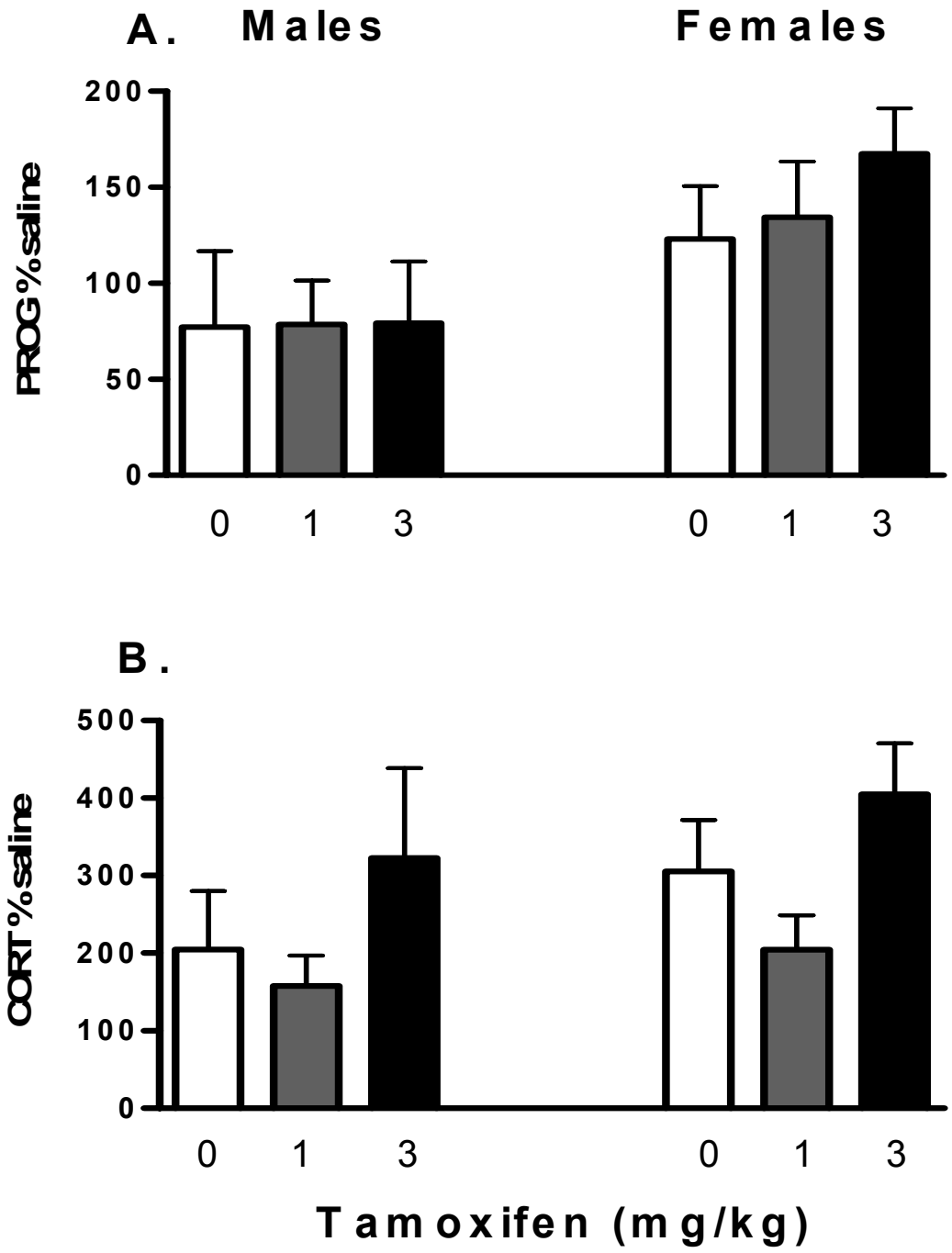


Figure 15. ***Dose effect of tamoxifen on cocaine-induced progesterone and corticosterone serum levels.*** Progesterone (PROG) and corticosterone (CORT) serum levels were normalized to saline to account for baseline effects for cocaine-treated (white bars), 1mg/kg of tamoxifen-treated (gray bars) or 3mg/kg of tamoxifen-treated (black bars) rats. The data was analyzed with a one-way ANOVA.

Discussion

As previously shown by Chin et al. (2001) and Festa et al. (2003), cocaine-treated female rats display greater rearing responses than do cocaine-treated male rats. However, we failed to observe the previously reported sexual dimorphic responses in ambulatory activities after cocaine administration {2133, 428}. This may be partly due to the DMSO pre-treatments in control groups, which have been previously shown to affect behavioral activities (Izzo et al., 2002).

In male rats, the attenuation of cocaine-induced ambulatory and rearing behavioral responses after pretreatment with lower doses of RU 486, suggests that activation of progesterone receptors may be a necessary step in mediating some of the cocaine-induced behavioral responses. RU 486's binding affinity for progesterone receptors has been shown to be dose dependent; lower doses of RU 486 are potent antiprogestins, whereas higher doses have both antiglucocorticoid and antiprogestin activities (Gaillard et al., 1984). The dose-dependent effects of RU 486 on the inhibition of ambulatory and rearing responses to cocaine may be partly explained by this pharmacological effect.

Moreover, progesterone receptor (PR)-A protein levels may mediate the effects of RU 486 on cocaine-induced behavioral response. PRs are expressed as two proteins, PR-A and PR-B, that are produced from a single gene at two distinct promoters (Mulac-Jericevic et al., 2000). PR-A has been reported to repress transcriptional activity, while PR-B functions as a strong transcription activator (Giangrande and McDonnell, 1999). Wu et al., (2005; unpublished data) observed an induction of PR-A protein levels in male rats after a similar dose of cocaine administration, suggesting that RU 486 may act

through PR-A to inhibit transcription of target genes that induce the behavioral response to cocaine. Further investigation is needed in male rats to delineate the RU 486 mechanism of action in attenuating locomotor activity when co-administered with cocaine.

It is interesting to note that RU 486 at 25 mg/kg has no effect on ambulatory or rearing behavior compared to vehicle-treated rats. Since RU 486 is both a progesterone and glucocorticoid antagonist, 25 mg/kg of RU 486 is expected to render similar inhibitory response as 3 mg/kg of RU 486. However, this was not observed. We speculate that an interaction between progesterone and glucocorticoid may account for this finding. Progesterone may inhibit the effects of glucocorticoid to facilitate behavior; therefore, we observed the behavioral response at equal levels to vehicle-treated rats. It is known that progesterone and adrenal glucocorticoid compete for the same steroid receptors in murine mammary cells (Shyamala, 1973; Wittliff, 1975). This may account for Ganguly et al., (1982) which reported antagonism of progesterone on casein (a major milk protein) gene expression through progesterone-mediated inhibition of glucocortiocid receptor binding in mammary cells. Moreover, Schmidt et al., (1998) suggested that progesterone inhibits glucocorticoid-dependent aromatase induction in human adipose fibroblasts through glucocorticoid competition for glucocorticoid receptor. The inhibitory effect of progesterone was not blocked by a progesterone antagonist, suggesting that the effect was not progesterone receptor-mediated.

Because female rats have higher intrinsic concentrations of progesterone receptors in areas important for cocaine-induced motor activation (Wu et al., 2005a; unpublished observations), it is feasible that the RU 486 doses used here were unable to completely

block progesterone receptors in the mesocorticolimbic areas and thus failed to alter the females' responses to cocaine.

Consistent with previous findings in humans, RU 486 dose-dependently increased corticosterone serum levels in control groups of both male and female rats (Bamberger and Chrousos, 1995). Pretreatment with DMSO has been shown to increase corticosterone levels (Matic et al., 2004). Moreover, the fact that no baseline effect was observed in tamoxifen-treated animals further confirms that the RU 486's baseline effects were in part vehicle induced.

RU 486 affected cocaine-induced corticosterone and progesterone responses in a dose dependent and sexually dimorphic manner. In male rats, 3 mg/kg of RU 486 increased cocaine-stimulated corticosterone and progesterone serum levels; in female rats, both 3 mg/kg and 25 mg/kg of RU 486 significantly attenuated cocaine's effects on corticosterone levels. Progesterone is a precursor of corticosterone. It is feasible that RU 486's effects on corticosterone and progesterone levels may be the result of a feedback control to counterbalance the observed baseline effects on corticosterone serum levels. However, the mechanism by which sex has an impact on the direction of these effects has yet to be determined. Because females have higher corticosterone and progesterone serum levels than males and both hormones are drastically induced in females after cocaine administration as compared with males, it is feasible that the pharmacokinetics of RU 486 in females differs from that in males (see chapter 2; 3; Festa et al., 2003; Walker et al., 2001c).

Progesterone has been shown to inhibit cocaine-induce behavioral response (Niyomchai et al., 2005). Progesterone serum levels were triple compared to

corticosterone levels at 3 mg/kg of RU 486. We speculate that high levels of progesterone may compete with glucocorticoid for steroid receptor binding to attenuate the behavioral response to cocaine. Moreover, progesterone and corticosterone serum levels are at equal levels at 25 mg/kg of RU 486, therefore, progesterone may counterbalance glucocortiocid's effect at this dose to render behavioral response similar to vehicle-treated rats.

Because tamoxifen neither altered cocaine-induced ambulatory and rearing activities nor had an effect on corticosterone or progesterone serum levels, we postulate that estrogen receptor activation may play a limited role in behavioral and neuroendocrinological responses to acute cocaine administration. Indeed, estrogen replacement consistently has been shown to have no effect on cocaine-induced behavioral responses after acute administration (Festa and Quinones-Jenab V, 2004). However, chronic estrogen replacement enhances cocaine behavioral responses regardless of concentrations and replacement paradigms (Hu and Becker, 2003; Perrotti et al., 2001b; Sell et al., 2002; Sircar and Kim, 1999). This, in turn, suggests that estrogen's effects may pertain only to behavioral responses after chronic cocaine administration. Taken together, our results suggest that while the role of progesterone receptor activation in cocaine-induced responses is sexually dimorphic, estrogen receptor activation plays a limited role in behavioral responses to acute cocaine administration.

CHAPTER 5: The effects of progesterone receptor activation in response to acute cocaine administration

Cocaine, a psychostimulant, is one of the most widely abused drugs in Western countries. Gender and estrous cycle effects in cocaine-induced behavioral and molecular alterations have been reported i.e., female rats have greater behavioral response to cocaine when compared to males (Glick et al., 1983a; Kuhn and Francis, 1997; Mc Monagle et al., 1999; Post et al., 1981; Quiñones-Jenab et al., 1999; Roberts et al., 1989; Walker et al., 2001b). Many studies have postulated that hypothalamic-pituitary-gonadal (HPG) axis regulation of cocaine-induced alterations is the basis for these gender and estrous cycle effects. For example, progesterone modulates different aspects of cocaine-induced behavioral alterations as well as some subjective effects of cocaine (Sofuoglu et al., 2002; Sofuoglu et al., 2003).

Cocaine also increased progesterone plasma levels in pregnant and intact females; animals in proestrus showed significantly higher cocaine-induced progesterone plasma levels than those in other stages of the cycle. In male rats, both single-and "binge"-pattern cocaine administration increased progesterone plasma levels (Quiñones-Jenab et al., 2000c; Walker et al., 2001d). Although progesterone has been implicated in drug addiction, little is known about molecular interactions between progesterone and cocaine.

Progesterone receptor isomers, A, B, and C, belong to a family of hormone-activated transcription factors when upon binding to the hormone, the receptor-hormone complex translocates into the nucleus and modulates target genes that contain progesterone response element (PRE) (De-Zhong et al., 1998; reviewed in Quinones-Jenab et al., 2001). The binding of the receptor-hormone complex to the target DNA

sequence regulates transcription. Administration of cocaine activates other DNA-binding regulated transcriptional mechanisms, such as CREB and ΔFosB/AP-1, which have been postulated to play a key role in the modulation of cocaine-induced molecular and cellular alterations.

We have previously demonstrated that RU 486, a progesterone receptor antagonist, attenuated cocaine-induced locomotor behavior. In this study, we hypothesis that alterations of progesterone receptor levels in the striatum may be involve in the behavioral effects of cocaine. The mesolimbic system, in particular, dopaminergic projections from the VTA (ventral tegmental area) to the nucleus accumbens has been implicated to be the key part of the brain's reward circuitry that affects cocaine addiction. However, we choose to study the striatum because it is also part of the reward circuitry and most prominently it regulates locomotor behavior. We speculate that the progesterone surge following cocaine administration will active progesterone receptor dimerization and translocation to the nucleus to affect transactivation of target genes that leads to cocaine-induced behavioral response.

Methods

Animals

Eight week-old intact male Fischer rats (Charles River; Releigh, NC) were individually housed in standard cages 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 a.m.) and handled for one week prior to experimental manipulations. Rats were randomly assigned to saline- or cocaine- treatment groups (n=3/group). All NIH guidelines for the care and

use of laboratory animals were strictly followed and were approved by the Institutional Animal Care and Use Committee of Hunter College (IACUC).

Cocaine administration

Thirty minutes after lights were on, rats received either saline or cocaine (15mg/kg or 30mg/kg; dissolved in 0.9% saline; intra-peritoneal) in their home cage and decapitated, following a brief exposure to CO_2 (20 seconds) at 10, 15, 30 or 60 minutes after drug administration. A separate cohort of rats was sacrificed without any treatment for baseline determinants.

Radioimmunoassays

Following decapitation, serums were collected after centrifuging the trunk blood at 3,000 rpm for 30 minutes and stored at −80 °C until use. Samples were analyzed using Coat-A-Count radioimmunoassay kits for progesterone from Diagnostic Products (Los Angeles, CA). Intra-assay coefficients of variation were less than 10.0% ± 1.0%. Hormone levels were determined by a log-logit analysis using GraphPad Prism Software (San Diego, CA). Serum levels of progesterone levels were expressed in ng/mL.

Whole Cell/Nuclear protein extracts

The striatum was dissected by freehand and stored at −80°C until use. Whole cell extracts were done as previously described by, with minor modifications (Jenab and Inturrisi, 2002). Briefly, tissues were homogenized in lysis buffer [20 mM HEPES pH 7.9, 10mM KCL, 1mM EGTA, 10% Glycerol, 0.2% NP-40] containing protease

inhibitors. After 15 minutes incubation, homogenates were centrifuged at 14,000 rpm for ten minutes at 4°C. To obtain nuclear extracts, pellets were resuspended in lysis buffer [20 mM HEPES pH 7.9, 10mM KCL, 1mM EGTA, 20% Glycerol, 0.2% NP-40, 0.4M NaCl], incubated for 30 minutes, and centrifuged at 14,000 rpm for 15 minutes at 4°C. For both preparation supernatant were collected and stored at -80°C until use.

Western blot analysis

Protein samples were analyzed for progesterone receptor (PR) protein levels using Western Blot techniques as previously described (Jenab and Morris, 1997). Briefly, 50µg of proteins were run in a denaturing gradient SDS-PAGE gel (Biorad, CA) and transferred to nitrocellulose membranes (Schleicher & Schuell, NH). Membranes were then blocked with 5% nonfat dry milk for 30 minutes and incubated with PR antibody (diluted 1:1000; Santa Cruz Biotechnology, CA) for one hour at room temperature. After washing with TBST (Tris-buffered saline-0.05% Tween-20 pH 7.4), membranes were incubated with the appropriate secondary antibody for 1 hour at room temperature. Band densities were detected with an enhanced chemiluminescence kit from Amersham (UK) and quantified with a Molecular Dynamic Computer Densitometer and Image Quant Program. α-tubulin antibody was used to normalized for protein loading.

Electro-mobility shift assays (EMSA)

Double-stranded progesterone response element (PRE) oligonucleotides (Santa Cruz Biotechnology, CA) were end labeled using T4 polnucleotide kinase (Promega, WI) and [γ-^{32}P] ATP (Perkin Elmer, MA). Fifty µg of protein extracts were incubated with

binding buffer [50mM Tris-HCL (pH7.5), 250mM NaCl, 2.5 mM DTT, 2.5 mM EDTA, 5mM $MgCl_2$, 20% glycerol, 0.25mg/ml poly (dl-dC)] at room temperature for 5 minutes. The mixture was then incubated for 15 minutes with radiolabeled PRE and run in a 5% nondenaturing polyacrylamide gel. Band intensities were quantified with a Molecular Dynamic Computer Densitometer and Image Quant Program. The presence of the PRE band has been previously confirmed with a supershift and competitive binding assays in our laboratory (Fabian et al.; unpublished).

Results

Dose dependent effect of cocaine-induced progesterone serum levels, protein levels, and DNA levels

Progesterone serum levels were increased after 15mg/kg or 30mg/kg of cocaine (Figure 14). However, 15mg/kg of cocaine induced progesterone serum levels after 10 minutes ($p=0.02$, Figure 14A), while 30mg/kg of cocaine induced progesterone serum levels after 15 minutes ($p=0.003$, Figure 14B). In both doses, cocaine effects on progesterone serum levels were transient. As shown in Fig. 2, progesterone receptor (PR) isomers PR-A and PR-B are at different levels in the striatum; PR-B are higher than PR-A protein levels, but it failed to reach statistical significance ($p>0.05$). In the nuclear extracts, induction of progesterone serum levels were closely followed by an upregulation of nuclear PR-A protein levels at 10 minutes (Figure 17B) and PR-DNA protein complex at 30 minutes after 15mg/kg of cocaine (Figure 21B; $p<0.05$). However, 30mg/kg of cocaine upregulated nuclear PR-A protein levels at 30 minutes (Figure 19B; $p=0.02$), but downregulated PR-DNA protein complex at 60 minutes (Figure 23B; $p=0.03$). In whole

cell extracts, regardless of drug treatment, dose, or time course, no changes were observed in PR protein or PR-DNA protein complex levels (Figure 16B and 18B; 20B and 22B, respectively).

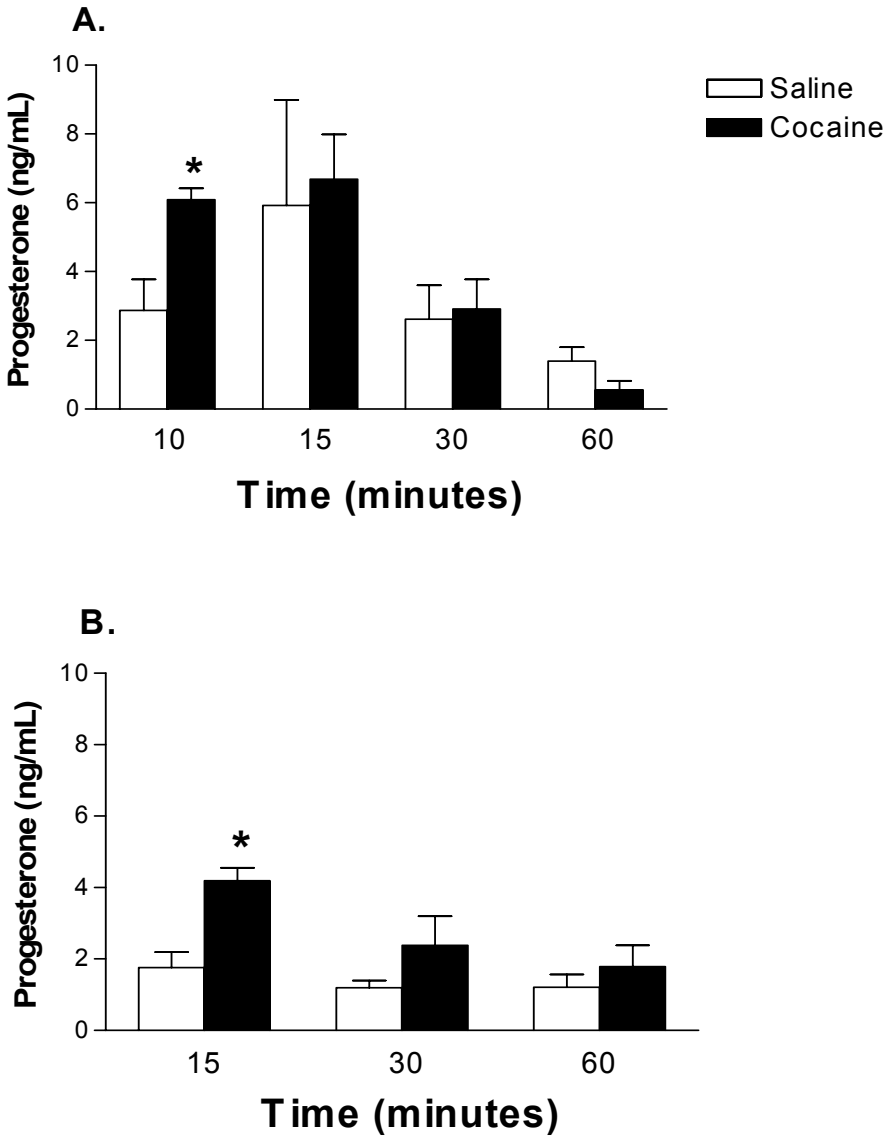


Figure 16. ***Progesterone serum levels 10, 15, 30, or 60 minutes after (A) 15mg/kg or (B) 30mg/kg of cocaine administration.*** Mean ± SEM serum levels of progesterone are expressed as ng/mL. * represent significant differences between saline- and cocaine-treated rats (N=3/group, $p<0.05$). The data was analyzed with a t-test.

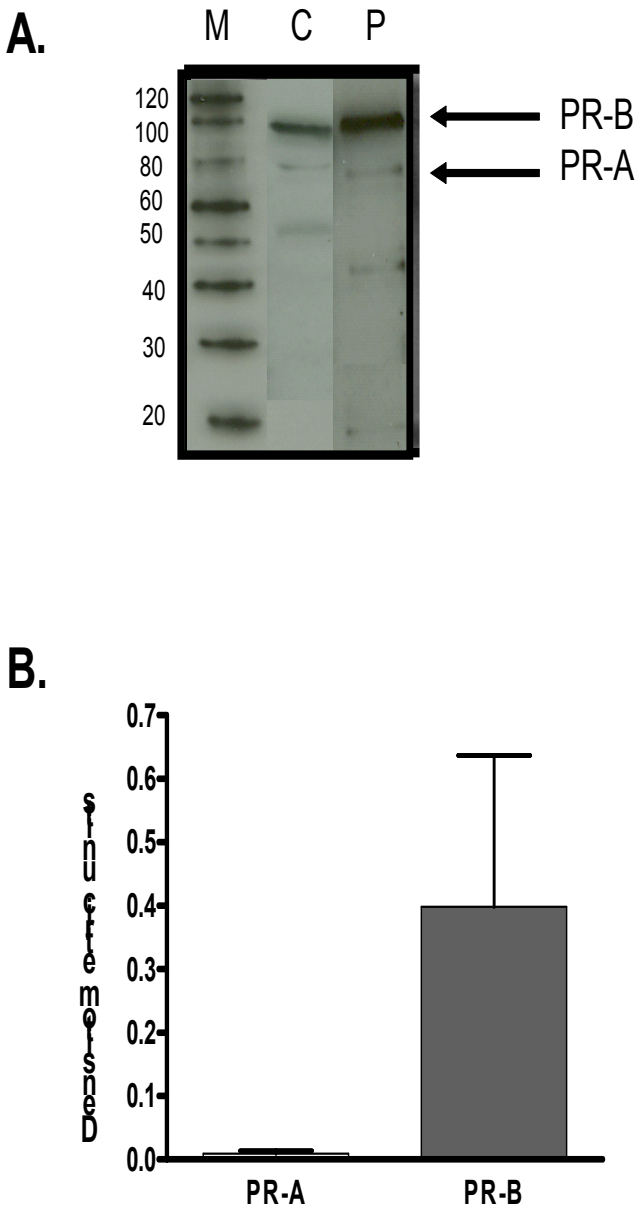
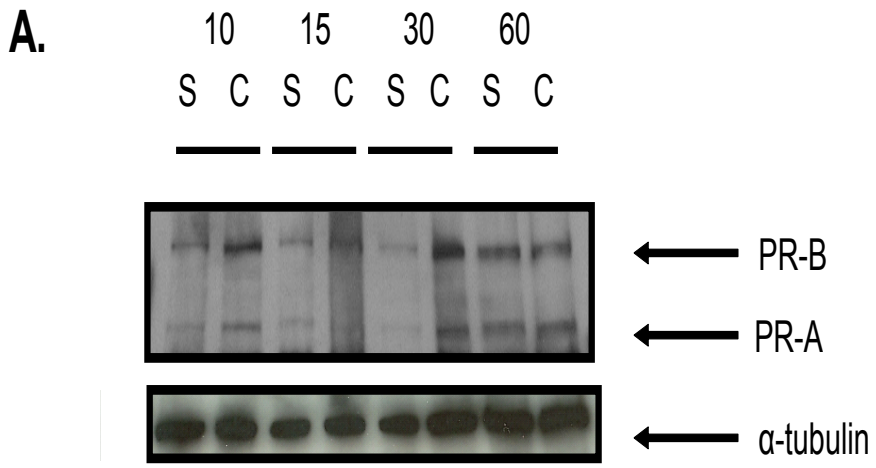


Figure 17. ***Localization of PR isoforms in the striatum of naïve animals.*** (A) PR-A and PR-B protein levels were detected using Western Blotting analysis and (B) quantified to densitomertic units after normalizing to α tubulin (N=3/group). MCF7 (M), a breast cancer cell line was used as a positive control. M=magic marker (standard), C=positive control, P=progesterone receptor. The data was analyzed with a t-test.



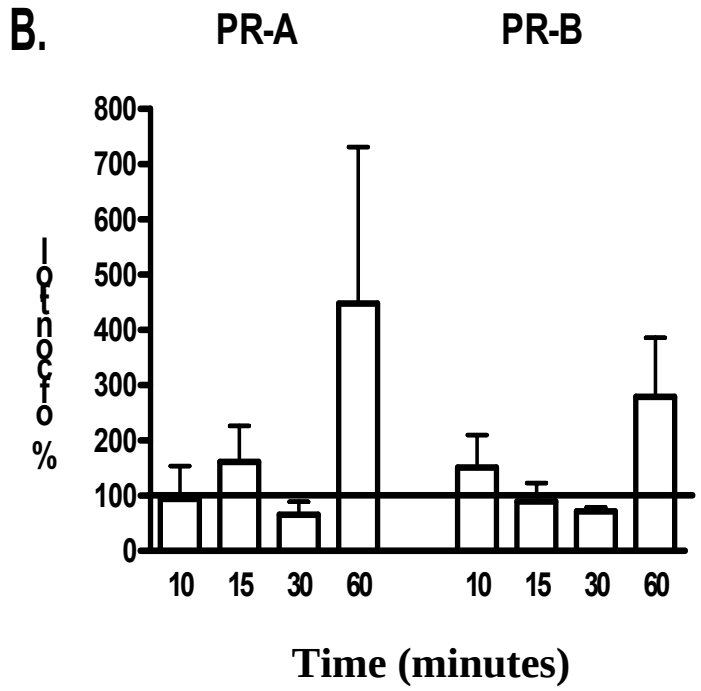


Figure 18. (A) ***Progesterone receptor levels in whole cell extracts of the striatum 10, 15, 30, or 60 minutes after cocaine (15mg/kg) or saline administration. (B)*** Progesterone receptor protein levels are presented as % change from control after normalizing to α tubulin (N=3/group). S=saline, C=cocaine. The data was analyzed with a t-test.

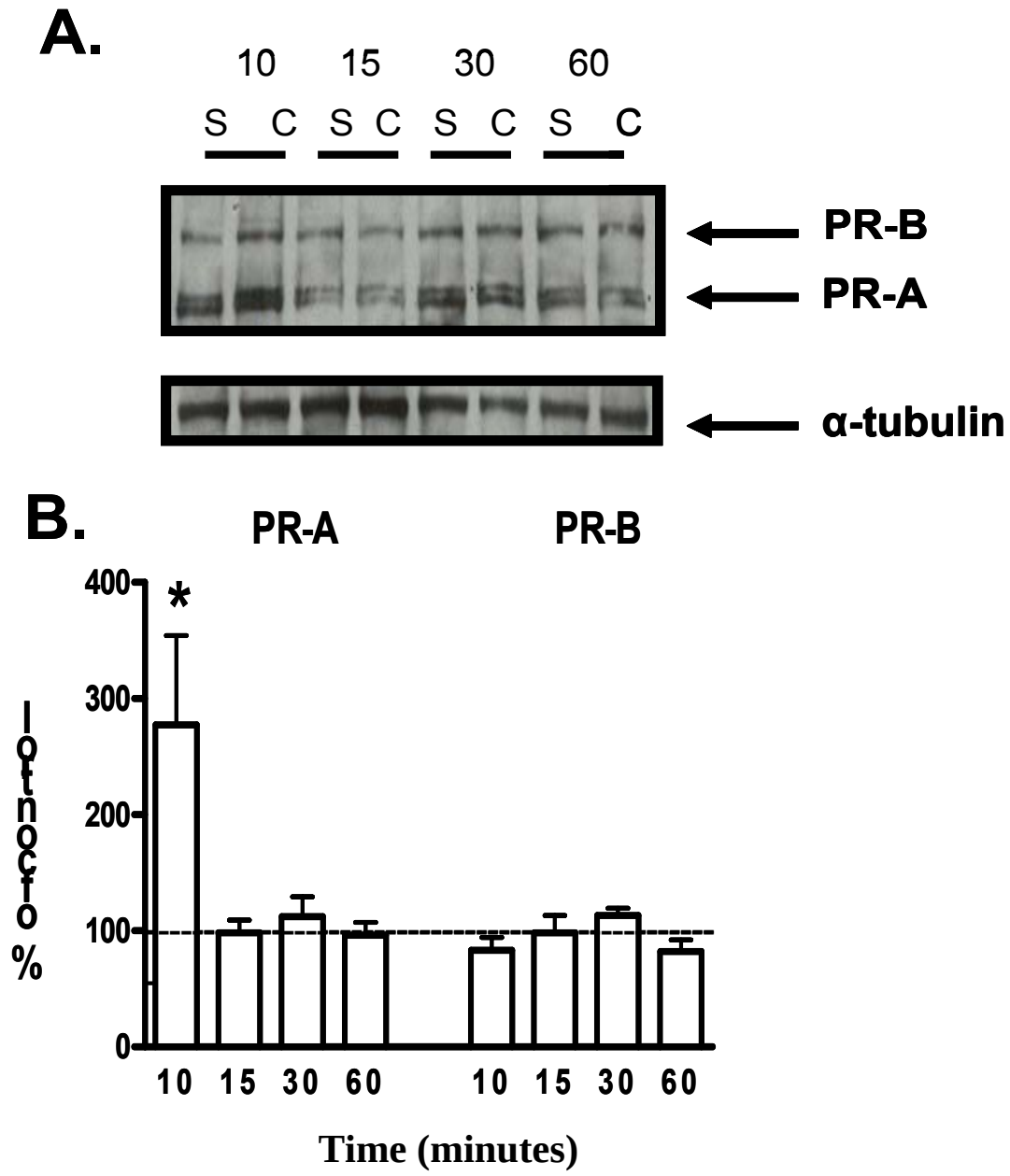


Figure 19. (A) ***Progesterone receptor levels in nuclear extracts of the striatum 10, 15, 30, or 60 minutes after cocaine (15mg/kg) or saline administration. (B)*** Progesterone receptor protein levels are presented as % change from control after normalizing to α tubulin. S=saline, C=cocaine. * represents significant differences from saline-treated controls (N=3/group, $p<0.05$). The data was analyzed with a t-test.

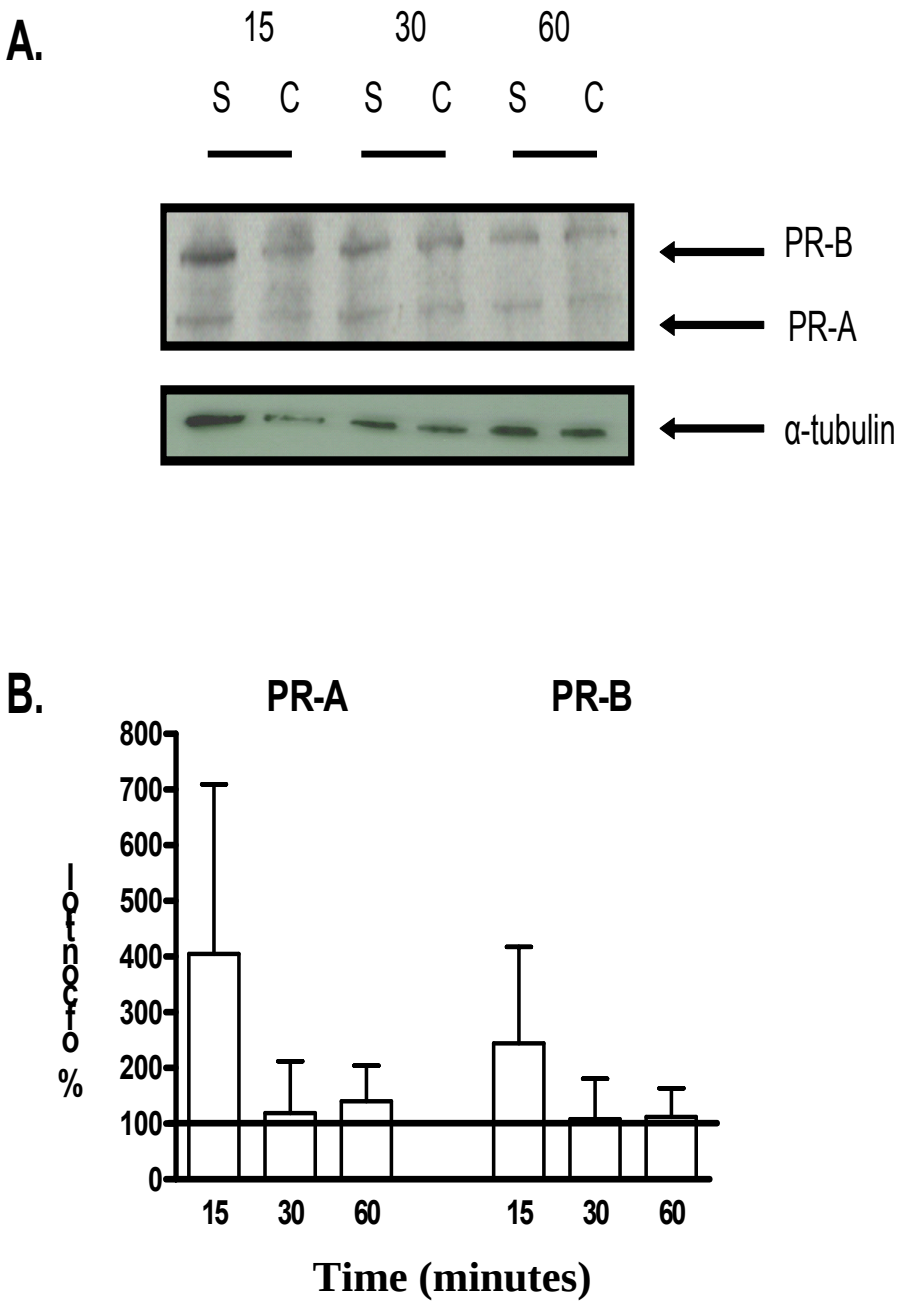


Figure 20. (A) ***Progesterone receptor levels in whole cell extracts of the striatum 15, 30, or 60 minutes after cocaine (30mg/kg) or saline administration. (B)*** Progesterone receptor protein levels are presented as % change from control after normalizing to α tubulin (N=3/group). S=saline, C=cocaine. The data was analyzed with a t-test.

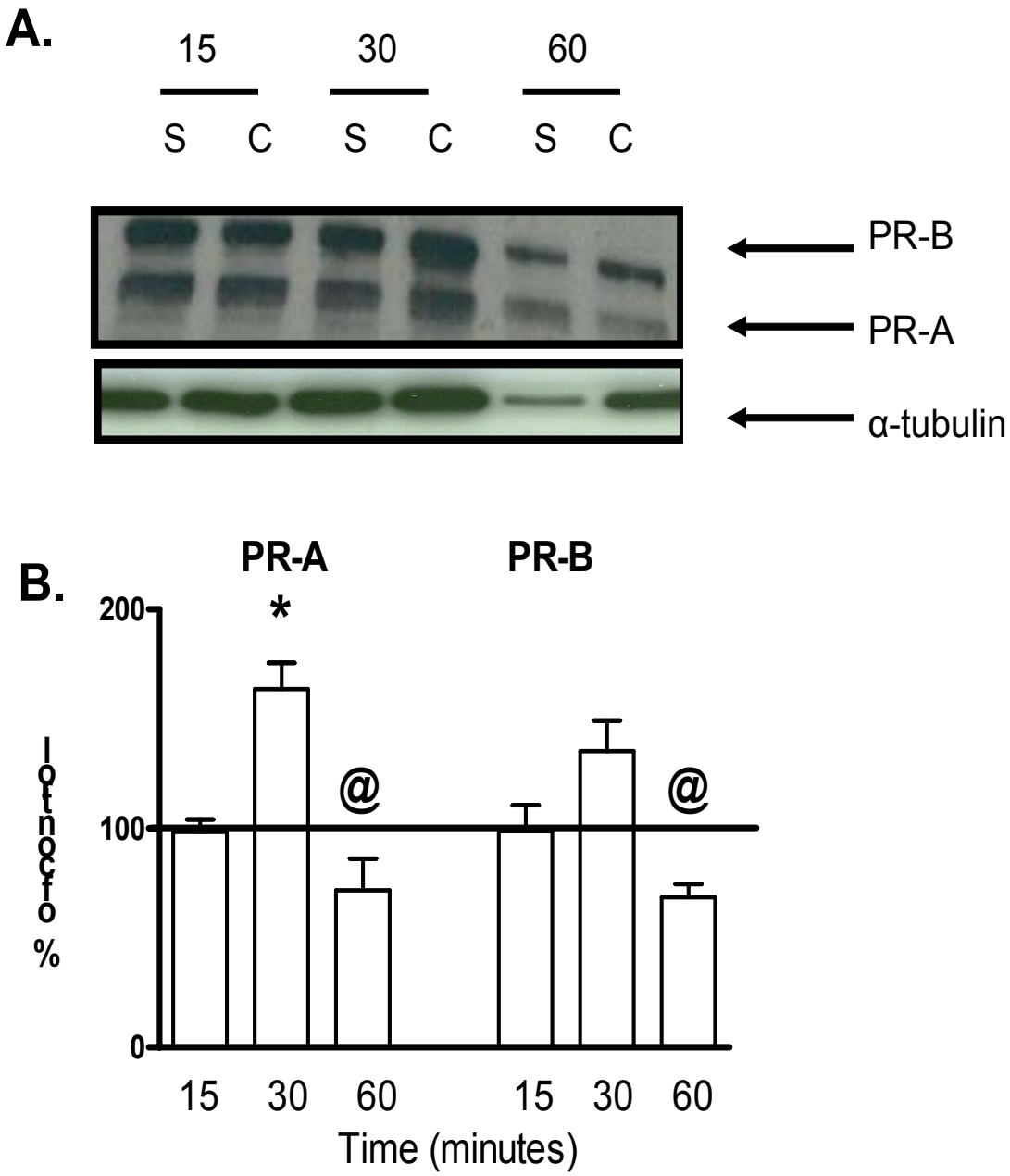


Figure 21. (A) ***Progesterone receptor levels in nuclear extracts of the striatum 15, 30, or 60 minutes after cocaine (30mg/kg) or saline administration. (B)*** Progesterone receptor protein levels are presented as % change from control after normalizing to α tubulin. S=saline, C=cocaine. * represents significant differences from saline controls compared to 15 and 60 minutes and **@** denotes differences from saline controls compared to 30 minutes (N=3/group, $p<0.05$). The data was analyzed with a t-test.

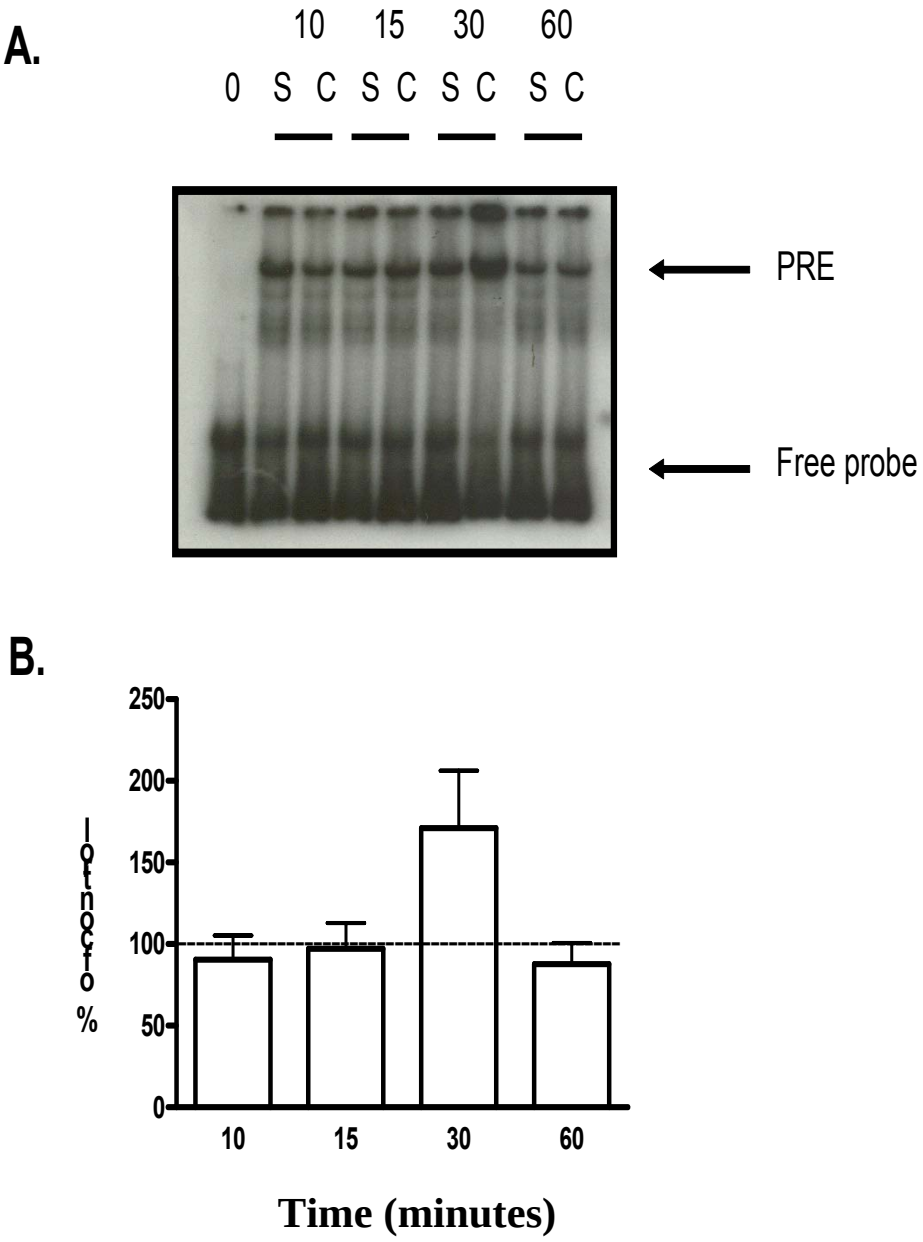


Figure 22. (A) ***Binding of PR protein complex to radio-labelled PRE in whole cell extracts of the striatum 10, 15, 30, or 60 minutes after cocaine (15mg/kg) or saline administration.*** Free probe represents the unbound DNA which lacks the PRE. ***(B)*** PRE levels are presented as % change from control group (N=3/goup). 0 represents a negative control, S=saline, C=cocaine. The data was analyzed with a t-test.

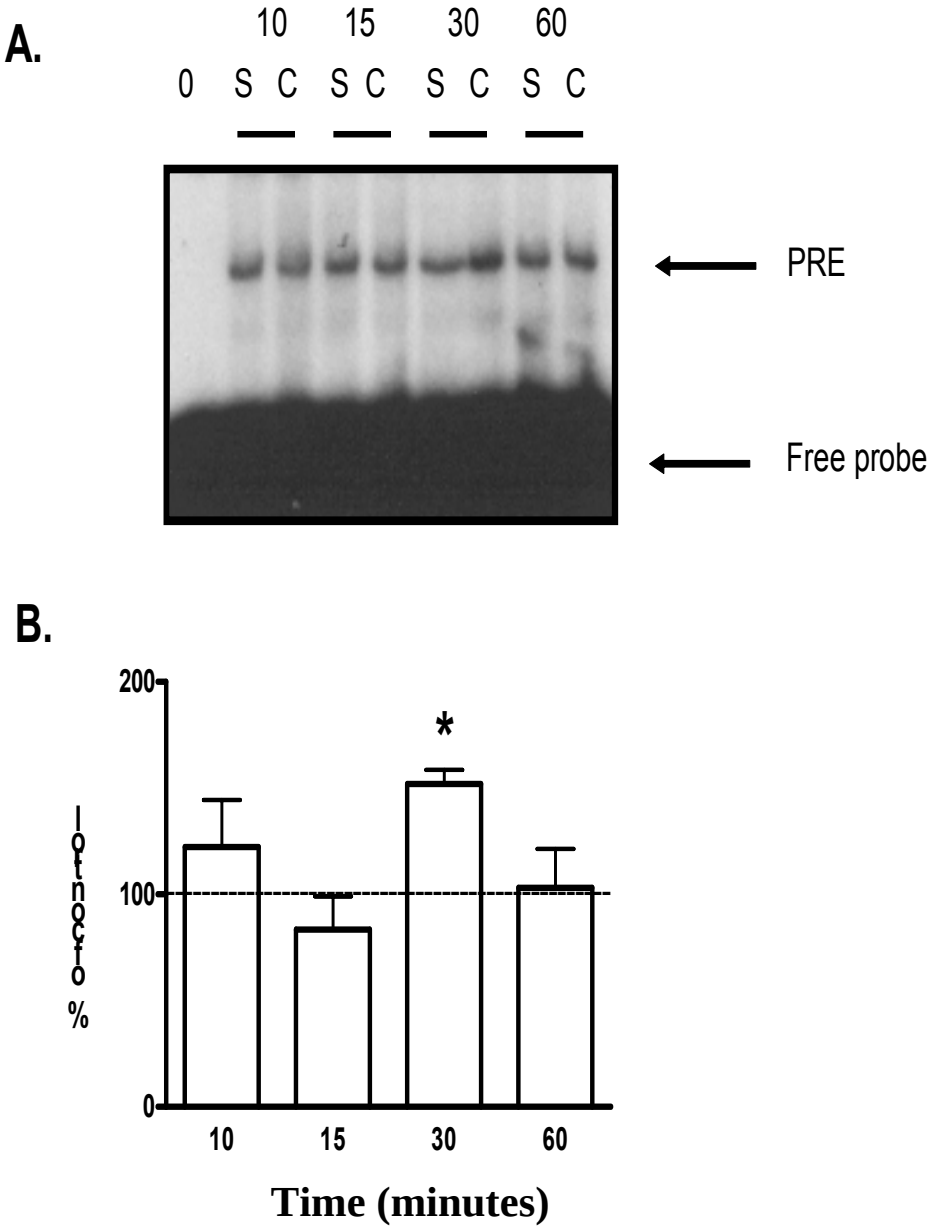


Figure 23. (A) ***Binding of PR protein complex to radio-labelled PRE in nuclear extracts of the striatum 10, 15, 30, or 60 minutes after cocaine (15mg/kg) or saline administration.*** Free probe represents the unbound DNA which lacks the PRE. ***(B)*** PRE levels are presented as % change from control group (N=3/goup). * represents significant differences from saline controls compared to 15 and 60 minutes. 0 represents a negative control, S=saline, C=cocaine. The data was analyzed with a t-test.

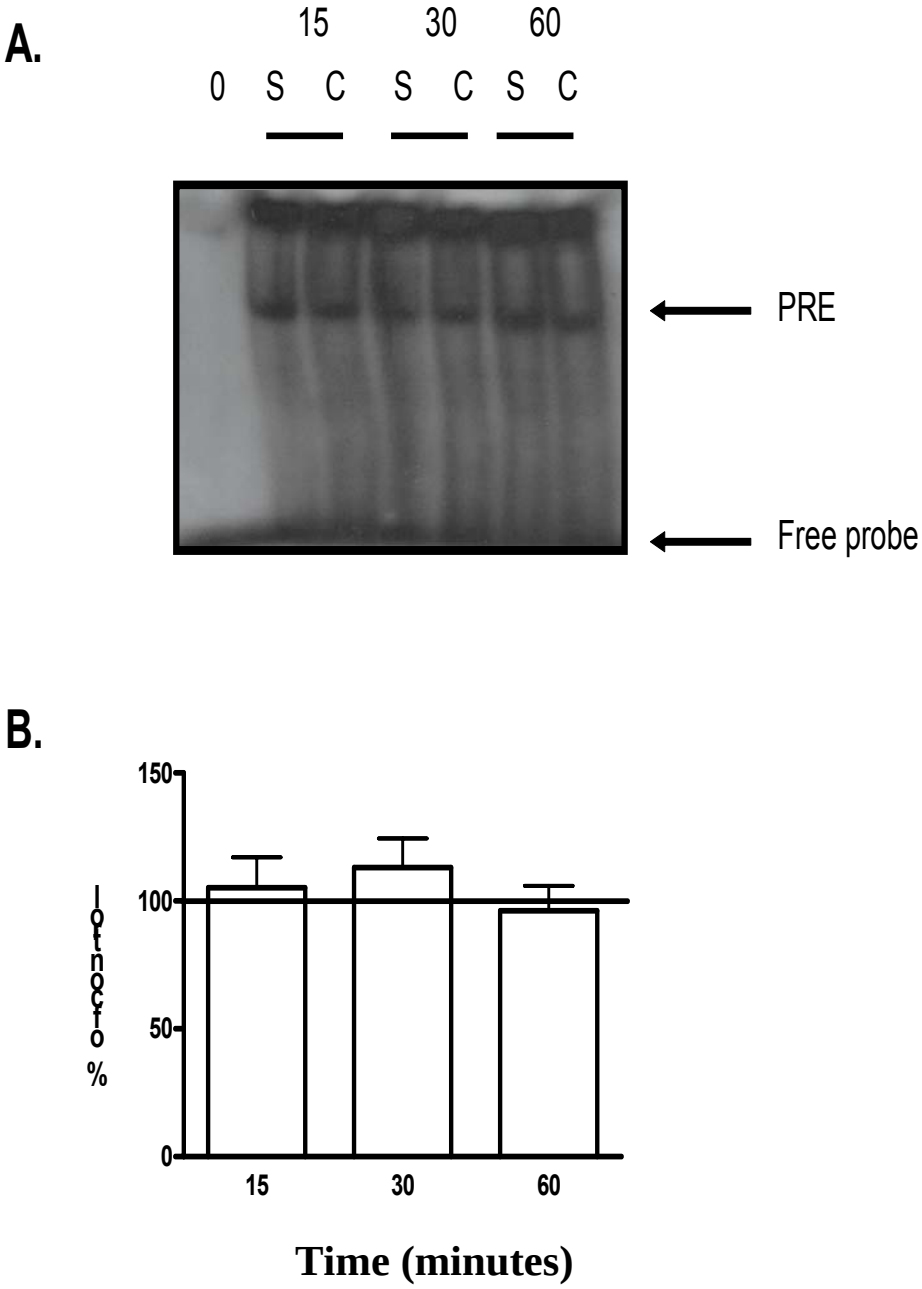


Figure 24. (A) ***Binding of PR protein complex to radio-labelled PRE in whole cell extracts of the striatum 15, 30, or 60 minutes after cocaine (30mg/kg) or saline administration.*** Free probe represents the unbound DNA which lacks the PRE. ***(B)*** PRE levels are presented as % change from control group (N=3/goup). 0 represents a negative control, S=saline, C=cocaine. The data was analyzed with a t-test.

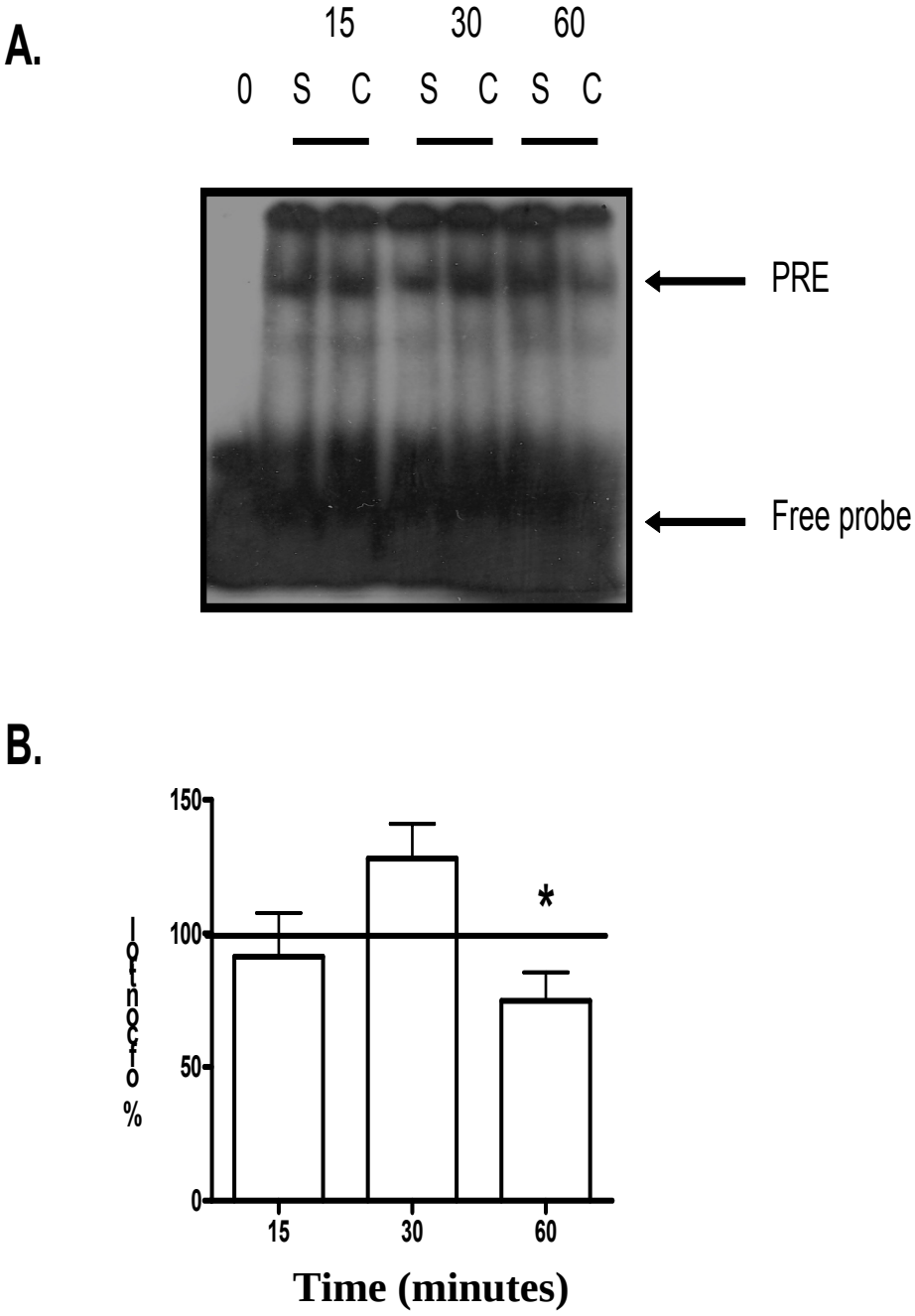


Figure 25. (A) ***Binding of PR protein complex to radio-labelled PRE in whole cell extracts of the striatum 15, 30, or 60 minutes after cocaine (30mg/kg) or saline administration.*** Free probe represents the unbound DNA which lacks the PRE. ***(B)*** PRE levels are presented as % change from control group (N=3/goup). * represents significant differences from saline controls compared to 60 minutes. 0 represents a negative control, S=saline, C=cocaine. The data was analyzed with a t-test.

Discussion

The observe induction of progesterone plasma levels by cocaine is consistent with that previously reported for intact and pregnant females and in male rats after "binge"- and single- cocaine administration paradigms. Walker et al., (2001d), reported an increase in progesterone levels in male Sprague-Dawley rats 30 minutes after acute cocaine administration using a 15mg/kg dose. Discrepancies between their study and our observations suggest possible strain differences on the temporal aspect of cocaine modulation of progesterone plasma levels in male rats. However, Goiny et al., (1986), demonstrated that in male dogs, dopamine agonists increased progesterone plasma levels with the same temporal pattern as that reported here.

It has been postulated that cocaine-induced alterations in progesterone plasma levels are probably due to an increase in secretion rates of progesterone rather than an acceleration of its transformation since cocaine treatment in ovariectomized rats given progesterone replacement does not alter progesterone levels (Quiñones-Jenab et al., 2000b). Cocaine effects on progesterone secretion may occur at any level of the HPG axis. At the hypothalamus or pituitary levels, cocaine, through its induction of extracellular dopamine, has been demonstrated to alter the release of both GnRH and LH/FSH secretion respectively (Grant, 1995; Canez et al., 1992; King et al., 1990; Mello et al., 1993). These hormones, in turn, may affect the release of progesterone by the gonads. Since it has been demonstrated that dopamine directly induces progesterone secretion by the gonads *in vivo* and *in vitro*, cocaine administration, via peripheral dopaminergic surges, may also directly alter progesterone's gonadal secretion (Mori et al., 1994).

Alternatively, since progesterone is a precursor of cortisol/corticosterone and dopamine agonist and antagonist affect progesterone secretion by the adrenal glands, cocaine may directly affect the adrenal glands either via stimulation of progesterone secretion or via ACTH regulation of corticosterone secretion (Bermudez and Lipsett, 1972; Satya et al., 1981). Indeed, it has been shown that the adrenal glands may be the source of cocaine induction of progesterone secretion in female rats, where after the adrenal removal, cocaine-induced alterations of progesterone levels were inhibited (Walker et al., 2001d).

In this study, a dose-dependent effect of cocaine on progesterone serum levels, receptor levels, and PR-DNA protein complex was observed. Cocaine at 30 mg/kg dose induced progesterone serum levels at a later time point compared to 15 mg/kg, suggesting that different doses of cocaine may activate different pathways. After 15 mg/kg of cocaine, induction of progesterone serum levels was closely followed by an induction of nuclear PR-A protein levels. On the other hand, 30 mg/kg of cocaine affected both PR-A and PR-B protein levels differentially. PR-A protein levels were upregulated at 30 minutes and both PR-A and PR-B protein levels were downregulated at 60 minutes. The progesterone surge after acute cocaine may rapidly bind and activate most of the available PR at 30 minutes, leaving very few unactivated PR at 60 minutes. Moreover, this process occurred so rapidly that the receptors may not be able to recycle in time for the incoming progesterone surge. In addition, upregulation of PR-A may explain RU 486's attenuation of locomotor response following cocaine administration. It has been shown that PR-A contains an IF (inhibitory function) region that inhibits transactivation, while PR-B contains the AF (activation function) region that activates transactivation

even in the presence of RU 486. PR-DNA protein complex formation was also affected dose-dependently. 15 mg/kg of cocaine increased binding of PR-DNA protein formation at 30 minutes, while 30 mg/kg of cocaine decreased PR-DNA protein binding with nuclear extracts in the striatum. Overall, whole cell extracts in the striatum had no effect on PR protein levels and PR-DNA protein formation after either 15 mg/kg or 30 mg/kg of cocaine, suggesting that the receptor was internalized and the binding formation occurred in the nucleus, respectively.

RU 486 dose-dependently blocked cocaine-induced behavioral response in male rats (Wu et al., 2005b; summitted data). Because cocaine induced PR protein levels and PR-DNA protein complex only in nuclear extracts, it is feasible to postulate that PR acts as a transcriptional mediator for cocaine-induced behavioral alterations. In turn, cocaine activation of the progesterone receptors may affect transcription of opioid peptides and receptors as well as dopamine receptors, which contain PRE sequences in their promoter regions. Based on the observed temporal aspects, cocaine induction of the progesterone receptor activation may play an important role in early cocaine-induced genomic alterations.

Due to the profound effects of these gonadal hormones in the modulation of CNS plasticity, the observed cocaine-induced alterations in the HPG/HPA axis via progesterone secretion and receptor activation may play a key role in the modulation of CNS neuronal functions and thereby be an important component in the cascade of events following the administration of cocaine or other psychostimulants. It has been postulated that interactions between the dopaminergic system and progesterone are needed to achieve integration of neuronal communication in the central nervous system

(Apostolakis et al., 1996; Mani et al., 1996; Mani et al., 1997; Phelps et al., 1998). Due to overlap of some reproductive and cocaine-induced behaviors, it is possible that the facilitation of some cocaine-induced psychomotor and subjective/rewarding effects may occur through progesterone receptor dependent mechanisms. Taken together, the activation of the progesterone system by cocaine may play a role in the sex differences observed in neurophysiological effects of cocaine and may explain the gonadal effects in behavioral as well as neurochemical cocaine-induced alterations.

CHAPTER 6: CONCLUSION

Although cocaine addiction has been studied extensively at various aspects, the underlying mechanism of action is not well understood. A plethora of evidence indicates that gonadal hormones, specifically estrogen and progesterone, may be the underlying basis for cocaine-induced behavioral sex differences. Progesterone and estrogen are steroid hormones, which exert their effects through receptor activation. However, little is known about cocaine's interaction with steroid receptor activation. In this thesis, we proposed that induction of steroid serum levels may mediate the behavioral responses to cocaine through activation of steroid receptors at different levels.

At the behavioral level, we have demonstrated cocaine-induced behavioral sex differences. Overall, cocaine-treated intact female rats exhibited higher ambulatory and rearing activities than cocaine-treated intact male rats. Furthermore, we expanded previous studies by demonstrating that sex differences occurred in certain aspects of cocaine-induced behavioral activation, development and sensitization. For example, cocaine-treated female rats had significantly higher ambulatory counts, developed sensitization with fewer injection days and maintained behavioral sensitization after a challenge dose of cocaine when compared with cocaine-treated male rats. Although cocaine-treated female rats reared more than male rats there were no statistically significant changes across the treatment conditions. This study also extends previous studies by demonstrating that although female rats' stereotypic responses to acute, chronic and sub-chronic cocaine administration was higher than male rats, no sensitization to cocaine-induced stereotypic activity was observed in either male or female rats. Thus, sensitization to cocaine occurs in only certain aspects of cocaine-

induced behavioral activity, i.e. locomotor behavior but not stereotypic activity. Taken together, differences in development and maintenance of sensitization may highlight differences in the pattern of chronic cocaine abuse between males and females.

To determine that the observed sex differences are gonadal hormone mediated, gonadectomized (GDX) male and female rats were administered with acute (day 1), subchronic (day 7), chronic (day 14), or a challenge dose of cocaine (day 21—a single dose of cocaine after 7 days withdrawal). We demonstrated that cocaine-treated GDX female rats had similar ambulatory counts as cocaine-treated intact male rats suggesting that ovarian hormones potentiate cocaine's effect in intact animals. This is consistent with observations that progesterone and estrogen generally produce opposite effects; estrogen potentiates, while progesterone inhibits cocaine-induced behavioral responses (Niyomchai et al., 2005; Perrotti et al., 2001c). Moreover, both intact and GDX female rats had higher rearing counts than intact and GDX male rats further indicating the influence of ovarian hormones. According to Walker et al. (2001), the adrenal gland is and the ovary is not the main source of cocaine-induced progesterone as adrenalectomy attenuated progesterone levels.

Both saline- and cocaine-treated GDX male rats increased ambulatory behavior after chronic injections of cocaine (14 days), suggesting that regardless of drug treatment, depletion of testosterone may play a minimum or stimulatory role on cocaine-induced behavioral sensitization. In addition, intact and GDX male rats exhibited significantly higher rearing behaviors after chronic cocaine administration (14 days) compared to acute administration, further implicating that testosterone has a minimum effect on

cocaine. Taken together, ovarian hormones may be a key player in cocaine-induced behavioral responses, while testosterone may play a limited role in the same processes.

Progesterone and estrogen are steroid receptors that mediate their actions through receptor activation. To determine the effects of progesterone or estrogen receptor activation in cocaine-induced behavioral alterations, RU 486 and tamoxifen (progesterone and estrogen antagonist, respectively) were administered to intact male and female rats. RU 486 and tamoxifen are pharmacological agents that are used to determine the influence of progesterone and estrogen receptors in regulating lordosis response. However, little is known on the impact of RU 486 and tamoxifen on the behavioral response to cocaine.

In this thesis, we observed sex differences in the attenuation of RU 486. We demonstrated that RU 486 did not affect cocaine-induced behavioral activity in female rats, but 3mg/kg of RU 486 significantly attenuated ambulatory and rearing behaviors in males. Although RU 486 can affect both progesterone and glucocorticoid receptors, at a low dose, RU 486 have been suggested to act primarily through progesterone receptor (PR), while at a higher dose through glucocortiocid receptor (Gaillard et al., 1984). In turn, we postulate that RU 486 acts through PR to attenuate cocaine-induced response in male rats. In female rats, it is feasible that the RU 486 doses used here were unable to completely block progesterone receptors and thus failed to alter the females' responses to cocaine. As a follow up experiment, an extensive dose response of RU 486 should be used to delineate the optimal dose to attenuate cocaine-induced motor response in female rats.

On the other hand, tamoxifen failed to potentiate or attenuate cocaine-induced ambulatory and rearing responses in either male or female rats. Indeed, estrogen replacement consistently has been shown to have no effect on cocaine-induced behavioral responses after acute administration (Festa and Quinones-Jenab V, 2004). As a follow up experiment, implants of Silastic capsules or chronic injections of tamoxifen may be useful as most effects of estrogen are observed after chronic administration or replacement. Taken together, our results suggest that although estrogen receptor may play a limited role, progesterone receptor activation is a necessary step in the cascade of events that may account for the behavioral and neuroendocrinological responses to acute cocaine administration.

Cocaine metabolites, benzoylecognine and ecgonine methyl ester, have been implicated to influence the hormonal status that may account for cocaine-induced sex differences in motor response (Van Haaren et al., 1997). However, we demonstrated herein that sex differences in BE do not completely explain the exaggerated locomotor and stereotypic responses after the different cocaine administration paradigms; nor the difference in sensitization to the behavioral effects of cocaine in female rats. Cocaine-treated female rats had higher levels of benzoylecognine compared with cocaine-treated male rats after acute cocaine administration, but not after chronic or a challenge dose of cocaine. However, other studies have reported changes or no change after acute cocaine administration or changes after chronic administration (Bowman et al., 1999b; Perrotti et al., 2000; Van Haaren and Meyer, 1991).

At the neuroendocrine level, sex differences in cocaine-induced HPA (hypothalamic-pituitary-adrenal) axis activation were also observed. Activation of the

HPA axis has been implicated to affect cocaine self-administration and relapse (Goeders, 2002). Overall, female rats have an exaggerated HPA axis compared with male rats. In female rats, we extend these results by demonstrating that chronic cocaine administration did not alter corticosterone plasma levels, indicating the possible development of tolerance of HPA activity. However, a challenge dose of cocaine in behaviorally sensitized female rats caused an increase in corticosterone levels. Thus, suggesting a desensitization of HPA activity after withdrawal of cocaine or a return to a hypersensitive HPA activity. On the other hand, no significant differences in corticosterone levels were observed in male rats across different administration paradigms. Taken together, sex differences in cocaine-induced corticosterone levels may indicate an interaction between ovarian hormones and corticosterone since progesterone is a precursor of corticosterone.

In RU 486-treated female rats, dimethyl sulfoxide (DMSO), the vehicle, significantly raised serum levels of corticosterone from baseline in saline-treated controls. It has been previously reported that DMSO administration rapidly doubled corticosterone serum levels to induce acute-phase response to inflammation (Matic et al., 2004). To account for baseline effects, progesterone and corticosterone data from RU 486 study were normalized to the percentage of saline. In female rats, we reported that both 3 mg/kg and 25 mg/kg of RU 486 significantly attenuated corticosterone levels, while in male rats, 3 mg/kg of RU 486 increased corticosterone levels in response to cocaine when compared with vehicle treatments.

Sex differences in cocaine-induced HPG (hypothalamic-pituitary-gonadal) axis activation were also observed. Consistent with previous reports, cocaine significantly induced progesterone levels in intact and pregnant females. After acute cocaine, we

observed a sex difference in cocaine-induced progesterone levels at different time course. In female rats, cocaine-induced progesterone levels at 30 minutes, while in male rats, cocaine-induced progesterone levels at an earlier time point of 10 minutes. Interestingly, higher doses of cocaine (30 mg/kg) delayed the surge of progesterone serum levels (15 minutes) suggesting that different pathways may be activated by the different doses used. However, in both doses, cocaine effects on progesterone serum levels were transient. Moreover, cocaine-induced progesterone levels were also observed after a challenge dose of cocaine in female rats. However, RU 486 treatment had no effect on cocaine-induced progesterone serum levels in female rats suggesting that RU 486 is attenuating cocaine's effect. On the other hand, in males, 3 mg/kg of RU 486 increased progesterone serum levels in response to cocaine when compared with vehicle treatments. In addition, testosterone levels are affected by cocaine administration in male rats, where we reported a reduction of testosterone plasma levels after withdrawal and challenge of cocaine administration, suggesting that testosterone has a limited impact on acute cocaine administration.

At the molecular level, cocaine-induced PR activation and PR-DNA protein complex in the striatum (regulates locomotor activity) of male rats were observed to be dose dependent. A small pilot study was conducted on naïve male rats to determine the presence of progesterone receptors in the striatum since this information is limited in the literature. We found a nonsignificant higher baseline level of progesterone receptor B (PR-B) protein levels compared to PR-A protein levels in the striatum. PR-A lacks the first 164 amino acid, which contains the IF (inhibitory function) region that inhibits

transactivation, thereby, alterations in this isoform may account for RU 486 attenuation of cocaine-induced locomotor activity.

A cocaine dose response and a time course study were conducted to compare the effects of 15mg/kg vs. 30 mg/kg of cocaine on PR protein levels and PR-DNA complex across time. Moreover, two types of extractions (whole cell extracts vs. nuclear extracts) were compared to determine the localization of PR in the presence of drug treatment. In a drug free environment, PR is found inside the nucleus and shuttles to and from the cytoplasm in an energy dependent manner. In whole cell extracts, regardless of drug treatment, dose or time course, no changes were observed in PR protein or PR-DNA protein complex formation. However, alterations in PR protein levels and PR-DNA complex were observed in nuclear extracts, suggesting that PR is internalized into the nucleus and the binding formation occurred in the nucleus. In the nuclear extracts, induction of progesterone serum levels at 10 minutes was closely followed by an upregulation of nuclear PR-A protein levels at 10 minutes and PR-DNA protein formation at 30 minutes after 15mg/kg of cocaine. However, 30mg/kg of cocaine upregulated nuclear PR-A protein levels at 30 minutes but downregulated both PR-A and PR-B protein levels at 60 minutes. Moreover, PR-DNA protein formation was affected dose-dependently. PR-DNA formation was upregulated at 30 minutes and downregulated at 60 minutes after 15 mg/kg and 30 mg/kg of cocaine, respectively. Taken together, the temporal pattern on serum levels, receptor levels, and DNA binding formation were dependent on cocaine dose; our laboratory reported 30 mg/kg of cocaine robustly increased behavioral responses compared with 15 mg/kg of cocaine (Festa et al., 2003). Moreover, PR-A activation inhibits transcription, therefore, upregulation of this

isoform may account for the observed RU 486 attenuation effect on cocaine-induced ambulatory and rearing activity in male rats.

The studies presented herein are conducted following acute cocaine administration. We speculate that chronic cocaine administration would render differential results as dopamine release in the mesocorticolimbic system would be affected. It has been reported that repeated administration of cocaine would result in a substantial increase of basal dopamine release in the mesocorticolimbic dopamine system, which may account for the tolerance effect of cocaine (Weiss et al., 1992). This suggests that animals with higher levels of basal dopamine release may act through a compensatory mechanism to decrease extracellular dopamine response to cocaine to develop tolerance. Moreover, repeated cocaine has also been demonstrated to diminish the efficacy of cocaine as a discriminative stimulus and to induce tolerance to the subjective and reinforcing effects of this drug (Fishman et al., 1985; Wood and Emmett-Oglesby, 1986; Emmett-Oglesby and lane, 1992). Taken together, further studies are needed to investigate the interaction of RU 486 and repeated cocaine administration on locomotor activities and progesterone receptor activation.

This thesis has extended our understanding of cocaine's effects on circulating steroid hormone levels, activated protein levels and DNA binding complexes that may play a crucial role in cocaine-induced behavioral responses. In conclusion, we have demonstrated that progesterone receptor activation is a necessary step in cocaine-induced behavioral alterations with RU 486. The hormonal status, types of hormone-based oral contraceptives used and the average drug dose intake should be crucial variables when designing new therapeutic and preventive models for cocaine addiction.

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