The Role of Estrogen and Progesterone in Neurochemical, Endocrinological and Behavioral Responses to Acute Cocaine

By

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Abstract

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By

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Estrogen and progesterone have been shown to alter responses to cocaine. We hypothesize that the concentrations of the hormones, time between hormonal surges, hormonally-mediated molecular and neurochemical mechanisms, and hormonal impact on cocaine metabolism are all factors that contribute to cycle-related fluctuations in cocaine effects. Utilizing different hormonal replacement paradigms we indeed found that estrogen and progesterone modulate responses to cocaine in a complex manner. Although estrogen did not affect cocaine-induced ambulatory and rearing behaviors dose-dependently, it affected stereotypic behaviors regardless of cocaine administration (animals receiving 50 μg had higher stereotypic counts than did the OVX group). In contrast, progesterone affected rearing activity dose-dependently: 50 and 500 μg of progesterone inhibited, whereas 100 μg and 250 μg stimulated, rearing in response to cocaine. The duration of hormonal exposure is also an important factor in cocaine-induced alterations; short-term estrogen replacement decreased cocaine-induced ambulations. Short-term progesterone decreased rearing, whereas long-term progesterone decreased ambulations. However, preprodynorphin mRNA levels were increased in the

caudate putamen of ovariectomized female rats pretreated with vehicle or a combination of estrogen and progesterone but not in ovariectomized female rats that were pretreated with either estrogen or progesterone alone. We also demonstrated that estrogen and progesterone either synergize to facilitate or antagonize to inhibit cocaine-induced behaviors. When progesterone was administered 1 and 48 hours after estrogen replacement, locomotor behavior was inhibited. On the other hand, when progesterone was administered 24 hours after estrogen replacement, behavior was enhanced. When animals were administered 50 μg E + 500 μg P (levels reflecting those observed during the late proestrus stage), total locomotor behavior was enhanced while levels of dopamine (DA) in the nucleus accumbens and the ratio of HVA to DA in the VTA were decreased. Administration of 10 μg E + 500 μg P (levels reflecting those observed during the diestrus stage) inhibited total locomotor activity and decreased baseline levels of DA. Estrogen and progesterone also affected cocaine pharmacokinetics: estrogen decreased brain levels of cocaine and norcocaine 30 minutes after cocaine administration in comparison to the group receiving vehicle at that time point and in progesterone-treated rats, levels of benzoylecgonine and ecgonine methylester were higher at 30 minutes post-administration than at 15 minutes. No changes were found in blood levels of the metabolites. These findings suggest that there are multi-faceted interactions between estrogen and progesterone in mediating the effects of cocaine.

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Chapter 1: Introduction

I. Background:

A. History of cocaine abuse

Cocaine is an active alkaloid found in the leaves of *Erythroxylon coca,* a tree indigenous to Peru and Bolivia. The drug has been used as a psychostimulant among the people of Colombia for 2,500-5,000 years (Platt, 1997). In the United States however, its use remained relatively limited until the late 1800s (Platt, 1997). Around this time, cocaine's popularity began to heighten when Sigmund Freud, a young physician, advocated its use as a treatment for a multitude of personality disorders and even morphine addiction (McKim, 1996). It was not long before reports of cocaine addiction threatened its newfound popularity and by 1894, the 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).

B. Overview and Rationale

Since the ban of this substance as a narcotic and potentially addictive substance, 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 1998, the national Household Survey on Drug Abuse reported that 33% of the 1.8 million Americans that currently use cocaine were women (Substance Abuse and Mental Health Services Administration, 2003). Although males are more likely than females to have an initial opportunity to use drugs, there seems to be no differences in the progression to chronic drug use following initial use (Van Etten and Anthony, 1999; Van Etten et al.,

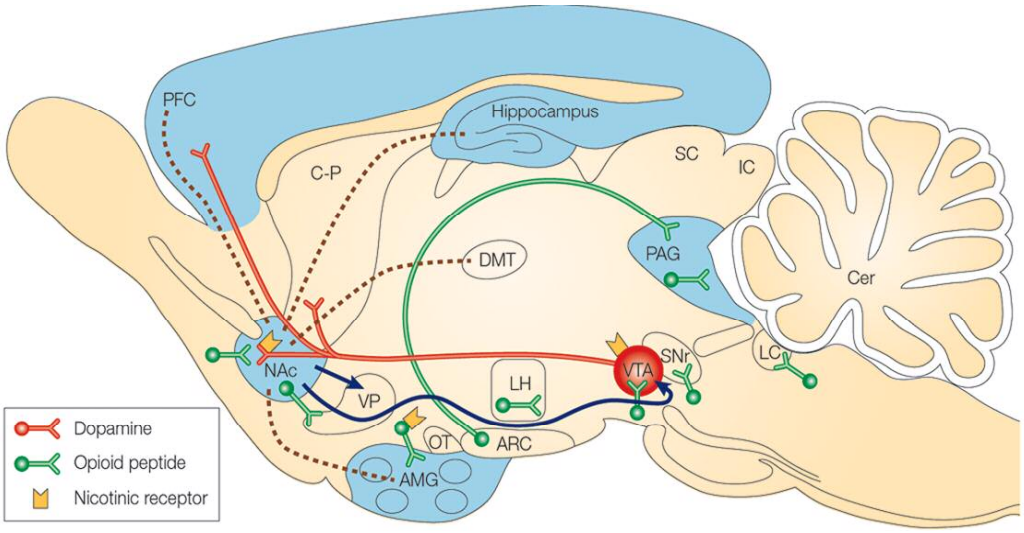
1999). Thus, sex differences in the pattern of drug abuse may be circumstantial, thereby providing males with greater opportunities to progress from initial to habitual use (Van Etten and Anthony, 1999; Van Etten et al., 1999).

Recent studies have shown that there are sex differences in cocaine-induced behavioral and neurochemical alterations in humans and animals (Becker and Ramirez, 1981; Chin et al., 2001; Lukas et al., 1996; Robinson et al., 1982a). The overall aim of this proposal is to obtain a further understanding of sex-related differences in the interactions between the endocrine, monoaminergic and glutamatergic systems. First, we aim to determine dose-dependent effects of estrogen and progesterone on behavioral and neuroendocrinological changes in response to cocaine. The mechanisms by which the hormones interact to modulate these alterations will also be ascertained. Finally, there will be a study to determine the effects of estrogen and progesterone co-administration where dopamine (DA), serotonin (5-HT) and GABA levels in different areas of the mesocorticolimbic system will be assessed. By examining the alterations in these systems, further comprehension of the underlying processes leading to sex and estrous cycle-dependent discrepancies in cocaine effects will be achieved.

C. The role of monoamines on cocaine effects:

Cocaine inhibits the reuptake of neuronal monoamines by binding to dopamine and 5-HT transporters, increasing their synaptic concentrations (Heikkila et al., 1975). Cocaine's effects occur within the mesocorticolimbic pathway, which has been shown to mediate the reinforcing properties of many drugs of abuse including cocaine,

amphetamine, and opioids (Koob, 1992). This pathway is comprised of dopaminergic cell bodies that project from the ventral tegmental area (VTA) to the frontal cortex, nucleus accumbens, and caudate putamen (Koob et al., 1993; Robbins and Everitt, 1996). Additionally, the amygdala has been implicated in drug reward and cocaine-seeking behaviors (Childress et al., 1999; Chin et al., 2001). Cocaine's effects on DA neurons in the prefrontal cortex, ventral pallidum, and olfactory tubercle also appear to be necessary for cocaine reinforcement (Goeders and Smith, 1983; Hubner and Koob, 1990; Koob, 1992; Kunko et al., 1998; Roberts et al., 1980).



(Nestler et al., 2001)

Figure 1: The reward circuit. Dopaminergic projections from the VTA terminating in the nucleus accumbens, caudate putamen, and the frontal cortex are of primary interest in drug abuse research. (Nestler et al., 2001)

Cocaine potentiates dopaminergic neurotransmission within the mesocorticolimbic pathway (Reith et al., 1997). The VTA projections to the nucleus accumbens have been postulated to play a pivotal role in the rewarding effects of cocaine. It has been shown that i.p. administration of cocaine increases extracellular DA concentrations in the VTA and the nucleus accumbens of freely moving rats, but with time course differences in DA release (Reith et al., 1997). Under normal physiological conditions in the VTA, there are GABAergic medium spiny neurons that express D1 DA receptors (Houdi et al., 1989). These GABA neurons project to the VTA and form inhibitory synaptic connections, where DA, acting on these GABA afferents inhibits VTA neurons (Houdi et al., 1989). Chronic cocaine can alter the normal physiological state by attenuating the GABA mediated inhibition, which in turn potentiates neuronal activity (Bonci and Williams, 1996). This potentiation of neuronal firing acts to increase DA release in the nucleus accumbens. It is generally assumed that changes within this neural circuit after chronic use of drugs of abuse underlies the behavioral changes we observed (McFarland and Kalivas, 2001). There are numerous studies indicating that there are semi-permanent cellular adaptations in these limbic nuclei after repeated administration of drugs of abuse (Nestler, 2001). However, it is still not well understood how alterations in the VTA and the nucleus accumbens contribute to the craving and relapse observed in chronic drug users.

Although the importance of DA in cocaine reinforcement is well-documented, studies using DA-transporter knock-out mice indicate that its presence is not necessary for place preference for cocaine and cocaine self-administration (Rocha et al., 1998; Sora

et al., 2001). Furthermore, cocaine reinforcement in DAT/SERT knockout mice is abolished (Sora et al., 2001). Serotonin activity has been shown to be an important mediator of cocaine effects (Muller et al., 2003). Barnes and Sharp (1999) (Barnes and Sharp, 1999) have shown that 5-HT2A and 5-HT2C receptors are localized to the mesoaccumbens DA pathway. In the VTA, a group of neurons that expresses 5-HT2C receptor is co-localized with tyrosine hydroxylase, the rate-limiting enzyme for DA (Doherty M.D. and Pickel, 2000). Elevated expression of 5-HT1B receptors in the nucleus accumbens increases cocaine-induced locomotor activity and increases conditioned place preference for cocaine (Neumaier et al., 2002). Antagonism of several 5-HT receptors leads to a dose-dependent decrease in the behavioral effects of cocaine (Carey et al., 2000; Carey et al., 2001; Herges and Taylor, 2000; McCreary and Cunningham, 1999; McMahon and Cunningham, 2001; Reith, 1990; Svingos and Hitzemann, 1992).

D. Sex differences in the behavioral and subjective effects of cocaine:

Several groups have reported that sex differences in humans in the behavioral response to cocaine maybe be attributed to hormonal profiles or administration paradigm (Lukas et al., 1996; Mendelson et al., 1999). However, there are contradictory results in human studies that compromise our understanding of sex-dependent responses. For example, Lukas et al. (1996) found that there are significant sex differences in response to acute cocaine administration in humans (Lukas et al., 1996). After subjects self administered a dose of intranasal cocaine, male subjects achieved a faster high and higher peaks of plasma cocaine levels, and reported more episodes of euphoria or “good-

feeling." Alternatively, Mendelson et al. (1999) reported no differences in the subjective response or plasma cocaine levels between the sexes after intravenous drug administration (Mendelson et al., 1999). The conflicting reports of subjective and physiological effects may result from the differences in administration paradigms (Sell et al., 2000; Van Etten et al., 1999).

Despite these inconsistencies, some studies provide evidence supporting the notion that females are more sensitive to the effects of cocaine. Robbins et al. (1999) report that female cocaine addicts experience a greater level of drug craving upon introduction to cocaine cues compared to male subjects (Robbins et al., 1999). Additionally, females begin using cocaine and enter treatment at earlier ages than do males (Griffin et al., 1989).

Sex differences in cocaine's effects have also been reported in rodents where females show enhanced locomotor behaviors after acute and chronic cocaine administration as compared to males (Chin et al., 2001; Kuhn and Francis, 1997; Walker et al., 2001a). Craft and Stratmann (1996) suggest few sex differences in the discriminative stimulus effects of cocaine, significant differences between males and females in the locomotor response (Craft and Stratmann, 1996). This difference may be attributed to the disparity in sex dependent responses to cocaine based upon the subjective effects experienced by males and females. Similarly, cocaine affects stereotypic behaviors differently between the sexes where females show more intense stereotypic behaviors after both acute and chronic cocaine administration (Chin et al.,

2001; Kuhn and Francis, 1997; Walker et al., 2001a). Females acquired cocaine self-administration more quickly and showed higher rates of cocaine self-administration, suggesting that females may be more sensitive to the reinforcing effects of the drug (Lynch and Carroll, 2000). Females are also more sensitive during the reinstatement phase of drug self-administration (Lynch and Carroll, 2000) and experience less toxicity to cocaine (Morishima et al., 1993).

Sex differences in behavioral sensitization to cocaine have been observed (Caihol and Morméde, 1999; Chin et al., 2002; Chin et al., 2001; Hu and Becker, 2003; Van Haaren and Meyer, 1991). Females experience a higher degree of sensitization of rotational behavior after cocaine administration (Caihol and Morméde, 1999; Hu and Becker, 2003). Chin et al. (2002) found that female rats become sensitized in cocaine-induced ambulatory and total locomotor activity. In contrast, males were sensitized only in total locomotor activity (Chin et al., 2002). Although females had significantly higher stereotypic and rearing activities after acute, chronic, or a challenge dose than did males after withdrawal from chronic cocaine administration, there was no sex-dependent behavioral sensitization in stereotypic and rearing activities (Chin et al., 2002; Chin et al., 2001).

E. Neuroendocrine effects of cocaine:

Cocaine's ability to modulate the hypothalamic-pituitary-gonadal (HPG) axis may contribute to sex dependent behavioral and neurochemical response and it can affect HPG activation at various levels. For example, cocaine modulates gonadotropin-releasing

hormone (GnRH) (Lebrethon et al., 2000; Parent et al., 1992). Cocaine also affects plasma progesterone levels in intact and pregnant female rats (Kuhn et al., 1999; Quiñones-Jenab et al., 2000a). Cocaine drastically increases plasma levels of progesterone during proestrus compared to other stages of the cycle (Quiñones-Jenab et al., 2000b). This modulation of progesterone appears to be transient and levels return to those seen in controls after three hours (Quiñones-Jenab et al., 1999). However, studies using primates show no changes in progesterone levels following cocaine administration (Mello et al., 1997).

During the follicular phase, but not the midluteal phase of the rhesus monkey, cocaine increases plasma levels of estradiol (Mello et al., 1997). In contrast, cocaine administration in female rats does not result in changes in estradiol levels (Walker et al., 1998). Further studies need to address the inconsistencies in the neuroendocrine response to cocaine in rats and monkeys.

There are also HPA effects of cocaine. Compared to male rats, females have been reported to have higher levels of corticosterone and a more prolonged response after cocaine administration (Walker et al., 2001; Chin et al., 2001; Chin et al., 2002; Kuhn and Francis, 1997). Ovariectomy has also been shown to affect corticosterone in that levels are higher after ovariectomy than they are in intact controls (Chin et al., 2002). In addition, levels of corticosterone vary throughout the estrous cycle of the rat; specifically, levels are highest during proestrus and lowest during diestrus (Walker et al., 2001). This

fluctuation suggests that endogenous ovarian hormones may modulate HPA responses to cocaine.

F. Gonadal hormone effects on cocaine-induced alterations:

Estrogen and progesterone are important mediators of synaptic plasticity. Therefore, changes in the HPG axis in response to cocaine may result in neuronal activation that alters behavioral response. Differences in the hormonal profiles between male and female rats may play an important role in the cascade of events that follow cocaine administration. Because of these disparities, the regulation of the HPG axis by cocaine may underlie estrus cycle differences and contribute to sex differences in cocaine effects.

There are significant cycle-dependent differences in rats and human subjects reported in studies of cocaine effects (Evans et al., 2002; Lukas et al., 1996; Mendelson et al., 1999; Quiñones-Jenab et al., 1999; Sell et al., 2002; Sofuoglu et al., 1999; Walker et al., 2001b). Women in the follicular phase have higher peak plasma cocaine levels compared to those measured during the luteal phase (Lukas et al., 1996). In rats, the estrous cycle influences an animal's motivation to self-administer cocaine, where rats in estrus demonstrate an increased motivation to self-administer cocaine compared to rats at other stages of the cycle (Roberts et al., 1989). Rats in estrus also experience greater locomotor activity and heightened intensity of stereotyped behavior (Quiñones-Jenab et al., 1999). Additionally, it has been shown that cocaine-induced activity is the lowest during the diestrus phase (Quiñones-Jenab et al., 1999; Sell et al., 2002; Walker et al.,

2001b). Since levels of progesterone are higher than estrogen during both the luteal and diestrus phases of the human and rat cycles, these studies suggest that high levels of progesterone may decrease the subjective and behavioral responses to cocaine. Cocaine's differential effects throughout the estrous/menstrual cycle provides evidence to support the postulate that gonadal hormones underlie sex-specific differences in responses to cocaine.

Ovariectomy decreases cocaine-induced activity (Chin et al., 2002), implicating estrogen and progesterone in the regulation of changes resulting from cocaine administration. As summarized in Table 1, although acute subcutaneous administration of estrogen or progesterone does not affect the overall responses to acute cocaine (Hu and Becker, 2003; Perrotti et al., 2001c), estrogen administered via SILASTIC capsules decreases cocaine self-administration (Lynch and Carroll, 1999) and has been shown to potentiate cocaine's behavioral effects (Perrotti et al., 2001c; Sell et al., 2002). In contrast, progesterone administered via silastic capsules attenuates cocaine-induced motor activity (Perrotti et al., 2001c; Sell et al., 2002). Ovariectomized rats treated with estradiol show enhanced behavioral sensitization to cocaine (Febo et al., 2003; Hu and Becker, 2003; Peris et al., 1991; Perrotti et al., 2001c; Sircar and Kim, 1999). However, Grimm and See (1997) report that estrogen replacement decreased cocaine self-administration. The role of progesterone in the modulation of cocaine effects have not been well studied (Grimm and See, 1997). Like estrogen, progesterone plays an important role in the control of the estrous cycle (Pfaff and Schwartz-Giblin 1995). It has been shown that a progesterone antagonist can decrease toxicity of cocaine in rats

(Sharma et al., 1993). Rats receiving co-administration of estrogen and progesterone demonstrated an increase in cocaine-induced locomotor activity and cocaine-CPP in comparison to ovariectomized controls, irrespective of the administration route (Perrotti et al., 2001c; Quiñones-Jenab et al., 2000a; Russo et al., 2003a; Sell et al. 2002; Sell et al., 2002). For example, Russo et al. (2003) showed that rats co-administered estrogen and progesterone developed a greater CPP for cocaine in comparison to the ovariectomized groups and those given either estrogen or progesterone (Russo et al., 2003a). In contrast, following binge or single dose cocaine administration, estrogen and progesterone co-administration has been shown to suppress cocaine-induced locomotor behavior, while each hormone alone has no effect (Quiñones-Jenab et al., 2000a).

Table 1: Effects of gonadal hormones on cocaine-induced activity following acute cocaine administration (Festa and Quinones-Jenab, 2004)

Hormone		Result	Cocaine Dose	Hormone Dose	Reference
Estrogen	↑	Cocaine-induced activity as compared to P-treated animals and OVX females	5 mg/kg i.p.	Silastic implant	Sell et al., 2000
	↑	Cocaine-induced activity compared to OVX females	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
	-	Does not affect cocaine-induced activity	15 mg/kg i.p.	2ug E s.c.	Sicar and Kim, 1999
	-	Does not affect cocaine-induced activity	15 mg/kg i.p. (x3)	50ug E s.c.	Quinones-Jenab et al., 2000
	-	Does not affect cocaine-induced activity	5-20 mg/kg i.p.	5ug E s.c.	Hu and Becker, 2003
Progesterone	↓	Cocaine-induced activity compared to OVX females	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
	-	Does not affect cocaine-induced activity	15 mg/kg i.p.	500ug P s.c.	Sicar and Kim, 1999
	-	Does not affect cocaine-induced activity	15 mg/kg i.p.	500ug P s.c.	Perrotti et al., 2000
	-	Does not affect cocaine-induced activity	15 mg/kg i.p. (x3)	500ug P s.c.	Quinones-Jenab et al., 2000
E+P	↑	Cocaine-induced activity compared to OVX females	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
	↑	Cocaine-induced activity compared to OVX females	15 mg/kg i.p.	2ug E/500ug P s.c.	Sicar and Kim, 1999
	↑↓	Inhibited cocaine-induced activity following 1st injection, increased activity following multiple injections.	15 mg/kg i.p. (x3)	50ug E/500ug P s.c.	Quinones-Jenab et al., 2000

G. Effects of ovarian steroids and estrous cycle variations on the dopamine system:

DA release, number of receptors and levels of DA transporter have been shown to vary due to estrous cycle effects and sex dependent differences in the DA system (Becker and Ramirez, 1981; Castner et al., 1993; Crowley, 1982; Di Paolo et al., 1979; Di Paolo et al., 1988; Di Paolo et al., 1982; Goetz et al., 1983; Houdi et al., 1989; Hruska, 1986; Hruska and Pitman, 1982; Joyce, 1983; Robinson et al., 1982b). It has been proposed that these effects are regulated by ovarian hormones. For example, estrogen can increase striatal DA turnover and produce changes in the density of striatal DA receptors (Becker, 1990; Di Paolo et al., 1979; Di Paolo et al., 1985; Hruska and Pitman, 1982; Hruska and Silbergeld, 1980). Additionally, estrogen can downregulate D2 class DA receptors (Bazzett and Becker, 1994). DA release has been shown to be enhanced after estrogen administration in the nucleus accumbens (Thompson and Moss, 1994). Similarly, estrogen can stimulate DA release in the caudate putamen (Becker, 1990). Ovariectomy causes a decline in methamphetamine-stimulated DA release in the striatum but chronic replacement of estrogen reversed the effect (Ohtani et al., 2001).

In estrogen-primed rats receiving progesterone, there was an increase in DA release in the striatum (Dluzen and Ramirez, 1984; Dluzen and Ramirez, 1987; Dluzen and Ramirez, 1991). Independent of estrogen effects, a physiological dose of progesterone has been shown to rapidly increase DA in this area (Petitclerc et al., 1995). The presence of progesterone amplifies the release of DA in response to NMDA in the striatum (Cabrera and Navarro, 1996). Allopregnanolone, a metabolite of progesterone,

was also able to produce this effect in rats in estrus which was abolished by ovariectomy (Dluzen and Ramirez, 1987). The ability of ovarian hormones to impact DA activity suggests a crucial role of estrogen and progesterone in modulating the effects of psychoactive drugs that act on the DA system.

H. Ovarian steroid hormone effects on the serotonergic system:

Steroid hormones have been shown to modulate the serotonergic system. The activity of 5-HT neurons is higher in male than in females (Klink et al., 2002). Ovariectomy induces a decrease in 5-HT2A receptor density which can be upregulated to control levels when estrogen replacement is administered (Cyr et al., 1998). Estrogen replacement increases 5-HT2C receptor mRNA levels in the midbrain (Zhou et al., 2002). Estrogen is also capable of exhibiting noncompetitive inhibition of 5-HT transport (Chang and Chang, 1999).

Firing rates of neurons in the dorsal raphe nucleus were correlated with plasma progesterone levels where higher progesterone corresponded to increased firing (Klink et al., 2002). However, in the hypothalamus, when progesterone was administered following estrogen priming, levels of 5-HT were reduced (Farmer et al., 1996; Gereau et al., 1993; Maswood et al., 1999). Maswood et al. (1999) suggest that the progesterone-induced decrease in extracellular 5-HT levels may be attributed to an alteration in a change in 5-HT autoreceptor function (Maswood et al., 1999). Our laboratory has previously shown that progesterone replacement increased levels of 5-HT in the prefrontal cortex after cocaine administration (Perrotti, L. I., Beck, K. D., Luine, V. N.,

and Quinones-Jenab, V00). Serotonin-hormone interactions have not been extensively studied but 5-HT has prominent role in drug reward and further studies of these interactions will result in an improved understanding of sex differences underlying drug effects.

I. Current problems in the hormone replacement literature and significance of work:

In the current literature, there are inconsistencies in the doses of estrogen and progesterone replacement in addition to the variation in the manner of hormone administration. It is difficult to compare the effects of the hormones across these studies due to these inconsistencies. Also, single hormone replacement paradigms (one dose, one time point of administration, and one length after OVX) and the use of doses producing higher than physiological-levels of hormone assume that estrogen and progesterone have single and distinctive effect on cocaine responses.

On the contrary, hormone effects on behaviors that occur during the estrous cycle are based on varying concentration and temporal interactions between estrogen and progesterone. These interactions can either inhibit or stimulate the components of reproductive behaviors. Due to the overlap of the reward and motor mechanisms of psychostimulant-induced and reproductive behaviors, cocaine-induced activity may be regulated by estrogen and progesterone interactions.

Table 2. Hormonal levels in intact rats and after various replacement paradigms after ovariectomy

Estrous cycle stage (approximate)	Levels of estrogen (pg/mL)	Levels of progesterone (ng/mL)
Proestrus	45	60
Estrus	10	10
Diestrus	20	22
Estrogen replacement		
Silastic capsules		
10%	60	-
20%	200	-
30%	125	-
40%	300	-
S.C. injection (μg)		
10	15	-
15	25	-
20	35	-
Progesterone replacement		
Silastic capsules (cm)		
1	-	7
1.5	-	10
3	-	20
9	-	20
S.C. injection (μg)		
10	-	10
50	-	10
100	-	12
500	-	20

I. Mechanisms of steroid action:

Steroids bind to intracellular receptors which then undergo a conformational change, allowing them to regulate gene expression as transcription factors (Evans, 1988; Rupprecht, 1997). They are capable of affecting the pattern of neurotransmitter receptor subunit expression. For example, progesterone alters mRNA levels of certain subunits of the GABA-A receptor in the hippocampus (Weiland and Orchinik, 1995). Finally, steroids can modulate ligand-gated ion channels and G-protein-coupled receptors which can regulate the expression patterns of downstream genes through the cAMP-PKA-CREB pathway (Wehling, 1997; Zakon, 1998).

In addition to the wide variety of actions steroids can exert on genomic alterations, it has been shown that they can mediate membrane channel activity, usually through calcium or calcium-dependent potassium channels (Kelly and Levin, 2001; Zakon, 1998). Steroids can also interact with neurotransmitter receptors to affect neuronal excitability (Majewska et al., 1986; Rupprecht, 1997). Beyer and Raab (1998) show that estradiol administration increases intracellular calcium levels in DA and GABA neurons (Beyer and Raab, 1998). Other evidence includes estrogen's ability to rapidly decrease mu-opioid activity in the hypothalamus (Lagrange et al., 1997) and inhibit episodic LH release (Condon et al., 1998) suggesting the involvement of a nongenomic mechanism. During pregnancy, progesterone acts directly on the oxytocin receptor, a member of the G-protein-coupled receptor family to decreases its sensitivity

to oxytocin which is crucial in maintaining pregnancy (Grazzini et al., 1998). Other examples of progesterone's membrane-mediated activities include the inhibition of cholesterol biosynthesis in the absence of progesterone receptor binding (Metherall et al., 1996) and the activation of protein kinase C via progesterone-stimulated calcium influx (Bonaccorsi et al., 1998). Allopregnanolone, a metabolite of progesterone is also capable of acting on the cell membrane. It acts as an allosteric agonist at the GABA-A receptor to enhance GABA release (Sullivan and Moenter, 2004; Uchida et al., 2002).

We hypothesize that there are hormone dependent modulations in cocaine-induced behavioral, endocrinological and neurochemical alterations. Specifically, estrogen and progesterone can either enhance or inhibit responses to cocaine based on temporal and concentration effects. To determine the possible modulation of response to cocaine by ovarian hormones, ovariectomized rats will be administered estrogen, progesterone or co-administered both hormones using a wide range of doses using different time courses. This design aims to simulate physiological hormonal fluctuations during the estrus cycle, allowing conditions under which hormone-drug interactions can be more accurately studied. The following specific aims are proposed:

Specific Aim 1:

We hypothesize that estrogen and progesterone will affect cocaine-induced behavior and endocrinological alterations in a dose dependent manner. To this end, behavioral activity after cocaine administration will be related changes in corticosterone levels. Additionally, we predict that there are steroid hormone dependent effects on cocaine metabolism.

Specific Aim 2:

We hypothesize that membrane and genomic-mediated mechanisms will enhance or inhibit cocaine-induced alterations. These mechanisms, in turn will activate receptor-mediated channels.

Specific Aim 3:

We hypothesize that co-administration of different ratios of estrogen and progesterone will enhance or inhibit behavioral and neurochemical alterations. This paradigm should follow reported behavioral patterns observed during the estrous cycle. To this end, besides the proposed measurement of different endocrine activity (i.e. CORT levels), we propose to measure DA and 5-HT levels in different areas of the mesocorticolimbic system. This will allow for a direct correlation of estrogen and progesterone effects and monoaminergic transmission.

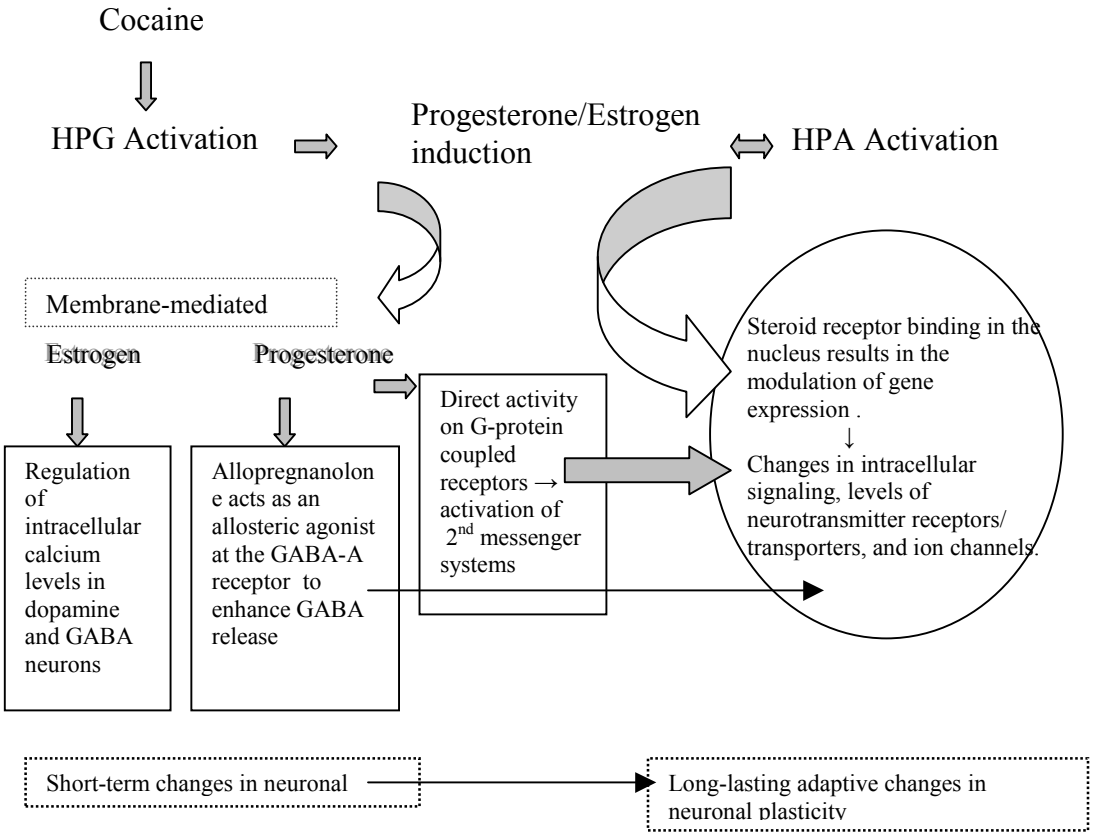


Figure 2: Hypothetical model of hormone signaling. Cocaine induced changes in gonadal steroid plasma levels will lead to changes in the HPA axis, including the biosynthesis of corticosterone and further progesterone release. Progesterone and estrogen induction will activate rapid membrane-bound non-genomic signaling, which may affect intracellular functions or lead to activation of transcription factors. Simultaneously, the hormones will bind to their receptors and interact with response elements containing promoters, leading to an up-regulation or down-regulation of gene transcription and subsequent tissue responses.

Chapter 2: Progesterone inhibits behavioral responses and estrogen increases corticosterone levels after acute cocaine administration

1. Introduction

Recent clinical and basic studies have demonstrated sex differences in the pattern of cocaine use and in the behavioral and subjective effects of cocaine. In human studies, females appear to be more sensitive to cocaine reward (Griffin et al., 1989; Lukas et al., 1996; Robbins et al., 1999). Similarly, female rats exhibit greater hyperactivity and exaggerated behavioral responses compared to male rats (Caihol and Morméde, 1999; Chin et al., 2002; Chin et al., 2001; Craft and Stratmann, 1996; Festa et al., 2003; Festa et al., 2004; Sircar and Kim, 1999; Van Haaren and Meyer, 1991). Female rats also display cocaine-induced locomotive sensitization sooner than males rats, and they maintain this sensitized response after a withdrawal period, whereas male rats do not (Chin et al., 2001). Additionally, female rats acquire conditioned place preference (CPP) for cocaine at lower doses and with fewer pairing sessions than do males (Russo et al., 2003b).

The estrous cycle influences behavioral responses to acute cocaine administration: cocaine-induced behavioral activity is lowest during diestrus in comparison with proestrus and estrus (Sell et al., 2000). It has been postulated that fluctuating levels of estrogen and progesterone modulate the locomotive response to acute cocaine administration (Hu and Becker, 2003). Steroid replacement paradigms have shown that estrogen enhances behavioral sensitization to cocaine, and estrogen has been found to

either decrease or increase cocaine self-administration depending on the concentration and manner of administration (Grimm and See, 1997; Lynch and Carroll, 1999; Perrotti et al., 2001b; Sell et al., 2002; Zhou et al., 2002). On the other hand, most studies show that progesterone has no effect on cocaine-induced locomotive activity in rats (Perrotti et al., 2001b; Sell et al., 2000; Sircar and Kim, 1999).

Similar to the sexually dimorphic cocaine-induced behavioral findings, there are also sex differences in hypothalamic-pituitary axis (HPA) responses to cocaine. Compared to male rats, females have been reported to have higher levels of corticosterone and a more prolonged response after cocaine administration (Walker et al., 2001; Chin et al., 2001; Chin et al., 2002; Kuhn and Francis, 1997). Ovariectomy has also been shown to affect corticosterone in that levels are higher after ovariectomy than they are in intact controls (Chin et al., 2002). In addition, levels of corticosterone vary throughout the estrous cycle of the rat; specifically, levels are highest during proestrus and lowest during diestrus (Walker et al., 2001). This fluctuation suggests that endogenous ovarian hormones may modulate HPA responses to cocaine.

Although estrogen and progesterone levels fluctuate during the estrous cycle, most published studies have used a single dose to determine how these ovarian hormones interact with cocaine to affect locomotive behaviors (Benmansour et al., 1992; Perrotti et al., 2001b; Sircar and Kim, 1999). A study on how hormonal fluctuations affect important components of cocaine-induced responses (behavioral activation, HPA responses and cocaine metabolism) has yet to be conducted. Determining these effects is

necessary to explain the biological basis of sex differences and estrous cycle influences in response to cocaine. The aim of the current study was to determine whether the dose of estrogen and progesterone affects cocaine-induced behavioral responses and corticosterone levels.

2. Methods

2.1 Animals

Eight-week-old OVX Fischer rats purchased from Charles River (Raleigh, NC) were individually housed in standard cages with access to food and water *ad libitum*. Rats were maintained on a 12-hour light/dark cycle with lights on at 10:30 AM. Rats were handled and weighed daily for 1 week before experimental manipulations. Experiments were conducted 2 weeks after ovariectomy. For all experimental groups, n ranged from 8 to 10. Each study consisted of at least two cohorts. All NIH guidelines for the care and use of laboratory animals were followed, and the experimental use of animals was approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Hormone Replacement

Rats received subcutaneous (s.c.) injections of either estrogen or vehicle (0, 5, 10, 20, or 50 μg) 48 hours, or progesterone or vehicle (0, 50, 100, 250, or 500 μg) 24 hours before administration of 15 mg/kg of cocaine or saline. In control groups, vehicle (sesame oil) was administered either 24 or 48 hours before exposure to cocaine. Administration of 20 and 50 μg of estrogen and 250 and 500 μg of progesterone

produces levels equal to those observed during the late proestrus stage; all other doses are representative of the fluctuating levels of the hormones throughout the cycle (Freeman, 1994; Pfaff and Schwartz-Giblin, 1995). Furthermore, these doses fall within the range of doses used in previously published studies that aimed to determine interactions between gonadal hormones and cocaine (Hu and Becker, 2003; Perrotti et al., 2001a; Quinones-Jenab et al., 2000; Sircar and Kim, 1999). The timing of progesterone administration was chosen on the basis of previous reports from our group showing that maximal behavioral alteration was observed when progesterone treatment was given 24 hours before cocaine administration (Perrotti et al., 2000).

2.3 Drug Administration

Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intraperitoneally (i.p.) at a volume of 1 mL/kg. Injections of 15 mg/kg of cocaine or saline were administered in the home cage 30 minutes after lights were turned on.

2.4 Behavioral Activity

Behavioral measurements were performed for each rat in its home cage for 30 minutes after saline or cocaine administration. Locomotive activity was monitored with a Photobeam Activity System from San Diego Instruments (CA), as previously described (Perrotti et al., 2001a). Ambulatory activity represents the number of counts produced by the interruption of two consecutive photobeams in the horizontal frame. Rearing activity represents the total counts of vertical motion.

To assess stereotypic activity, rats were videotaped for 40 seconds, 15 and 30 minutes after cocaine or saline administration. The videotapes were later analyzed for behavioral stereotypy by three trained observers blinded to each animal's treatment group. The rating for cocaine-induced stereotypic behavior was based on a modification of the Creese and Iversen scale (Creese and Iversen, 1974). 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 (Table 2). A score of 10 was never observed during the course of this experiment. Because no significant differences were observed in stereotypy between 15 and 30 minutes, only the stereotypic data recorded at 15 minutes after cocaine administration are presented here.

Table 3: 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 hind limbs

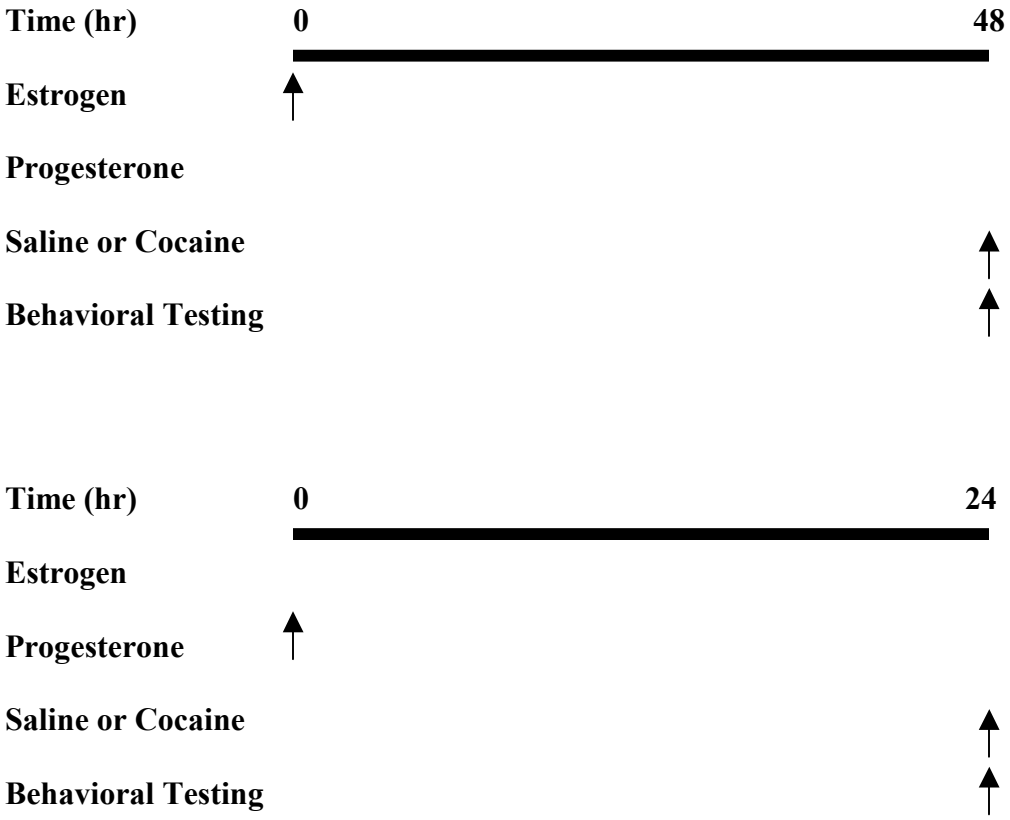


Figure 3. Dose response administration paradigm. Arrows represent times at which hormones and cocaine was administered.

2.5 Serum Levels of Benzoylecgonine and Corticosterone

Thirty minutes after cocaine or saline administration, the rats were sacrificed by decapitation, following a brief exposure (20 seconds) to CO_2. Trunk blood was collected and centrifuged at 3,000 RPM for 15 minutes at 4ºC. Serum was collected and stored at -80ºC. Serum was analyzed with Coat-A-Count radioimmunoassay kits for benzoylecgonine and corticosterone (National Diagnostic, San Diego, CA). Because we have previously determined that BE is not detected in saline-treated animals, in this study we did not perform the analysis on these groups. Intra-assay coefficients of variation were less than 10.0% ± 1.0%. Results for these assays were determined by a log-logit analysis within GraphPad Prism (GraphPad Software, Inc, San Diego, CA, USA). Serum levels of corticosterone and benzoylecgonine are expressed as ng/mL.

2.6 Statistical Analysis

Ambulatory, rearing, and corticosterone data are presented as mean ± standard error of the mean. Stereotypic data are presented as median score ± semi-interquartile range. To analyze locomotive activity, we used two-way analyses of variance to determine the effects of cocaine and hormone on locomotive behavior as follows: drug (saline or cocaine) X hormone (vehicle, estrogen, or progesterone). For all analyses, separate ANOVAs were performed on estrogen- and progesterone-treated groups, and comparisons were made with their respective controls. When significant interactions were obtained, Fisher LSD post hoc tests were used to assess differences between cocaine

groups and their respective saline controls within each hormone group. To analyze stereotypic behavior, we used a Kruskal-Wallis test, followed by a Dunn's post hoc analysis, to assess the effects of hormone dose or cocaine treatment. A p-value of <0.05 was considered significant in all statistical analyses.

3. Results

3.1 Estrogen Effects on Cocaine-Induced Behaviors and Serum Levels of Corticosterone and Benzoylecgonine

Overall, cocaine significantly increased the ambulatory, rearing, and stereotypic activities following cocaine administration as compared with those of saline-treated controls ([F (1,95) = 21.240, p = 0.0001], Figure 4A; [F (1,95) = 38.520, p = 0.0001], Figure 4B; and [H (1,74) =17.725, p = 0.0001], Figure 4C). There was a significant effect of estrogen dose on stereotypic behavior in that animals receiving 50 μg had higher stereotypic counts than did the group receiving only sesame oil [H (4,74) =10.255, p = 0.0139]. After 30 minutes, cocaine-induced stereotypic activity was similar to that observed at 15 minutes (data not shown). None of the estrogen doses altered baseline behavioral activity in saline-treated controls.

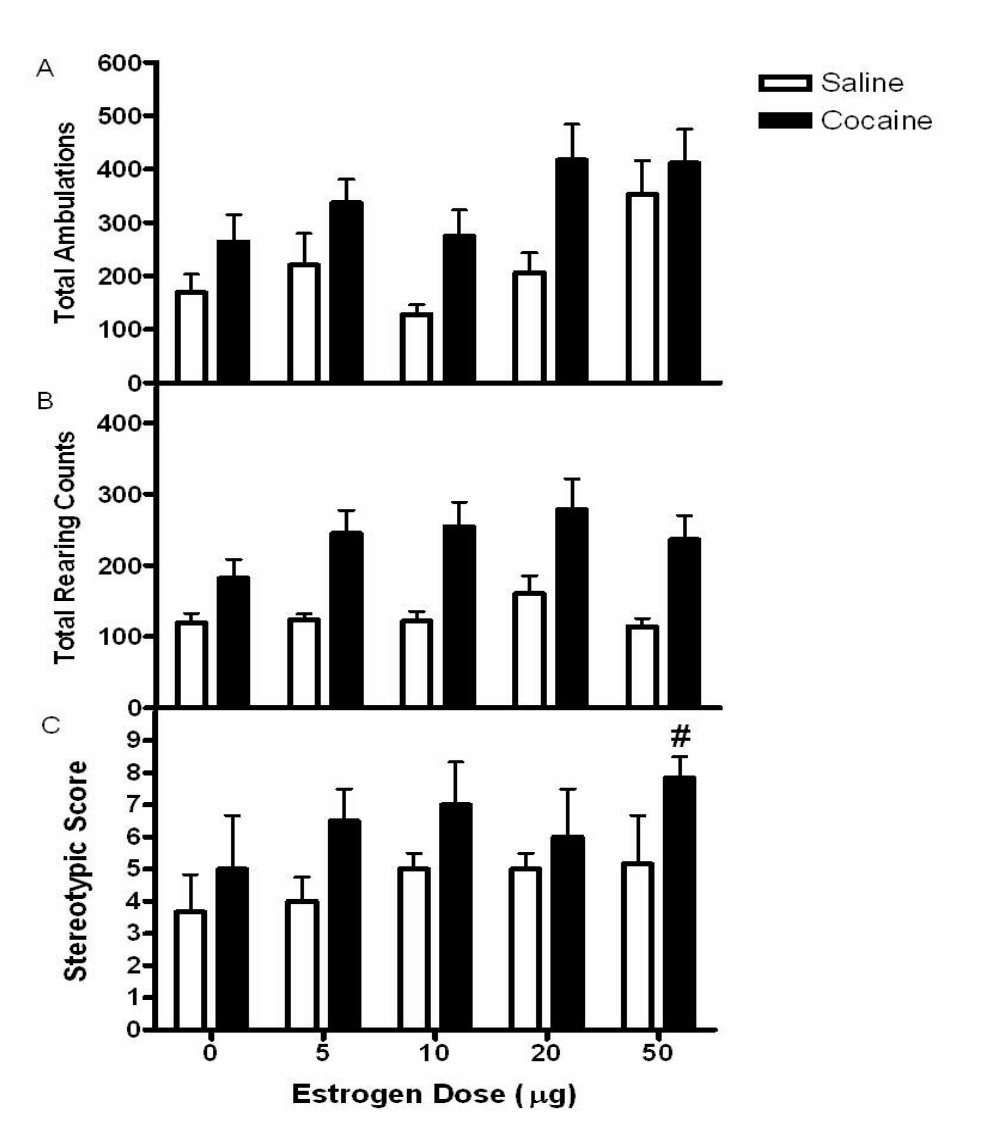


Figure 4: Influence of estrogen dose on cocaine-induced (A) ambulatory, (B) rearing, and (C) stereotypic activities. Graphs summarize behavioral activity after administration of saline (white bars) or cocaine (solid bars) for OVX Fischer rats pretreated for 48 hours with estrogen (0, 5, 10, 20, or 50 μg). Data are represented as cumulative ambulatory counts for the 30 minutes of behavioral testing. #Represents statistically significant differences between the vehicle- and hormone-treated groups (p <0.05).

Estrogen administration affected baseline serum levels of corticosterone in saline-treated controls ([F (4,33) = 2.858, p = 0.0388], Figure 5); saline-treated rats receiving 50 µg of estrogen had higher serum levels of corticosterone than rats receiving vehicle, 5, or 10 µg of estrogen (p = 0.0091, p = 0.0179, p = 0.0424, respectively). To account for the estrogen effect on baseline corticosterone levels, data were normalized to the percentage-change in reference to the respective controls where we observed an effect of estrogen dose [F (4,38) = 5.537, p = 0.0013]. Estrogen administration of 5 or 10 µg led to a higher percentage-increase in serum levels of corticosterone after cocaine administration than was found in the hormone group receiving only sesame oil (p = 0.0032 and p = 0.0004, respectively) or 50 µg (p = 0.0257 and p = 0.0035, respectively). No significant differences in serum levels of benzoylecgonine were observed among the cocaine-treated groups ([F (4,41) = 0.5533, p = 0.6977], Table 4).

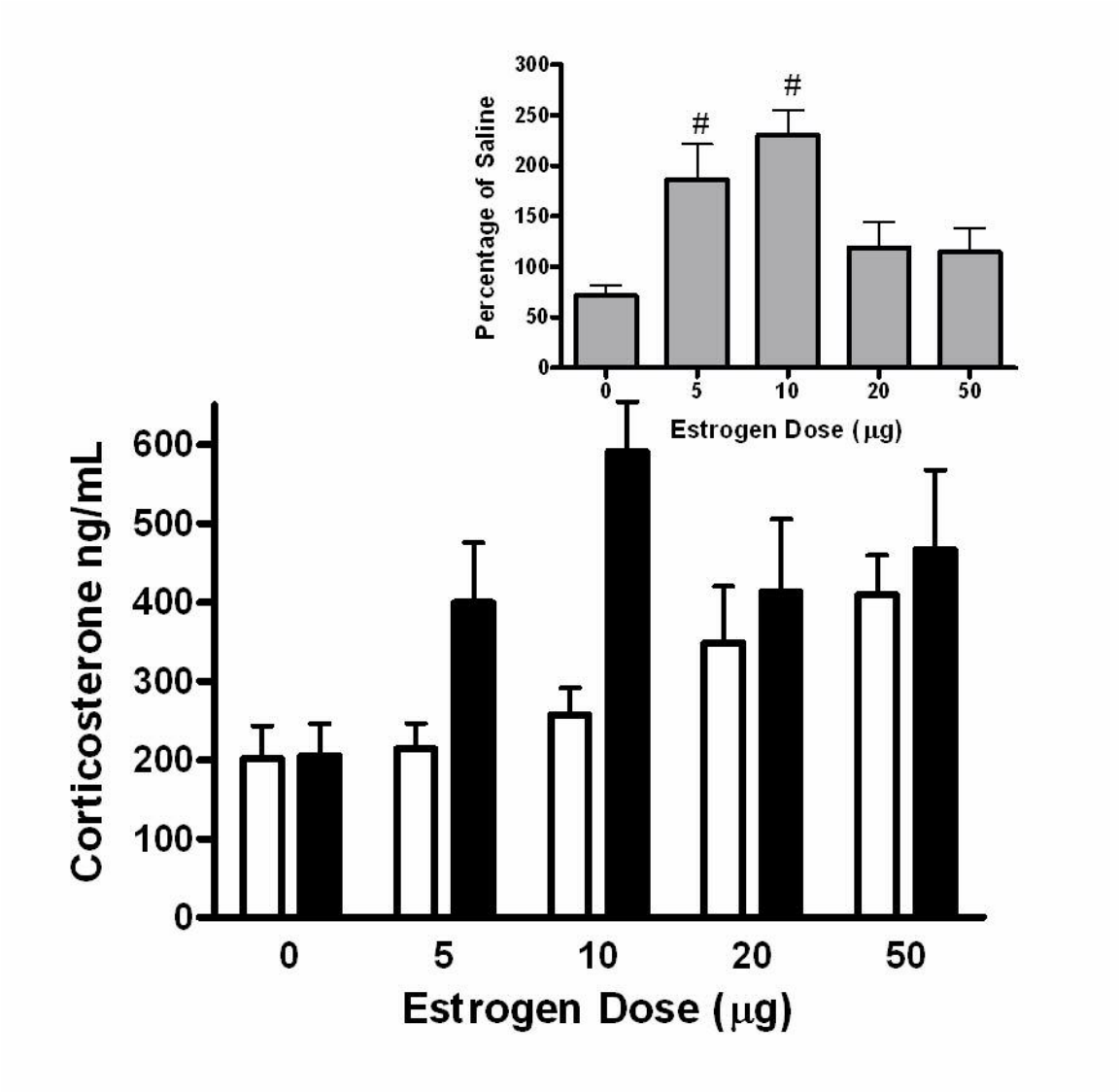


Figure 5: Effect of estrogen replacement on serum corticosterone levels after saline or cocaine administration. Mean ± SEM serum levels of corticosterone (expressed as ng/mL) for cocaine- (black bars) and saline-treated (white bars) rats. The inset shows the percentage change. #Represents statistically significant differences between the vehicle- and hormone-treated groups (p <0.05).

Table 4. Serum levels of benzoylecgonine (BE) after hormonal pretreatment and cocaine administration

Estrogen		**Progesterone**	
Dose (µg)	**Serum levels (ng/mL)**	**Dose (µg)**	**Serum levels (ng/mL)**
0	45.33 ± 6.98	0	46.26 ± 4.18
5	50.00 ± 9.27	50	39.97 ± 6.14
10	50.84 ± 11.95	100	60.64 ± 15.48
20	56.04 ± 13.29	250	49.60 ± 8.09
50	66.45 ± 11.37	500	33.17 ± 1.49

3.2 Progesterone Effects on Cocaine-Induced Behaviors and Serum Levels of Corticosterone and Benzoylecgonine

There was a significant interaction between cocaine administration and progesterone dose in rearing counts: groups receiving 50 and 500 μg displayed an inhibition and those receiving 100 and 250 μg exhibited an enhancement in cocaine-induced rearing [F (4,76) = 7.106, p = 0.0001]. Additionally, cocaine administration significantly increased rearing and stereotypic activity as compared with that of saline-treated controls ([F (1,76) = 23.123, p = 0.0001], Figure 6B; and [H (1,75) = 34.039, p = 0.0001], Figure 6C, respectively). Progesterone did not affect baseline activity in any of the behavioral measurements, nor did it affect baseline corticosterone levels ([F (4,35) = 1.408, p = 0.2517], Figure 7). Moreover, progesterone administration did not affect the cocaine-induced alteration of corticosterone and benzoylecgonine levels ([F (4,76) = 0.852, p = 0.4971], Figure 7; and [F (4,33) = 1.003, p = 0.4971], Table 4, respectively).

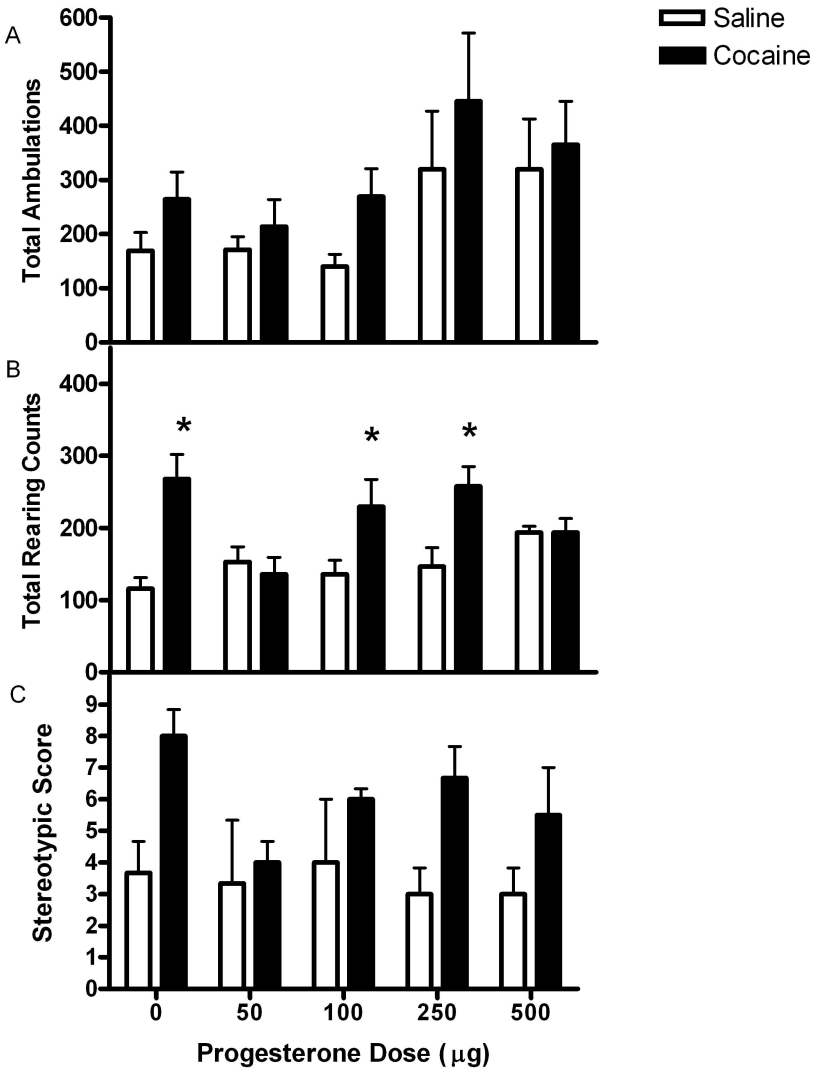


Figure 6: Influence of progesterone dose on cocaine-induced (A) ambulatory, (B) rearing, and (C) stereotypic activities. Graphs summarize behavioral activity after administration of saline (white bars) or cocaine (solid bars) for OVX Fischer rats pretreated for 24 hours with progesterone (0, 50, 100, 250, or 500 μg). Data are represented as cumulative counts for the 30 minutes of behavioral testing. *Indicates a statistically significant difference from saline-treated control, $p < 0.05$.

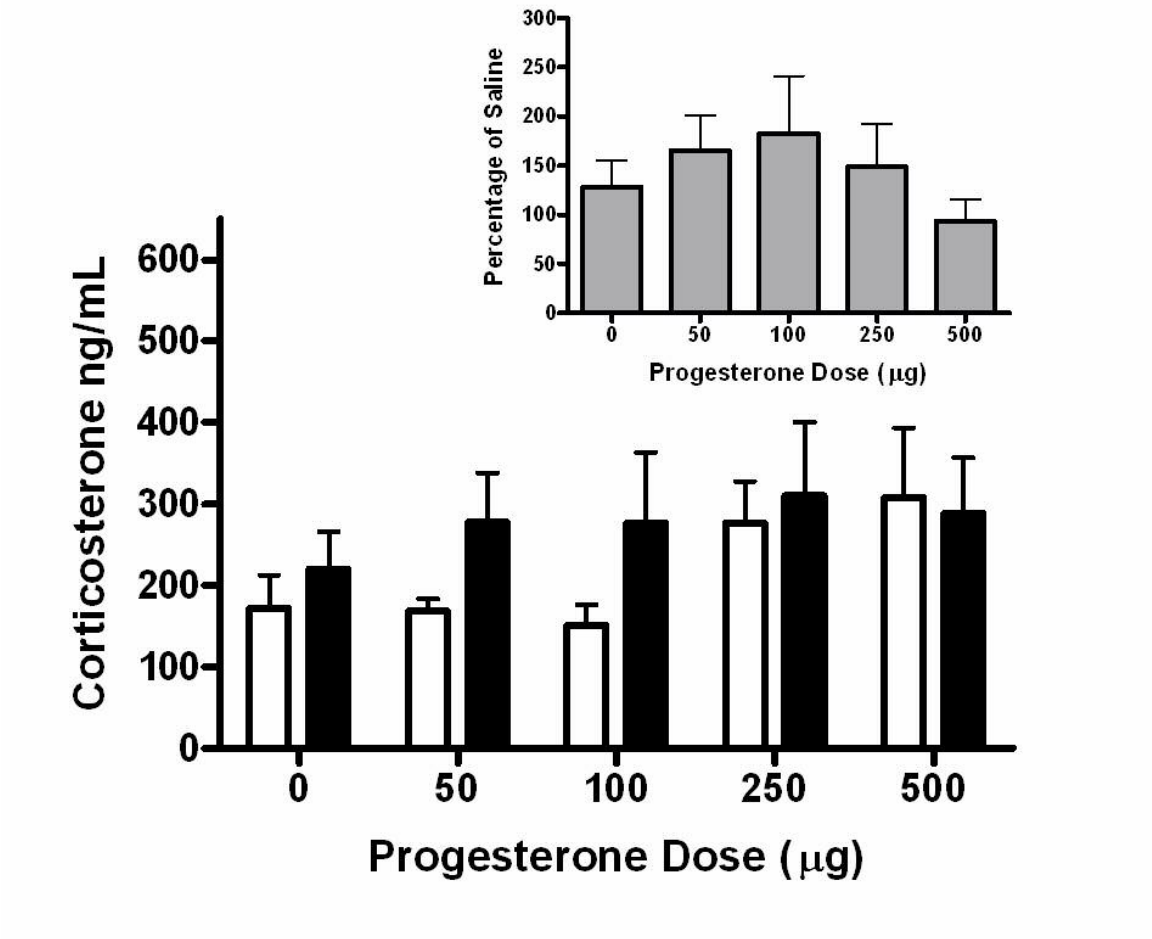


Figure 7: Effect of progesterone replacement on serum corticosterone levels after saline or cocaine administration. Mean ± SEM serum levels of corticosterone (expressed as ng/mL) for cocaine- (black bars) and saline-treated (white bars) rats. The inset shows the percentage change.

4. Discussion

Similar to findings of past studies, we observed that cocaine increased locomotive behavior in OVX rats (Chin et al., 2002; Sell et al., 2000; Walker et al., 2001a). Consistent with previously published reports, this study found an interaction between estrogen, progesterone, and some cocaine-induced behavioral responses (Hu and Becker, 2003; Perrotti et al., 2001b; Sell et al., 2000). However, we extend current knowledge by demonstrating that both estrogen and progesterone differentially affect behavioral and hypothalamic-pituitary-adrenal (HPA) responses to cocaine dose-dependently. Also consistent with past studies, our results show that s.c. estrogen administration does not affect ambulatory and rearing responses to acute cocaine administration (Hu and Becker, 2003; Quinones-Jenab et al., 2000; Sircar and Kim, 1999). Previous studies showed that estrogen, administered via SILASTIC capsules, potentiates cocaine-induced behavioral effects (Perrotti et al., 2001b; Sell et al., 2000). Thus, it is possible that estrogen's effects on cocaine-induced activity vary as a result of prolonged exposure to estrogen rather than a single surge. Festa and Quiñones-Jenab (2004) postulated that variations in estrogen effects after s.c. and SILASTIC administration may be attributed to different mechanisms of actions, such as long- versus short-term effects of estrogen in the central nervous system (CNS). Further studies are needed to address whether genomic and/or membrane effects may underlie the heterogeneity of reported estrogen effects on cocaine-induced behavioral responses.

It has previously been shown that progesterone administration (500 μg, 4 hours before cocaine administration) has no effect on cocaine-induced behavioral responses (Perrotti et al., 2001b; Quinones-Jenab et al., 2000; Sell et al., 2000; Sircar and Kim, 1999). In the present study, however, administration of 50 and 500 μg but not 100 and 250 μg of progesterone 24 hours before cocaine administration inhibited rearing responses. Thus, progesterone-dose effects on cocaine-induced rearing activity may be more complex than previously expected. It is feasible that at different doses of progesterone separate mechanisms may be activated. However, the mechanisms underlying this dose-dependent effect have yet to be determined. Russo et al. (2003a) showed that progesterone replacement via SILASTIC capsules attenuated cocaine CPP. Our results are consistent with these observations and further demonstrate that, at certain concentrations, progesterone attenuates or inhibits some cocaine-induced behavioral effects in female rats. It is possible that inconsistencies between our observations and those of others (Perrotti et al., 2001b; Quinones-Jenab et al., 2000; Sell et al., 2000; Sircar and Kim, 1999) may be attributed to differences in the route of administration and the time at which progesterone was administered.

The mechanisms by which these hormones differentially affect behavioral responses to cocaine are not well understood. Progesterone has been shown to decrease anxiety (Fernandez-Guasti and Picazo, 1995; Rodriguez-Sierra et al., 1984). Additionally, allopregnanolone, a metabolite of progesterone that interacts with the GABA-A receptor, exhibits anxiolytic effects (Bitran et al., 1993; Bitran et al., 1995; Fernandez-Guasti and Picazo, 1995; Kokate et al., 1999; Majewska, 1992; Reddy DS et al., 2004). Thus,

progesterone-mediated inhibition of rearing activity may, in part, be the result of anxiolytic effects resulting from GABA-A activation.

Several studies have shown that the estrous cycle influences both behavioral outcome and the animals' motivation to self-administer cocaine (Bless et al., 1997; Lynch et al., 2000; Roberts et al., 1989; Walker et al., 2001b). In rats, cocaine-induced behavioral responses during diestrus are lower than those of rats in other stages of the cycle (Sell et al., 2000; Walker et al., 2001b). In women, subjective effects of cocaine were also shown to fluctuate during the menstrual cycle (Sofuoglu et al., 1999). Female cocaine users had an attenuated subjective response to cocaine during the luteal phase of the menstrual cycle as compared with those in the follicular phase (Sofuoglu et al., 1999). In addition, Evans et al. (2002) demonstrated that women in the luteal phase had less desire to smoke cocaine than they did in the follicular phase. Since the luteal and diestrus phases of the cycle are characterized by higher levels of progesterone, these reports suggest that the increase of progesterone serum levels decreases the subjective effects of psychostimulants in humans. This decrease may partially be attributed to the anxiolytic effects of progesterone.

Estrogen may modulate cocaine-induced behavior through alternate pathways. For example, estrogen increases striatal dopamine turnover and produces changes in the density of striatal dopamine receptors (Becker, 1990; Di Paolo et al., 1981; Di Paolo et al., 1985; Hruska and Pitman, 1982; Hruska and Silbergeld, 1980). Additionally, estrogen has been shown to increase dopamine release in the mesolimbic pathway (Becker, 1990;

Thompson and Moss, 1994). Thus, an estrogen-mediated increase in dopamine activity may underlie increases in stereotypic behavior. However, since no effect of estrogen was observed in locomotor activity, it is feasible that estrogen affects various components of behavioral responses via differential modulation of CNS pathways. For example, it has been postulated that stereotypic and locomotive responses are controlled by different pathways in the CNS, as well as by differential activation of the D1/D2 dopamine receptors (Capper-Loup et al., 2002; De Jonge et al., 1986; McCreary and Marsden, 1993).

On the basis of our observations, we postulate that the transition from inhibition to potentiation of behavioral responses to cocaine in female rats during the estrous cycle is mainly derived from alterations in hormone levels. We showed that although hormonal fluctuations affect cocaine-induced responses, the magnitude of their impact on behavioral outcome was not dramatic. Indeed, ambulatory activity was not affected by either hormone. It is possible that the presence of both hormones may lead to a more robust behavioral effect than that observed after replacement of a single hormone. In a recent publication by our group, we showed that estrogen and progesterone co-administration produced a complex interaction wherein increases or decreases in behavioral outcomes varied according to temporal interactions between the hormones (Perrotti et al., 2000). The degree to which the fluctuation of ovarian hormone levels affects these phenomena remains to be elucidated.

A second aim of this report was to examine the effect of ovarian hormones on the activation of the HPA axis. The post-cocaine surge of corticosterone has been postulated to be essential for the control of behavioral cocaine alterations. For example, manipulation of corticosterone levels has been shown to influence locomotive responses to cocaine (Marinelli et al., 2000; Marinelli et al., 1994) as well as the development of sensitized responses after cocaine administration (Rough-Pont et al., 1995). After acute cocaine administration, female rats have exhibited greater activation of the HPA axis than male rats (Chin et al., 2001; Festa et al., 2003; Kuhn and Francis, 1997). Moreover, cocaine-induced increases in levels of corticosterone were present in rats receiving estrogen replacement but not in OVX controls (Perrotti et al., 2001b). Our results are consistent with these findings. Additionally, we have expanded on these observations by demonstrating that estrogen, but not progesterone, affects corticosterone levels dose-dependently. The observed estrogen effects occurred at two levels. On one level, an alteration in baseline corticosterone levels was observed: namely, there was an observed three-fold increase in corticosterone levels in estrogen-treated rats in response to cocaine. Our finding is consistent with a study that reported an increase in corticosterone levels in OVX rats after estrogen replacement (Burgess and Handa, 1992). This observation strongly suggests that fluctuating levels of estrogen during the estrous cycle are potentially involved in creating a differential predisposition to cocaine-induced responses in HPA activity. On a second level, there was a cocaine-estrogen interaction wherein percentage increases in levels of corticosterone were higher at certain doses. However, it remains to be elucidated whether administration of higher doses of estrogen results in a ceiling effect that diminishes cocaine-induced increases in corticosterone levels at these

doses. Based on our findings, we postulate that fluctuations of estrogen during the estrous cycle affect HPA-mediated responses to cocaine, whereas progesterone-HPA interactions play a limited role.

Taken together, our results demonstrate that ovarian hormones interact with cocaine in a multifaceted manner, both inhibiting and enhancing behavioral and endocrinological responses to cocaine. Important observations can be drawn from this study. According to the findings of our dose-response study, the use of contraceptives in varying concentrations of estrogen and progesterone may differentially affect cocaine-induced effects in women. Studies delineating how hormonal interactions affect cocaine-induced responses and subjective effects are necessary to improve treatment methods developed for cocaine addiction in females.

Chapter 3: Effects of short- and long- term estrogen and progesterone replacement on behavioral responses and c-fos mRNA levels in female rats after acute cocaine administration

1. Introduction

Previously published studies have shown that female rats exhibit greater hyperactivity a more exaggerated behavioral responses to cocaine than do male rats (Caihol and Morméde, 1999; Chin et al., 2002; Chin et al., 2001; Craft and Stratmann, 1996; Festa et al., 2003; Festa et al., 2004; Sircar and Kim, 1999; Van Haaren and Meyer, 1991). These sexually dimorphic patterns have been attributed to fluctuating levels of estrogen and progesterone during the female reproductive cycle; cocaine-induced behavioral activity is lower during diestrus than during proestrus or estrus (Sell et al., 2000). Recent studies have shown that while estrogen replacement has no effect on behavioral responses to acute cocaine, progesterone replacement either attenuates or does not affect cocaine-induced locomotor activity (Grimm and See, 1997; Lynch and Carroll, 1999; Perrotti et al., 2001b; Sell et al., 2002; Zhou et al., 2002). As yet, the underlying causes of the discrepancies in behavior after hormone replacement and the mechanisms by which steroids alter behavioral activity have not been determined.

Recent findings have demonstrated that gonadal hormones may alter behavioral responses via two distinct mechanisms. First, upon steroid binding to an intracellular receptor, these complexes act as transcription factors that regulate the expression of genes (Beato et al., 1996; Beato and Klug, 2000; Evans, 1988). For example, mRNA levels of c-*fos*, an immediate-early gene activated after cocaine administration (Daunais and

McGinty, 1995; Graybiel et al., 1990; Hope et al., 1992) is upregulated by estrogen and progesterone (Hyder et al., 1999). Second, both estrogen and progesterone are capable of exhibiting rapid nongenomic effects through activation of plasma membrane receptors (Pappas et al., 1995; Towle and Sze, 1983). For example, there is a rapid attenuation in the potency of μ-opioid hyperpolarization after estrogen administration (Lagrange et al., 1997). Similarly, progesterone has been shown to modulate GABAergic membrane ion channels (Bitran D et al., 1993; Bitran et al., 1995; Fernandez-Guasti A. and Picazo O., 1995; Kokate TG et al., 1999; Majewska, 1992; Reddy DS et al., 2004). It is yet to be established whether estrogen and progesterone modulate cocaine-induced alterations through activation of membrane and/or genomic effects. The aim of this study was to test this possibility.

2. Methods

2.1 Animals

Eight-week-old ovariectomized (OVX) Fischer rats purchased from Charles River (Raleigh, NC) were individually housed in standard cages with access to food and water *ad libitum*. Rats were maintained on a 12-hour light/dark cycle with lights on at 10:30 AM. Rats were handled and weighed daily for 1 week before experimental manipulations. Experiments were conducted 2 weeks after ovariectomy. Each study consisted of at least two cohorts with an n ranging from 8 to 10. All NIH guidelines for the care and use of laboratory animals were followed, and the experimental use of animals was approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Hormone Replacements

Before administration of cocaine or saline, rats received subcutaneous injections of either 50 μg of estrogen (30 minutes or 48 hours before) or 500 μg of progesterone (30 minutes or 24 hours before). To control for differences in hormonal pretreatment, four separate groups received vehicle treatment at 30 minutes (2 groups), 24 hours, or 48 hours before drug treatment. The time points used to assess the effects of long-term exposure to estrogen (48 hours) and progesterone (24 hours) administration were chosen on the basis of previous studies by our group, in which a maximal behavioral alteration was observed at these time points (Perrotti et al., 2003). The 30-minute time point was used to assess nongenomic activity, in accord with previous studies demonstrating a behavioral effect 30 minutes after hormone administration (Frye and Gardiner, 1996). Furthermore, 50 μg of estrogen and 500 μg of progesterone were chosen because they produced the most pronounced effects on behavioral activity after cocaine administration (Chapter 2). These doses fall within the range of doses used in previously published studies that aimed to determine the role of gonadal hormones in response to cocaine (Hu and Becker, 2003; Perrotti et al., 2003; Quinones-Jenab et al., 2000; Sircar and Kim, 1999).

2.3 Drug Administration

Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intraperitoneally at a volume of 1 mL/kg. Injections of 15 mg/kg of cocaine or saline were administered in the home cage 30 minutes after lights were turned

on. This dose of cocaine was chosen on the basis of a previously conducted dose response study in which an optimized level of behavioral activation was observed (Festa and Quinones-Jenab, 2004). Thirty minutes after cocaine or saline administration, the rats were sacrificed by decapitation, after a brief exposure (20 seconds) to CO_2.

2.4 Behavioral Activity

Behavioral measurements were performed for each rat in its home cage for 30 minutes after saline or cocaine administration. Locomotive activity was monitored with a Photobeam Activity System from San Diego Instruments (CA), as previously described (Perrotti et al., 2001a). Total locomotor activity is the sum of all beam breaks. Ambulatory activity represents the number of counts produced by the interruption of two consecutive photobeams in the horizontal frame. Rearing activity represents the total counts of vertical motion.

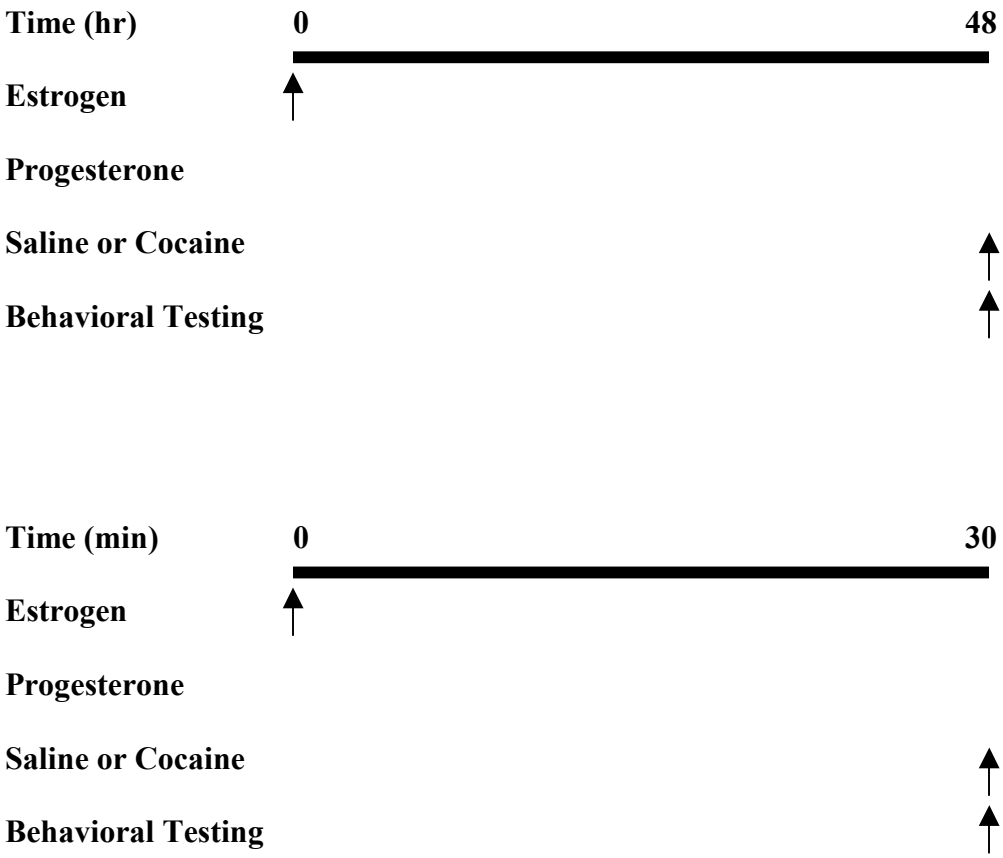


Figure 8. Short- and long-term estrogen administration paradigm. Arrows represent times at which hormones and cocaine was administered.

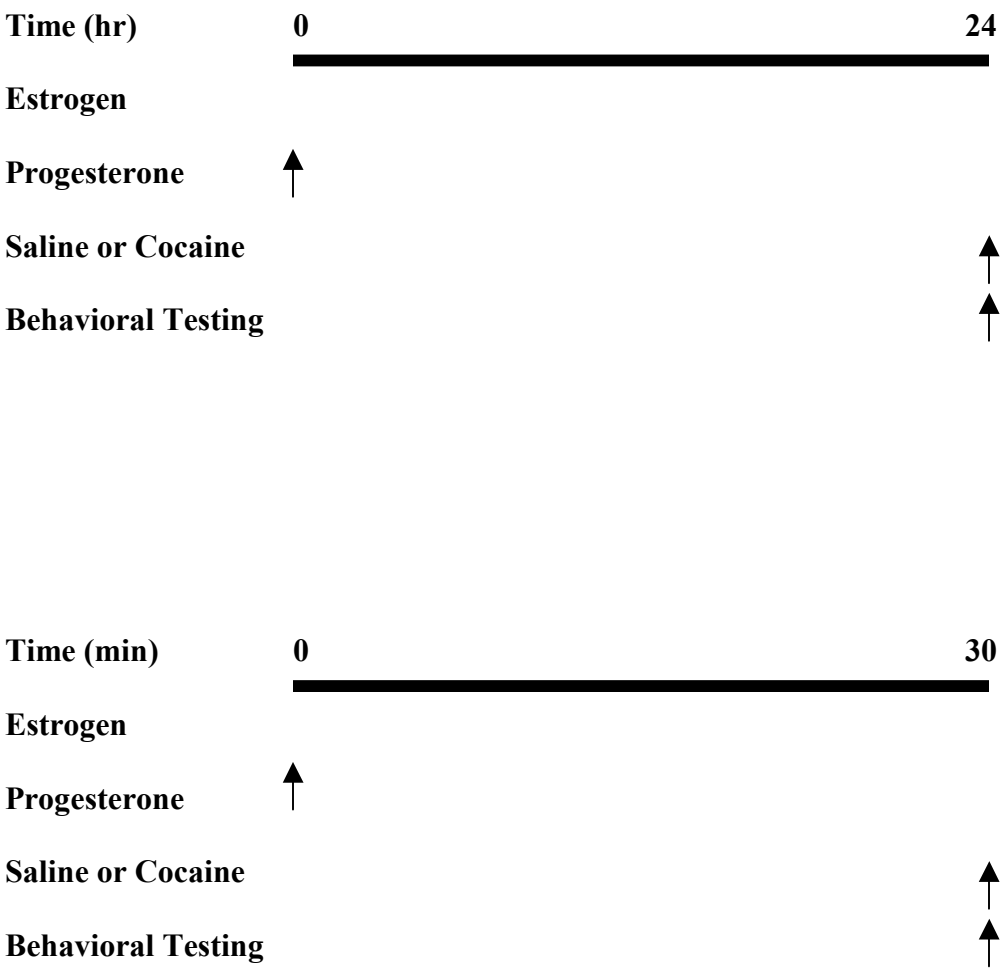


Figure 9. Short- and long-term progesterone administration paradigm. Arrows represent times at which hormones and cocaine was administered.

2.5 RNA Analysis

From coronal slices, the caudate/putamen were dissected as previously described (Civelli et al., 1985; Jenab and Morris, 1997). The c-*fos* mRNA levels were measured by solution hybridization assay. Briefly, aliquots of total RNA extracts were hybridized overnight to ^{32}P-riboprobes in hybridization buffer (10 mM EDTA, 0.3 M NaCl, 0.5% sodium dodecylsulfate and 10 mM *N*-Tris[hydroxymethyl]methyl-2-amino-ethanesulfonic acid, pH 7.4). The mixture was then subjected to 40 μg/mL ribonuclease A and 2 μg/mL ribonuclease T1 for 1 hour in 0.3 M NaCl, 5 mM EDTA, and 10 mM Tris–HCl (pH 7.4). TCA precipitable dcmswere counted by liquid scintillation (Beckman Coulter, Fullerton, CA). Comparisons were made with c-*fos* standard calibration curves to quantify the levels of each respective mRNA.

2.6 Serum Levels of Corticosterone

After the animals were sacrificed, their trunk blood was collected and centrifuged at 3,000 RPM for 15 minutes at 4ºC. Serum was later analyzed with Coat-A-Count radioimmunoassay kits for corticosterone (National Diagnostic, San Diego, CA). Intra-assay coefficients of variation were less than 10.0% ± 1.0%. Results for these assays were determined by a log-logit analysis within GraphPad Prism (GraphPad Software, Inc, San Diego, CA). Serum levels of corticosterone are expressed as ng/mL.

2.7 Statistical Analysis

Ambulatory, rearing, total locomotor activity, and corticosterone data are presented as mean ± standard error of the mean (SEM). To analyze locomotive activity,

two-way analyses of variance (ANOVAs) were used to determine the effects of cocaine and hormone on locomotive behavior as follows: drug (saline or cocaine) X hormone (vehicle, estrogen, or progesterone). To analyze changes in serum corticosterone and levels of c-*fos* mRNA, separate ANOVAs were performed on estrogen- and progesterone-treated groups, and comparisons were made with their respective controls. When significant interactions were obtained, Fisher LSD post hoc tests were used to assess differences between cocaine groups and their respective saline controls within each hormone group. A value of $p<0.05$ was considered to be significant in all comparisons.

3. Results

3.1 Effects of Time of Estrogen Administration on Cocaine-induced Alterations

As shown in Figure 10, cocaine increased ambulatory activity, rearing, and total locomotor activity ([$F(1,48)=31.227$, $p<0.001$], [$F(1,48)=18.261$, $p<0.001$], [$F(1,48)=24.106$, $p<0.001$], respectively). Because estrogen affected baseline behavioral responses ([$F(25,3)=4.560$, $p=0.011$], wherein activity was the highest among rats receiving a 48-hour pretreatment with estrogen, behavioral activation is presented and analyzed as percentages of their respective controls. Cocaine's effects on ambulatory activity were lower in animals pretreated with estrogen for 30 minutes than in the control group ([$F(3,23)=3.348$, $p<0.037$], Table 5). Although cocaine increased serum levels of corticosterone and c-*fos* mRNA in the CPu ([$F(3,50)=7.477$, $p=0.008$], Figure 11 and [$F(1,28)=7.4778$, $p=0.017$], Table 6, respectively), none of the estrogen replacements had an effect on either measure.

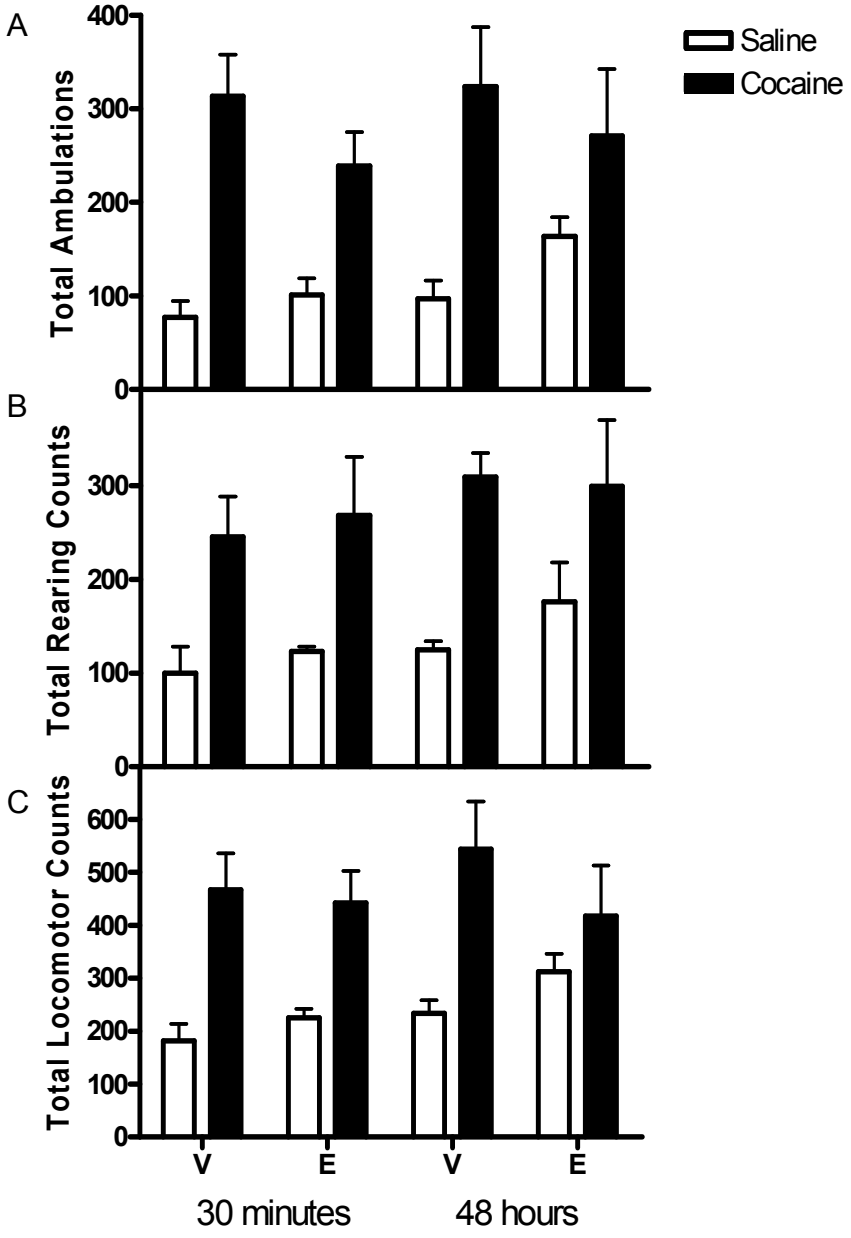


Figure 10: Influence of short- and long-term estrogen replacement on cocaine-induced (A) ambulatory, (B) rearing, and (C) total locomotor counts. Graphs summarize behavioral activity ± SEM after administration of saline (white bars) or cocaine (black bars) for OVX Fischer rats pretreated for 30 minutes or 48 hours with vehicle or estrogen (50 μg). Data are represented as cumulative ambulatory counts for the 30 minutes of behavioral testing.

Table 5. Cocaine-induced behavioral alterations after short- and long-term hormone replacement

Hormone Replacement	Ambulations	Rearing	Total Locomotor
Vehicle (30 min)	407.42 ±57.78	170.21±40.04	257.94±36.81
Estrogen (30 min)	**236.77±36.06***	249.42±20.81	196.69±26.88
Vehicle (30 min)	280.69±86.17	368.65±89.95	222.79±41.23
Progesterone (30 min)	181.65±22.29	**188.32±25.08***	161.89±16.08
Vehicle (48 hours)	334.02±65.97	218.35±50.60	232.29±38.48
Estrogen (48 hours)	165.24±43.88	245.12±43.32	133.74±30.67
Vehicle (24 hours)	271.87±54.62	196.27±36.14	196.53±29.68
Progesterone (24 hours)	**109.51±16.45***	136.90±22.13	143.85±20.89

Data are represented as percentage change from saline ± SEM, * represents a significant change as compared with the respective control group, $p<0.05$ is considered to be significant for all analyses.

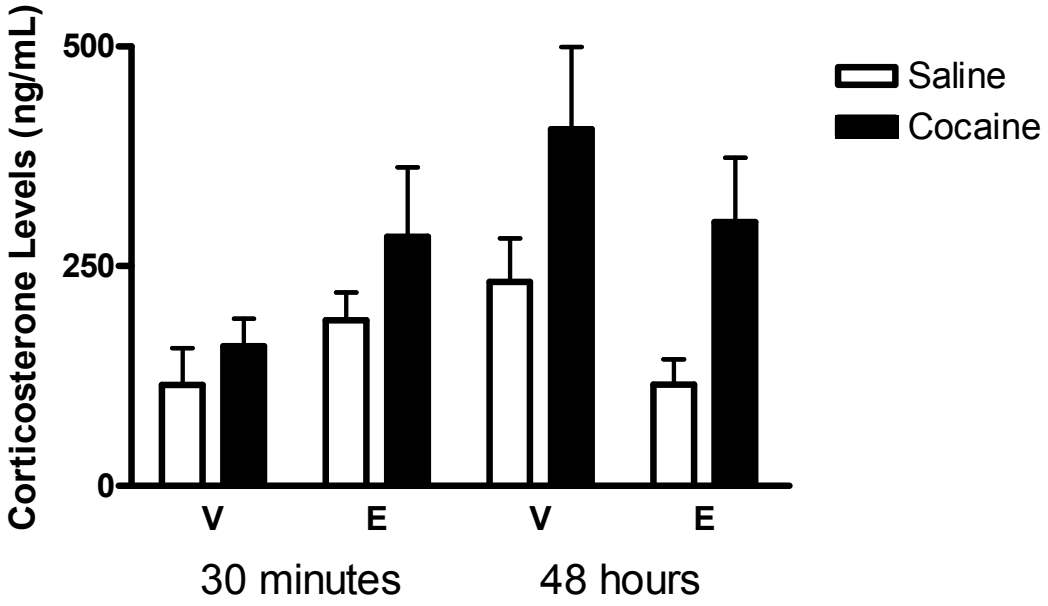


Figure 12: Effect of short- and long-term estrogen replacement on serum corticosterone levels after saline or cocaine administration. Mean ± SEM serum levels of corticosterone (expressed as ng/mL) for saline- (white bars) or cocaine-treated (black bars) rats. The inset shows the percentage change. #Represents statistically significant differences between the vehicle- and hormone-treated groups (p <0.05).

Table 6. Levels of c-fos mRNA in the caudate putamen after cocaine administration and hormone replacement

Hormone Replacement	Saline	Cocaine
Vehicle (30 min)	1.01±0.08	2.31±0.61
Estrogen (30 min)	1.50±0.42	2.58±0.29
Vehicle (30 min)	0.92±0.12	3.02±0.62
Progesterone (30 min)	1.11±0.22	3.83±1.52
Vehicle (48 hours)	1.58±0.24	5.00±1.36
Estrogen (48 hours)	1.91±0.59	2.20±1.57
Vehicle (24 hours)	1.13±0.15	3.36±1.47
Progesterone (24 hours)	1.67±0.19	3.87±0.80

Data are represented as mean±SEM. C-fos levels are expressed as pg mRNA/μg of total RNA.

3.2 Effects of Time of Progesterone Administration on Cocaine-induced Alterations

As shown in Figure 12, ambulatory, rearing, and locomotor activities increased after acute cocaine administration ([F(1,48)=31.227, p<0.001], [F(1,48)=18.261, p<0.001], and [F(1,50)=24.106, p<.0001], respectively). Because progesterone affected baseline behavioral responses ([F(3,26)=4.710, p<0.007], where activity was the highest in rats receiving a 24-hour pretreatment with progesterone), behavioral activation is presented and analyzed as percentages of their respective controls. Pretreatment with progesterone 24 hours before cocaine administration attenuated only ambulatory activity when compared with its control group ([F(1,26)=6.666, p<0.016], Table 5). Additionally, pretreatment with progesterone 30 minutes before cocaine administration decreased cocaine-induced rearing activity in comparison with the control group ([F(1,28)=4.532, p<0.007], Table 12). Pretreatment with progesterone for 30 minutes increased serum levels of corticosterone compared with controls ([F(3,48)=3.200, p<0.028], Figure 13). Cocaine, but none of the progesterone pretreatments, increased mRNA levels of c-*fos* in the CPu([F(28,1)=13.093, p=0.001)], Table 6).

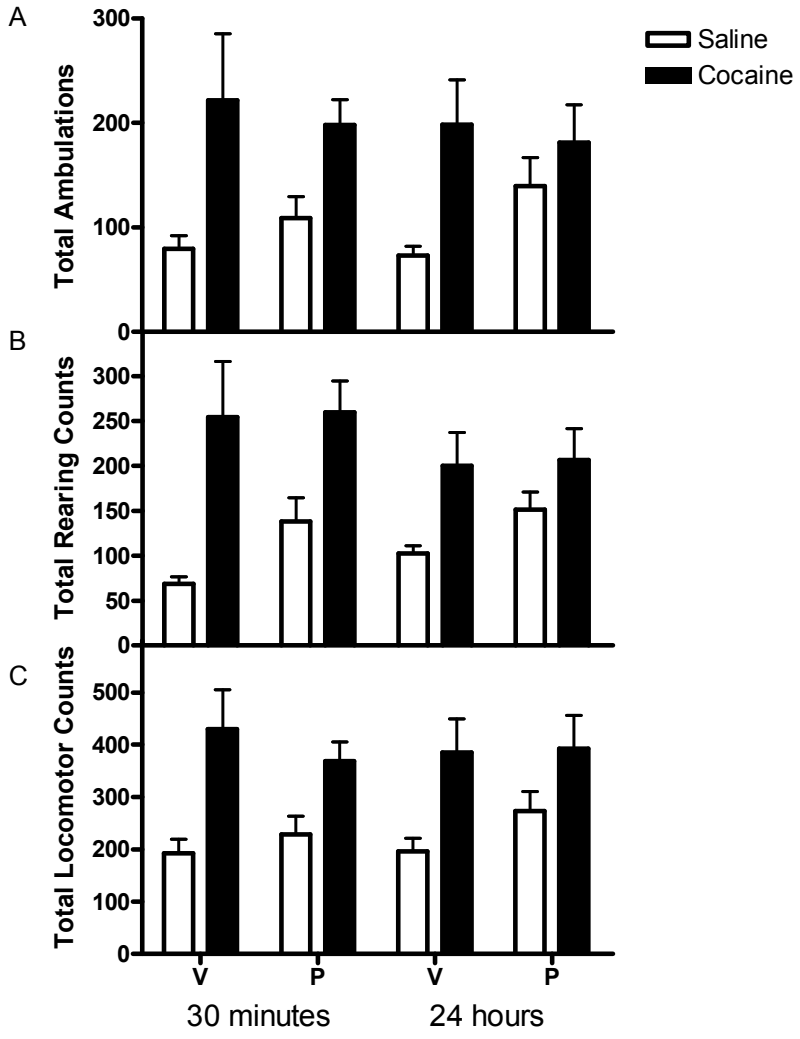


Figure 12: Influence of short- and long-term progesterone replacement on cocaine-induced (A) ambulatory, (B) rearing, and (C) total locomotor counts. Graphs summarize behavioral activity ± SEM after administration of saline (white bars) or cocaine (black bars) for OVX Fischer rats pretreated for 30 minutes or 24 hours with vehicle or progesterone (500 μg). Data are represented as cumulative ambulatory counts for the 30 minutes of behavioral testing.

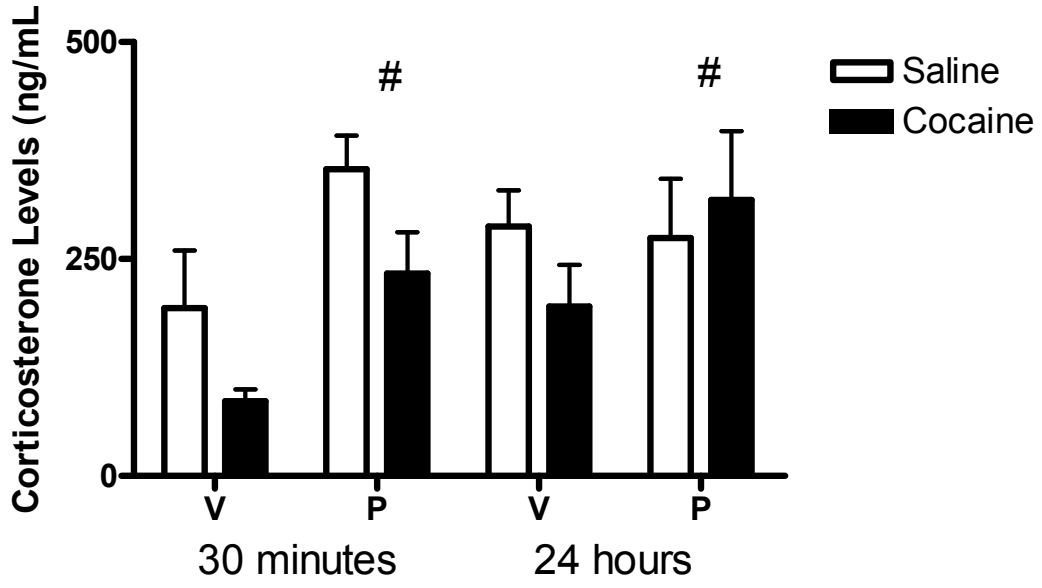


Figure 13: Effect of short- and long-term progesterone replacement on serum corticosterone levels after saline or cocaine administration. Mean ± SEM serum levels of corticosterone (expressed as ng/mL) for saline- (white bars) or cocaine-treated (black bars) rats. #Represents statistically significant differences between the vehicle- and hormone-treated groups (p <0.05).

4. Discussion

Using long-term estrogen replacement paradigms (Silastic capsules and 48 hours s.c., before acute cocaine administration), previous studies have shown that estrogen has no effect on behavioral responses to cocaine after acute administration (Grecksch et al., 1999; Hu and Becker, 2003; Peris et al., 1991; Perrotti et al., 2000; Quiñones-Jenab et al., 2000c; Sircar and Kim, 1999). The current study extends present knowledge by demonstrating that short-term estrogen replacement (30 minutes) attenuates cocaine-induced activity. This differential effect of long- versus short-term estrogen replacement implicates estrogen membrane receptor involvement in cocaine-induced responses. Indeed, recent studies have shown that shortly after exposure to estrogen, lordosis behavior in rats can be modulated, suggesting that activation of genomic receptors are not solely involved in producing changes in steroid-mediated behavioral responses (Kow and Pfaff, 1998; Vasudevan et al., 2005).

Consistent with previous findings, long-term progesterone replacement decreases ambulatory activity after cocaine administration. We further demonstrate that short-term progesterone replacement also attenuates cocaine-induced rearing activity, a finding that suggests both genomic and membrane receptors may be involved in progesterone-mediated responses to cocaine. Progesterone and alloprenanolone, its bioactive metabolite, produce anxiolytic effects through interaction with nongenomic mediated mechanisms, such as the inhibitory GABA-A receptor (Bitran D et al., 1993; Bitran et al., 1995; Fernandez-Guasti A. and Picazo O., 1995; Kokate TG et al., 1999; Majewska,

1992; Reddy DS et al., 2004). The attenuation of rearing activity after co-administration of cocaine and progesterone (30 minutes prior to cocaine) may, in part, be attributed to activation of these membrane-mediated mechanisms. Cocaine-induced ambulatory activity was altered after 24 hours of progesterone and rearing behavior after 30 minutes of progesterone; thus, it is possible that progesterone mediates different components of cocaine-induced behavioral responses through distinct intracellular responses.

Levels of corticosterone were higher in rats administered progesterone than in control rats. Since corticosterone is derived from progesterone (McShane and Fencl, 1983), exogenous administration of progesterone may indeed alter basal serum corticosterone levels. Consistent with previous findings, no interactions between cocaine and hormonal treatment on levels of corticosterone were observed (Chapter 2). Furthermore, similar to the report of Jenab et al. (2002), although cocaine increased c-*fos* mRNA levels in female rats, estrogen and progesterone replacement did not differentially affect increases in *c-fos*. These observations suggest that although HPA activation and c-*fos* mRNA induction are critical responses to cocaine administration, they may play a limited role in estrogen- and progesterone-dependent behavioral alterations and molecular adaptations.

Taken together, these observations suggest that besides previously reported concentration and temporal effects of estrogen and progesterone on cocaine-induced activity (Chapter 2; Perrotti et al., 2003), a third component regulating gonadal hormone control of cocaine-induced behavioral responses may reside in differential intracellular

mechanisms of estrogen and progesterone activation. Thus, the activation of membrane and/or genomic receptors may differentially contribute to the complex neurochemical and behavioral responses reported during the female reproductive cycle. Indeed, previously reported discrepancies in estrogen and progesterone effects on cocaine-induced responses (reviewed in Festa et al., 2004) may, in part, be explained by variations among studies in the length of estrogen and progesterone replacement paradigms. Because these multiple effects of estrogen and progesterone may interact to regulate different components of behavioral and neuroendocrinological responses to cocaine in females, more systematic studies are needed to further elucidate the role of hormones in the development and maintenance of addiction to this potent psychostimulant in human females, a sector of the population that is increasingly engaging in addictive patterns.

Chapter 4: Estrogen and progesterone affect cocaine pharmacokinetics in female rats

1. Introduction

Numerous studies have shown that there are sex differences in cocaine-induced behavioral activation (Chin et al., 2002; Russo et al., 2003b; Sell et al., 2000; Van Haaren and Meyer, 1991; Walker et al., 2001a). Specifically, females display a greater degree of locomotor and stereotypic responses, self-administer cocaine at higher rates, and have higher levels of corticosterone after chronic and acute cocaine administration (Chin et al., 2002; Chin et al., 2001; Lynch and Carroll, 2000; Walker et al., 2001a). Additionally, females require fewer pairings and lower doses of cocaine to develop cocaine conditioned place preference (Russo et al., 2003b). Fluctuations in estrogen and progesterone during the estrous cycle have been postulated to underlie these sex differences in behavior (Bless et al., 1997; Hu and Becker, 2003; Lynch et al., 2000; Lynch and Carroll, 2000; Perrotti et al., 2001b; Quinones-Jenab et al., 2001; Quinones-Jenab et al., 2000; Quiñones-Jenab et al., 1999; Roberts et al., 1989; Sell et al., 2002; Walker et al., 2001c; Zhou et al., 2002). In female rats, cocaine-induced behavioral responses during diestrus are lower than those of rats in other stages of the cycle (Quiñones-Jenab et al., 1999; Sell et al., 2000; Walker et al., 2001c). Estrogen replacement has been shown to enhance locomotor and stereotypic behaviors in response to cocaine (Chapter 2; Perrotti et al., 2001b; Sell et al., 2000). Although progesterone has a limited role in cocaine-induced locomotive activity in rats, it has been postulated to attenuate hyperactivity and the rewarding effects of cocaine (Chapter 2; Perrotti et al., 2001b; Russo et al., 2003a; Sell et al., 2000; Sircar

and Kim, 1999). The mechanisms that underlie estrogen's and progesterone's effects on cocaine-induced alterations remain unclear.

After administration, cocaine is rapidly metabolized to norcocaine by liver enzyme CYP2B1 and carboxylesterases, non-enzymatic degradation in plasma converts cocaine to benzoylecgonine (BE), and ecgonine methylester (EME) is produced by plasma cholinesterase (Hoffman et al., 2004). Norcocaine and BE are bioactive metabolites; norcocaine self-administration in monkeys and both metabolites induce behavioral hyperactivity in rats (Boelsterli et al., 1992; Schuelke et al., 1996; Spealman and Kelleher, 1981). Although EME has a limited role in behavioral activation, it has been reported to decrease cocaine toxicity (Bowman et al., 1999; Dean et al., 1995; Hoffman et al., 2004; Mets et al., 1999; Misra et al., 1975; Schuelke et al., 1996; Stewart et al., 1979).

Sex differences in cocaine pharmacokinetics have been postulated to contribute to sexually dimorphic behavioral responses. For example, levels of butyrylcholinesterase, an enzyme that contributes to cocaine hydrolysis, is ten times more abundant in females than in males (Edwards J.A. and Brimijoin S., 1983). Additionally, female rats have greater brain and blood levels of norcocaine and EME than do male rats (Festa et al., 2004). Although most studies have shown sex differences in BE levels after cocaine administration, the outcomes are conflicting in that males have been shown to have similar, higher, or lower levels than do females (Bowman et al., 1999; Chin et al., 2001; Festa et al., 2004; Van Haaren et al., 1997). It has been hypothesized that some of these

sex differences in cocaine pharmacokinetics are, in part, mediated by effects of gonadal hormones on cocaine metabolism (Festa et al., 2004). Indeed, estrogen and progesterone have been shown to affect CYP enzymes (Pond et al., 1992). For example, there is a downregulation of CYP enzymes in the liver and brain of rats after ovariectomy compared with intact controls (Anakk et al., 2003; Wang and Strobel, 1997). Estrogen replacement was able to reverse this effect (Anakk et al., 2003). Additionally, long-term, but not short-term, progesterone administration decreases levels of CYP in the liver (Anakk et al., 2003). At present, no study has yet examined the direct effects of estrogen and progesterone on cocaine pharmacokinetics. The aim of this study was to test this possibility.

2 Methods

2.1 Animals

Eight-week-old ovariectomized (OVX) Fischer rats (purchased from Charles River, Kingston, NY) were individually housed for 1 week prior to experimental testing. Rats had free access to standard lab chow and water and were maintained on a 12-hour light/dark cycle. Two cohorts were run with a total of 8 rats per group. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Hormone Replacement

Rats received subcutaneous injections of either estrogen (50 μg) 48 hours or progesterone (500 μg) 24 hours before administration of cocaine or saline. Two separate groups received vehicle treatment either 24 or 48 hours before drug treatment to control for the time at which hormone was administered. Since no statistically significant differences were observed between vehicle-treated control groups, the experimental groups were combined for statistical analyses. The timing of both estrogen and progesterone administration were chosen on the basis of previous studies by our group, in which a maximal behavioral alteration was observed at these time points (Perrotti et al., 2003). Furthermore, 50 μg of estrogen and 500 μg of progesterone produced the most pronounced effects on behavioral activity after cocaine administration (Chapter 2). Moreover, these doses fall within the range of doses used in previously published studies that aimed to determine the role of gonadal hormones in responses to cocaine (Hu and Becker, 2003; Perrotti et al., 2003; Quinones-Jenab et al., 2000; Sircar and Kim, 1999).

2.3 Drug Administration

Cocaine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO). Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intra-peritoneally at a volume of 1 mL/kg. Rats received a single injection of cocaine (15 mg/kg) and were sacrificed at 15 or 30 minutes.

2.4 Behavioral Activity

Behavioral activity was measured in animals, who were sacrificed at 30 minutes post-cocaine administration, in the rats' home cages. Total locomotor activity was

monitored using a Photobeam Activity System from San Diego Instruments (San Diego, CA) as previously described (Chin et al., 2002). Locomotor activity represents the number of counts produced by the interruption a photobeam in the horizontal frame.

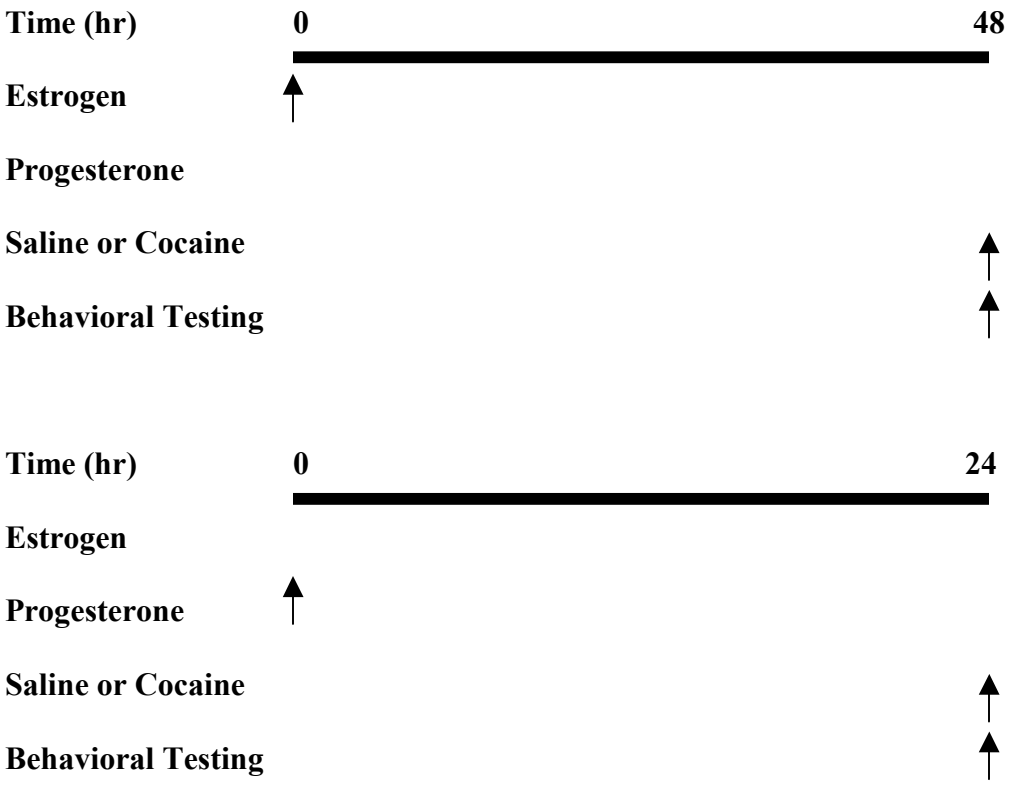


Figure 14. Cocaine metabolism administration paradigm. Arrows represent times at which hormones and cocaine was administered.

2.5 Analysis of Levels of Cocaine and Its Metabolites

Fifteen and thirty minutes after drug treatment, rats were sacrificed by decapitation, following a brief exposure (20 seconds) to CO_2, and their brains were removed and rapidly frozen in methylbutane (-40°C). Trunk blood was collected and immediately placed on ice. Blood was centrifuged at 3000 rpm for 20 minutes at 4°C and serum was collected. Both brain tissue and serum were stored at -80°C until further analyses were run. Cocaine and its metabolites were extracted as previously described with the following modifications (Lin et al., 2001). Briefly, rat brains were weighed and homogenized in a measured volume of 0.1 M sodium phosphate buffer (pH 7.0). A solution containing cocaine-d_3, BE-d_3, EME-d_3, and norcocaine-d_3 was added to each sample of rat brain homogenates and serum to give a concentration of 25 ng/mL of each internal standard. Four milliliters of 0.1 M acetate buffer (pH 4.0) were added to the homogenate and serum samples. After vortex mixing and centrifugation at 2400 rpm for 10 minutes, the supernatants were subjected to solid-phase extraction (SPE) using Clean Screen extraction columns purchased from United Chemical Technologies, Inc. (Bristol, PA). Immediately following SPE, the retained analytes were eluted with 3 mL of methylene chloride/isopropanol/NH_4OH (80:20:2), and the extracts were collected and dried. The residues were reconstituted with 100 μL of methanol/0.1% formic acid in Milli-Q water (10:90) and transferred to autosampler vials.

LC/MS/MS was performed as previously described with the following modifications (Lin et al., 2001). Briefly, analyses were performed using a ThermoQuest

model TSQ 7000 triple-stage quadruple MS equipped with an Xcalibur data system (San Jose, CA). The LC system consisted of an Agilent 1100 with a 5μ ODS-3 Inertsil LC column (100 mm x 2.0 mm) purchased from Metachem Technologies, Inc, Torrance, CA. The LC was interfaced to the MS by means of an atmospheric pressure chemical ionization source. Approximately 20 μL of each extracted sample was injected into the LC/MS/MS. The LC was operated isocratically with a flow rate of 150 μL/min, and the mobile phase consisted of 45% methanol and 55% water with 0.1% formic acid. MS conditions for the TSQ 7000 were as follows: corona current, 5 μA; vaporizer temperature, 375 °C; heated capillary tube temperature, 150 °C; and sheath gas pressure, 40 psi. The following selected reaction monitoring (SRM) transitions were used to quantitate the analytes and internal standards: cocaine: m/z 304–182; cocaine-d_3: m/z 307–185; BE and norcocaine: m/z 290–168; BE-d_3 and norcocaine-d_3: m/z 293–171; EME: m/z 200–182; EME-d_3: m/z 203–185. Although the SRM transitions are the same for BE and norcocaine, each metabolite had a different retention time. Before each run, the instrument performance was evaluated by injecting 20 μL of check solution consisting of 10 pg/μL each of cocaine, BE, EME, norcocaine, and the corresponding internal standards. Data were quantified using Xcalibur's LCquan software. Brain and serum levels of cocaine, BE, EME, and norcocaine are expressed as ng/g of tissue and ng/mL of serum.

2.6 Statistical Analysis

All comparisons were made with their respective control groups. Separate 2-way ANOVAs were used to analyze behavioral and pharmacokinetic effects of estrogen- and

progesterone-treated rats. Additionally, 1-way ANOVAs were used to analyze differences among hormone groups within each time point. When significant interactions were obtained, Fisher LSD post hoc tests were used to assess differences among treatment groups. Significance in all cases was considered to be $p<0.05$. Cocaine, norcocaine, BE, and EME are presented as ng/g of brain tissue and ng/mL of serum.

3. Results

A main effect of cocaine was observed; overall, cocaine increased locomotor counts in comparison with controls receiving saline [estrogen, $F(1,29)=8.499$, $p=0.0057$ and progesterone, $F(1,29)=5.221$, $p=0.0298$, Figures 15A and 15B, respectively]. Additionally, a significant interaction between hormone and cocaine treatment was observed; progesterone inhibited cocaine-induced total locomotor activity [$F(1,29)=4.534$, $p=0.0418$].

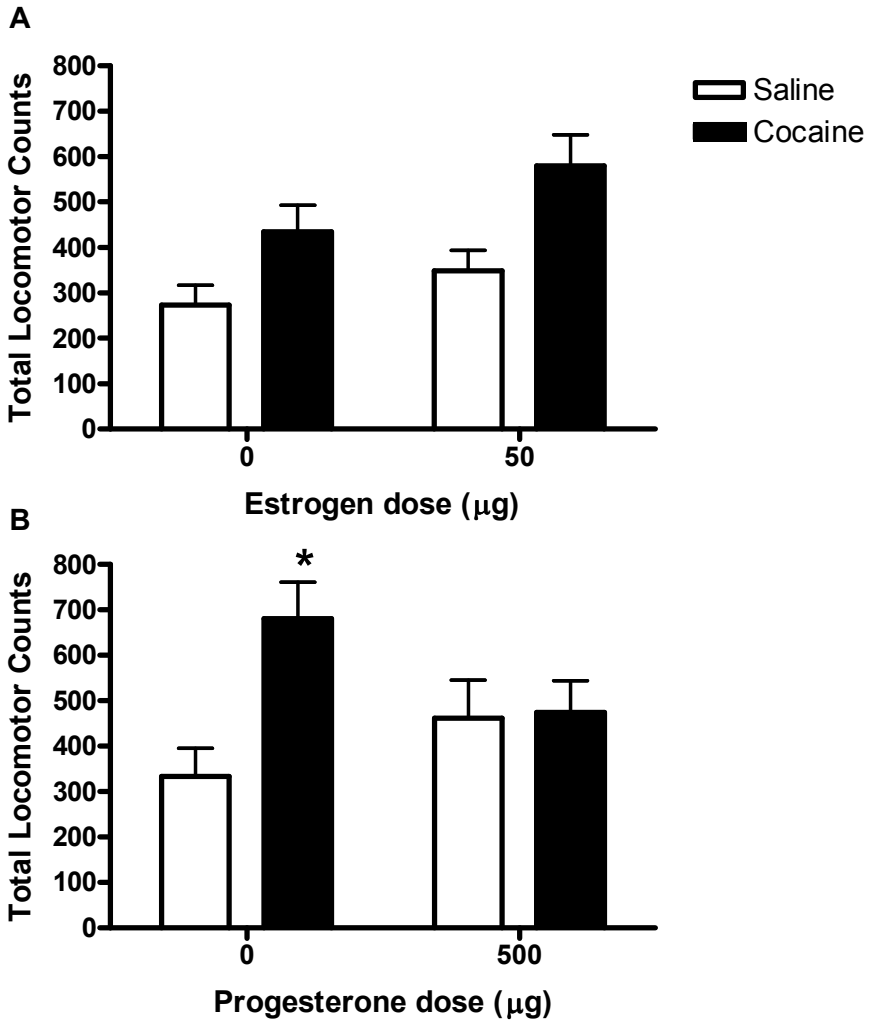


Figure 15: Influence of estrogen (A) and progesterone (B) on cocaine-induced locomotor activity . Graphs summarize behavioral activity after administration of saline (white bars) or cocaine (solid bars) for OVX Fischer rats pretreated for 48 hours with estrogen (50 μg) or progesterone (500 μg). Data are represented as cumulative locomotor counts (mean ± SEM) for the 30 minutes of behavioral testing. *Indicates a statistically significant difference from saline-treated control, $p < 0.05$.

Regardless of the hormone replacement paradigm, levels of cocaine and norcocaine were lower at 30 minutes than at 15 minutes [$F(1,48)=6.120$, $p=0.0170$ and $F(1,48)=11.350$, $p=0.0001$, respectively, Figure 16]. Additionally, 30 minutes after cocaine administration, estrogen administration lowered cocaine and norcocaine levels when compared with vehicle-treated groups [$F(2,26)=4.650$, $p=0.0188$ and $F(2,26)=5.104$, $p=0.0135$, respectively]. A significant interaction between hormone and time after cocaine was also observed for BE levels; in progesterone-treated groups, BE levels were higher 30 minutes after cocaine administration than 15 minutes after [$F(2,47)=3.644$, $p=0.034$]. Moreover, an interaction was also observed between time after cocaine and hormone treatment on levels of EME. While there were no differences in EME levels from 15 to 30 minutes after estrogen replacement, levels increased in both vehicle- and progesterone-treated groups [$F(2,47)=5.065$, $p=0.010$]. Furthermore, when assessing differences within groups at 30 minutes, EME levels were lower in estrogen-treated than vehicle-treated rats [$F(2,23)=6.750$, $p=0.005$]. There were no significant effects of estrogen or progesterone on serum levels of cocaine or its metabolites (Table 7).

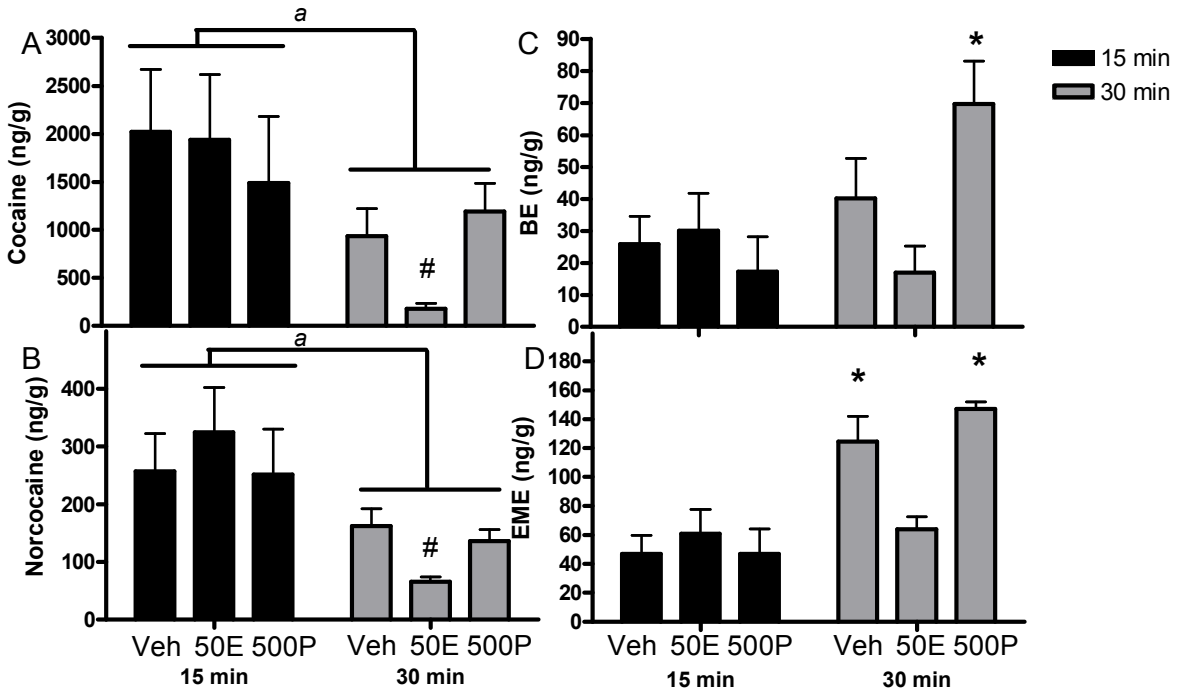


Figure 16: Effect of estrogen and progesterone replacement on brain levels of cocaine (A), norcocaine (B), BE (C), and EME (D). Mean ± SEM levels of cocaine and its metabolites after 15 minutes (black bars) and 30 minutes (gray bars). *a* Represents an overall difference in levels between 15 and 30 minutes. 50E represents the group receiving 50 µg of estrogen and 500P represents the group receiving 500 µg of progesterone. * Indicates a statistically significant difference between 15 and 30 minutes within the same hormonal group. # Represents statistically significant differences between the vehicle- and hormone-treated groups within the same time group ($p < 0.05$).

Table 7. Serum levels ± SEM of cocaine and metabolites after estrogen and progesterone pre-treatment (ng/mL)

Cocaine	**15 min**	**30 min**
Vehicle	473.6±156.2	206.5±63.9
E	439.7±143.3	67.5±16.8
P	392.3±176.9	238.4±53.5
Norcocaine		
Vehicle	57.6±6.69	26.6±3.52
E	97.7±8.03	12.6±2.01
P	18.0±7.09	33.0±2.80
BE		
Vehicle	688.7±157.1	750.4±151.6
E	777.2±165.4	428.5±75.3
P	686.2±219.5	1027.8±146.3
EME		
Vehicle	194.6±40.5	212.9±27.3
E	200.4±36.2	128.2±10.5
P	140.1±26.9	198.6±8.3

E, estrogen; P, progesterone; BE, benzoylecgonine; EME, ecgonine methylester

4 Discussion

As shown by Sell et al. (2000) and in chapter 2, acute progesterone attenuated cocaine-induced behavioral activity. However, a study using a similar administration paradigm previously demonstrated that progesterone did not attenuate ambulatory activity in OVX rats (Chapter 2). Similarly, Sircar and Kim (1999), Perrotti et al. (2000), and Quinones-Jenab et al. (2000) found no effect of progesterone on ambulations when administered 4 hours prior to exposure to cocaine. Discrepancies in progesterone's effects on behavioral responses may be, in part, due to the type of behavioral activity measured. For example, total behavioral activity includes all aspects of locomotor activity, including rearing, whereas ambulations consist only of forward movement. Different doses and/or timing of progesterone replacement prior to exposure to cocaine may also differentially affect the various behaviors via activation of distinct mechanisms.

Similar to the findings of Hu and Becker (2003), Mori et al. (1994), Sircar and Kim (1999), Perrotti et al. (2000), and in Chapter 2, estrogen administration had no effect on cocaine-induced behavioral responses. However, estrogen replacement via Silastic capsules has been shown to potentiate cocaine's behavioral effects (Perrotti et al., 2001b; Sell et al., 2000). These inconsistencies in estrogen's effects on behavioral effects has been attributed to the method of hormonal replacement: whereas estrogen replacement via Silastic capsules provides relatively steady levels of estrogen, subcutaneous injections produce transient increases in levels of estrogen. It is possible that acute and chronic estrogen replacement may induce differential mechanisms of action (genomic versus

nongenomic mechanisms, respectively). Further studies are necessary to investigate this theory.

We have previously demonstrated that female rats have higher levels of norcocaine and EME than do male rats. We extended this observation by showing that hormone replacement affected cocaine metabolites in the brain but not in the serum, where estrogen overall reduced levels of cocaine and norcocaine. There were also non-significant reductions in levels of BE and EME in estrogen-treated rats. Although previously reported sex differences in levels of cocaine and its metabolites may indeed reflect hormonal effects on pharmacokinetics, a direct relationship between cocaine metabolism and behavioral responses was not observed. For example, in progesterone-treated rats, although behavioral responses to cocaine decreased, there was no corresponding pattern of alterations in levels of metabolites. On the other hand, though estrogen had no effect on behavioral responses, it decreased overall levels of cocaine and norcocaine in the brain. This finding suggests that while progesterone has an impact on behavior, pharmacokinetic effects may have a limited role in mediating these behavioral responses. To further explore the possible contribution of estrogen and progesterone in cocaine metabolism, more than two time points may be necessary to obtain a clearer pharmacokinetic profile.

There is considerable evidence of sex-specific differences related to cocaine's health effects. Cocaine is the second most commonly found drug in the systems of patients treated in emergency rooms, and since 1999 the total number of drug-related

hospital visits by females has increased 9% whereas there has been no such increase for males (www.nida.nih.gov). Moreover, there are also reports of gender-related differences in relapses, craving, rate of drug use, and subjective effects (Chen and Kandel, 2002; Kosten et al., 1996; Robbins et al., 1999). Differences in cocaine pharmacokinetics in males and females may account for sex differences in cocaine effects reported in the clinical literature. Fluctuating levels of estrogen and progesterone in females may differentially affect a woman's subjective response to cocaine. Indeed, sex differences and fluctuations in levels of cocaine metabolites during the menstrual cycle have been demonstrated. Therefore, the use of estrogen- or progesterone-based contraceptives in varying concentrations can affect the experience associated with cocaine use in females. These important clinical observations require further consideration.

Chapter 5: Effects of cocaine on c-fos and preprodynorphin mRNA levels in intact and ovariectomized Fischer rats

I. Introduction

Cocaine is known to increase the synaptic concentrations of monoamines by binding to their transporters and thus, preventing the reuptake of these monoamines into presynaptic neurons (Heikkila et al., 1975). Neurochemical and behavioral studies suggest that cocaine's effects on these monoamines as well as the opioid systems may underlie motivational and emotional behaviors and the control of locomotor activity in rats [Reviewed in (Koob, 1992; Koob and LeMoal, 1997; Unterwald, 1995)]. Further studies have demonstrated that some of cocaine effects on genomic changes are modulated by dopamine or serotonin receptor blockers and/or in knockout mice, indicating that both monoamines may contribute to cocaine effects in the CNS (Bhat and Baraban, 1993; Lucas et al., 1997; Spangler et al., 1997b; Young et al., 1991).

Several studies have demonstrated that in male rats while chronic or repeated binge cocaine administration increases PDYN, mRNA levels and dynorphin immunoreactivity in the nucleus accumbens and dorsal striatum, a continuous infusion of cocaine or cocaine withdrawal decreases PDYN mRNA levels in the hypothalamus and striarum, respectively (Daunais and McGinty, 1995; Romualdi et al., 1996; Spangler et al., 1993; Spangler et al., 1997a; Spangler et al., 1997b; Svensson and Hurd, 1998). Additionally, cocaine administration decreases kappa opioid receptor mRNA levels and increases mu opioid receptor mRNA levels in nigrostriatal and mesolimbic dopamine pathways, respectively (Azaryan et al., 1996; Spangler et al., 1996; Yuferov et al., 1999).

Several cocaine administration paradigms also consistently cause an increase in *c-fos* mRNA and in *fos* protein levels in various CNS loci (Daunais and McGinty, 1995; Graybiel et al., 1990).

In the United State about 36% of cocaine users are women and accumulating evidence suggests that there are gender differences in behavioral response to cocaine (Bowman et al., 1999; Caihol and Morméde, 1999; Craft and Stratmann, 1996; Glick et al., 1983; Kuhn and Francis, 1997; Lynch and Carroll, 2000; Sircar and Kim, 1999; Substance Abuse and Mental Health Services Administration 03; Van Haaren and Meyer, 1991). Overall, female rats are more hyperactive after cocaine administration, show a longer lasting and more robust behavioral sensitization to cocaine and may display exaggerated behavioral responses to cocaine (Chin et al., 2001; Glick et al., 1983; Kuhn and Francis, 1997; Post et al., 1981). Furthermore, ovarian hormones have been postulated to underlie certain aspects of cocaine-induced behaviors (Perrotti et al., 2001a; Quinones-Jenab et al., 2000; Sell et al., 2000; Sircar and Kim, 1999). However, little is known on how the interaction between cocaine and the ovarian hormones alters molecular events. To the best of our knowledge, there is no published studies regarding the effects of cocaine on *c-fos* and PDYN gene expression in female rats. Therefore, the aim of this study was to determine cocaine effects on *c-fos* and PDYN mRNA levels in intact Fischer rats and in ovariectomized (OVX) female rats and whether estrogen and progesterone replacement therapy could affect alterations in these genes, which may consequently influence cocaine-induced behaviors.

2. Methods

2.1 Animals

Eight-week-old intact male and female or OVX female Fischer rats (Charles River, Kingston, NY, n=8-12 per treatment) were individually housed in cages with free access to food and water and were maintained on a 12-hour light/dark cycle with lights on at 9 a.m. Intact male and female rats received one single injection of saline or cocaine (i.p.15 mg/kg in 0.9% saline) for 30 min or 3 hrs or one daily single injection of saline or cocaine (i.p.15 mg/kg in 0.9% saline) for 14 days. Two weeks after ovariectomy, the OVX rats were injected with vehicle (sesame oil), estradiol benzoate [s.c., 50 μg dissolved in sesame oil, 48 hrs before saline or cocaine (i.p.15 mg/kg in 0.9% saline) injection] and/or progesterone [s.c., 500 μg dissolved in sesame oil, 4 hrs before saline or cocaine (i.p.15 mg/kg in 0.9% saline) injection]. Previous reports have shown that this timing and dosing of estrogen and progesterone replacement therapy are optimal in cocaine-induced behavior, lordosis behavior and gene expression (Chung et al., 1991; Lauber et al., 1991; Quinones-Jenab et al., 2000). All NIH Guidelines for the Care of Laboratory Animals were followed.

2.2 RNA analysis

At indicated times after the last saline or cocaine injections, the animals were decapitated, coronal slices were cut out in a matrix (American Scientific Instruments) and the caudate/putamen were dissected out on a cold glass plate. Tissues were homogenized in Trizol reagent (Life Technologies, Grand Island, NY) and total RNA was extracted following the manufacture's instructions. *C-fos* and PDYN mRNA levels were measured

by solution hybridization assay as previously described (Civelli et al., 1985; Jenab and Morris, 1997). Briefly, aliquots of total RNA extracts were hybridized to P^{32}-riboprobes in a hybridization buffer (10 mM EDTA, 0.3 M NaCl, 0.5% sodium dodecylsulfate and 10 mM N-Tris[hydroxymethyl]methyl-2-amino-ethanesulfonic acid, pH 7.4) overnight and the mixture was subjected to 40 μg/ml ribonuclease A and 2 μg/ml ribonuclease T1 for 1 hr in 0.3 M NaCl, 5 mM EDTA and 10 mM Tris-HCl pH 7.4 and the TCA precipitable dcms were counted by liquid scintillation (Beckman Coulter, Foullerton, CA). Comparisons were made with *c-fos* or PDYN standard calibration curves to quantify the levels of each respective mRNA. Each cocaine group was paired with its own saline group and the data were subjected to a t-test at the 0.05 level of significance.

3. Results

Figure 1 shows that one single injection of cocaine for 30 min or 3 hrs or one daily injection of cocaine for 14 days increased c-fos mRNA levels in intact male or female rats, while Figure 18 shows that cocaine did not changed PDYN mRNA levels in either intact male or female rats at these time courses. As shown in Figure 19, one single injection of cocaine increased the *c-fos* mRNA levels in the caudate/putamen of OVX female rats that were pretreated with vehicle (V), estrogen (E), progesterone (P), or estrogen plus progesterone (E+P). However, this treatment only increased PDYN mRNA levels in the vehicle (V) and estrogen plus progesterone pre-treated OVX (E+P) animals when compared to their respective saline-treated groups (Figure 20).

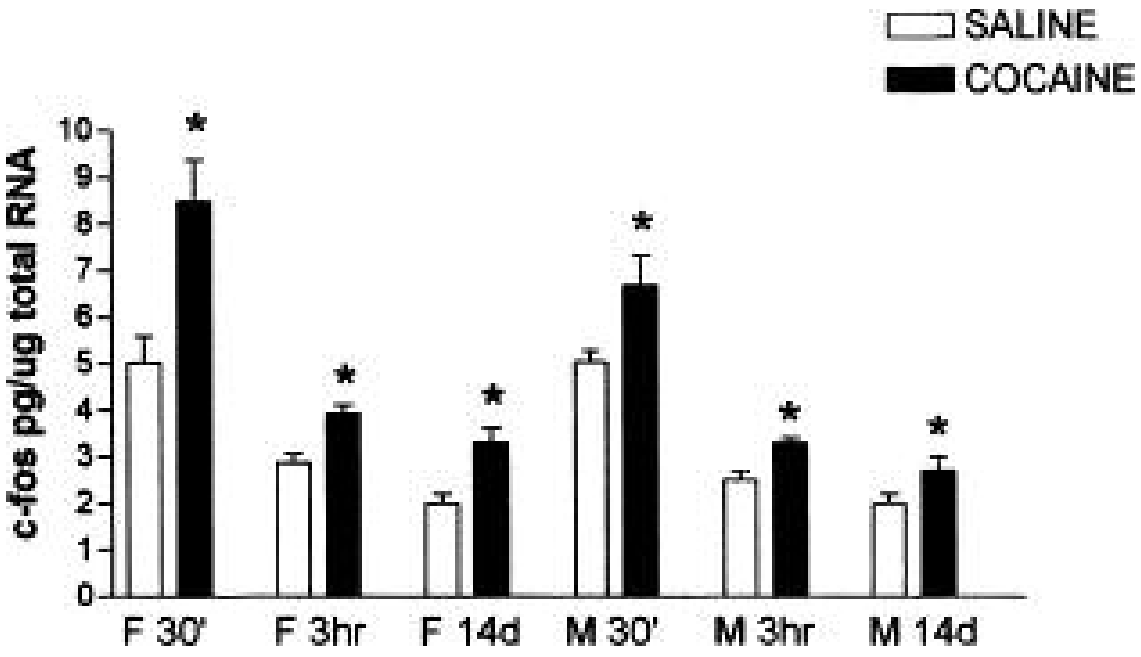


Figure17: *c-fos* mRNA levels in the caudate/putamen after saline or cocaine administration in intact male or female Fischer rats. Total RNA samples from rats that were injected with one single saline or cocaine administration for 30 min or 3 hrs or one daily injection for 14 days were subjected to solution hybridization assays using *c-fos* riboprobe. The data are represented as mean $\pm$ SEM. The *asterisks* indicate significant changes compared to saline treated animals ($p<0.05$).

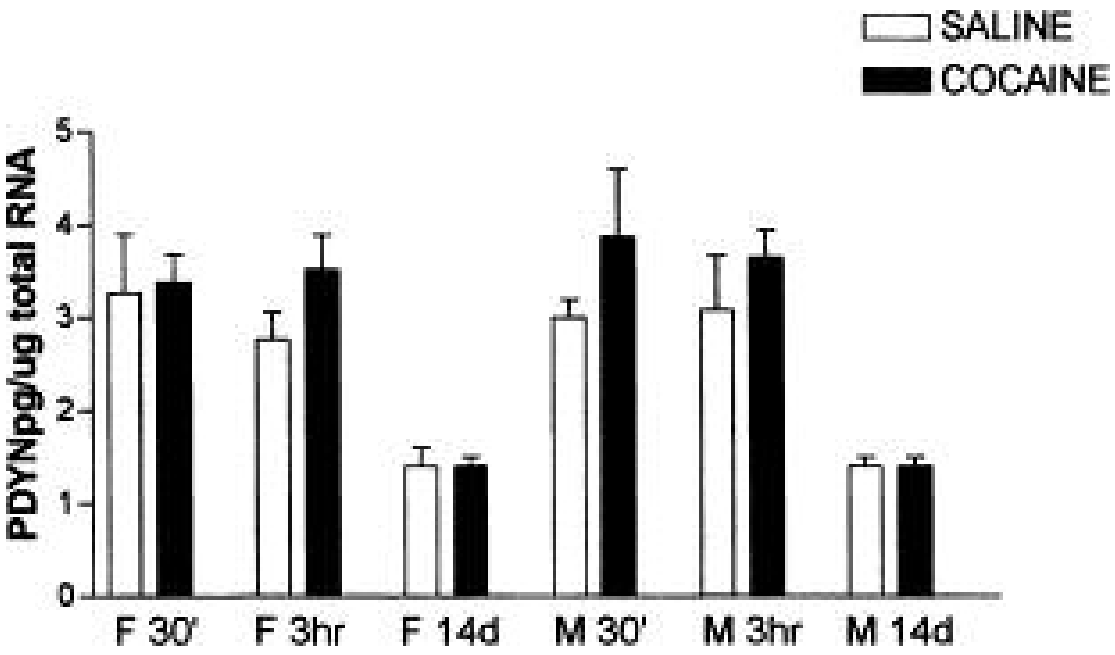


Figure18: PDYN mRNA levels in the caudate/putamen after saline or cocaine administration in intact male or female Fischer rats. Total RNA samples from rats that were injected with one single saline or cocaine administration for 30 min or 3 hrs or one daily injection for 14 days were subjected to solution hybridization assays using PDYB riboprobe. The data are represented as mean $\pm$ SEM.

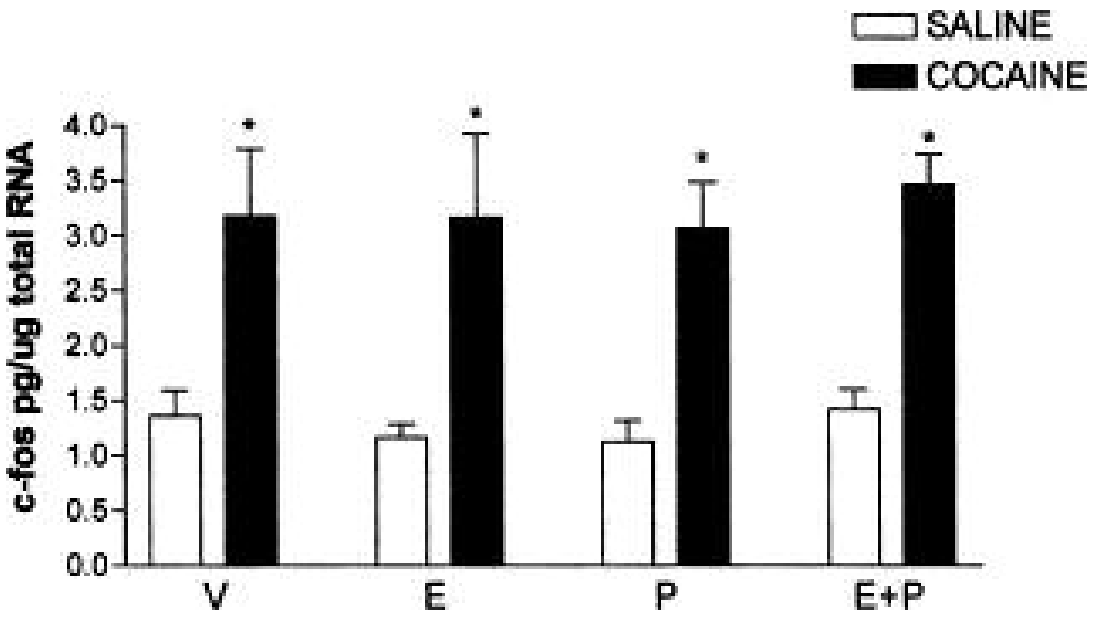


Figure 19: *c-fos* mRNA levels in the caudate/putamen after saline or cocaine administration in OVX Fischer rats. Total RNA samples from each of the four hormone pretreatment groups: vehicle-control (V), estrogen (E), progesterone (P), or estrogen plus progesterone (E+P) after a single saline or cocaine administration were subjected to solution hybridization assays using *c-fos* riboprobe. The data are represented as mean $\pm$ SEM. The *asterisks* indicate significant changes compared to saline treated animals ($p<0.05$).

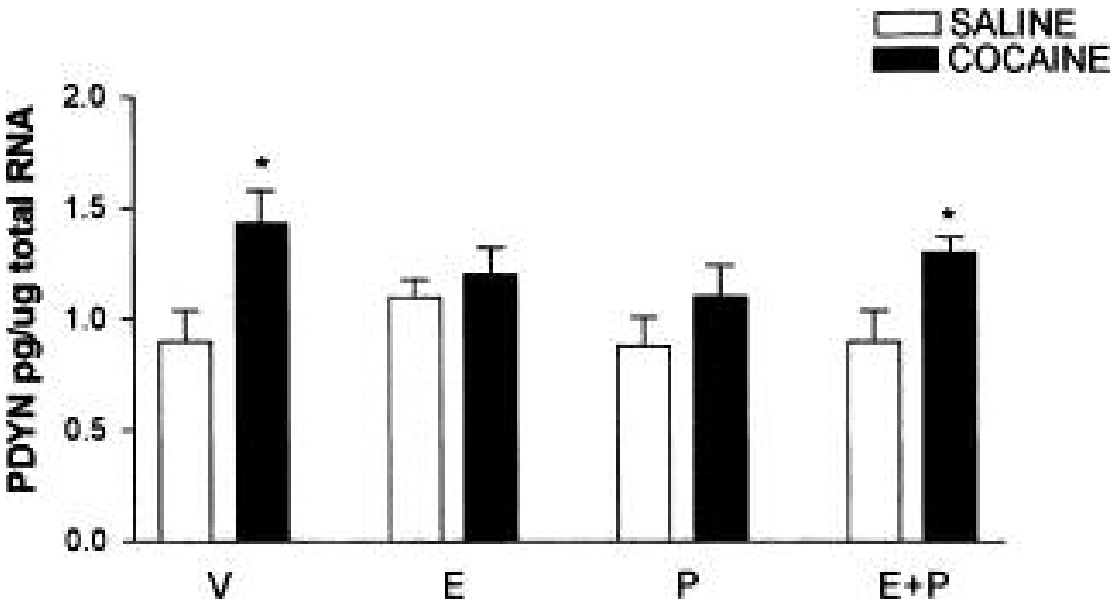


Figure 20: PDYN mRNA levels in the caudate/putamen after saline or cocaine administration in OVX Fischer rats. Total RNA samples from each of the four hormone pretreatment groups: vehicle-control (V), estrogen (E), progesterone (P), or estrogen plus progesterone (E+P) after a single saline or cocaine administration were subjected to solution hybridization assays using PDYN riboprobe. The data are represented as mean $\pm$ SEM. The *asterisks* indicate significant changes compared to saline treated animals ($p<0.05$).

4. Discussion

Previous studies have demonstrated that acute and chronic binge cocaine administration increases PDYN mRNA levels in the caudate/putamen of male rats (Daunais and McGinty, 1995; Spangler et al., 1993; Spangler et al., 1997a; Spangler et al., 1997b; Turchan et al., 1998). In this study, a baseline fluctuation in mRNA levels in intact males and females which may be attributed to the time at which injections were given. Additionally, one single injection of cocaine did not affect PDYN mRNA levels in either intact male or female rats acutely or chronically. Similarly, recent autoradiographic studies have shown that single injections of cocaine produce no effects or less effects on dopamine D1 and mu and kappa opioid receptor bindings than multiple cocaine dosing does in the striatum of male Fischer rats (Unterwald et al., 2001), suggesting that the dosing and the manner of cocaine administration affects the activation of the opioid systems. However, our study suggests that there are hormonal modulation of PDYN mRNA levels by cocaine in OVX female Fischer rats as PDYN mRNA levels were increased after one single cocaine administration in the caudate/putamen of OVX rats pretreated with vehicle or estrogen plus progesterone but not in OVX rats that were pretreated with either estrogen or progesterone. These data indicate that either estrogen or progesterone alone inhibit cocaine's upregulation of PDYN mRNA levels in OVX rats.

Estrogen and progesterone functionally interact in a temporal, synergistic or antagonistic manner, complicating the physiological and behavioral outcomes in response to estrogen and progesterone replacement therapies. In reproductive and cocaine induced behaviors, behavioral activity is either inhibited or facilitated according to the length of

interaction between estrogen and progesterone (Satou and Yamanouchi, 1996). In lordosis behavior the interaction between estrogen and progesterone is bimodal i.e. progesterone can inhibit or synergize with estrogen effects on sexual receptivity. While progesterone modulates different aspects of cocaine alterations than estrogen does, it is not known how the combination of both hormones modulate cocaine induced behaviors (Castner et al., 1993; Hruska and Pitman, 1982; Morishima et al., 1993; Perrotti et al., 2001a; Sell et al., 2000).

Molecular mechanisms by which cocaine effects female rat behavior is complicated since cocaine changes both the hormonal levels and the opioid systems. Cocaine is known to increase progesterone, corticosterone, and ACTH but not estrogen blood levels in female rats while increasing PDYN and mu opioid receptor mRNA levels and decreasing kappa opioid receptor mRNA levels in male rats (Azaryan et al., 1996; Quiñones-Jenab et al., 2000b; Spangler et al., 1996; Spangler et al., 1993; Walker et al., 2001c; Yuferov et al., 1999). Consequently, cocaine can differentially effect the opioid system through several distinct transcriptional pathways.

One mechanism may involve rapid phosphorylation of second messenger transcription factors such as the AP-1proteins that bind to the promoters of targeted genes including the opioids and their receptors (Cochran, 1993; Nestler, 2001). Our data show that in the caudate/putamen of all the experimental groups, cocaine consistently increased one of the components of AP-1 complex, the *c-fos*, mRNA levels. Since PDYN was increased only in OVX rats treated with vehicle or estrogen plus progesterone treatment

groups, further studies are needed to determine the roles of *c-fos* and the AP-1 factors in modulation of PDYN mRNA levels in these experimental groups.

A second mechanism by which cocaine changes PDYN mRNA levels in female rats may involve changes in the plasma levels of the ovarian hormones. The effects of estrogen and progesterone, which regulate many cellular and biological functions such as reproduction, differentiation, homeostasis and growth, are mediated through their respective receptors and may include many cellular factors, including dopamine and dynorphin (Mani et al., 1996; Mani et al., 1997; Pfaff and Schwartz-Giblin, 1995; Spaminato et al., 1995; Tsai and O'Malley, 1994; Wagner et al., 1994). Estrogen has been shown to regulate the opioid system, such that it decreases PDYN and increases mu opioid receptor mRNA levels in CNS areas of OVX female and male rats (Quiñones-Jenab et al., 1997; Spaminato et al., 1995). Since dynorphin peptides and non peptide kappa opioid receptor agonists have been shown to decrease dopamine release in the striatum, it is hypothesized that cocaine-induced increases in PDYN functions as a negative feedback mechanism to reduced cocaine-induced dopamine increase (DiChiara and Imperato, 1988; Manzaneres et al., 1991; Spanagel et al., 1990; Spangler et al., 1997a). We and others have shown that cocaine increases progesterone but not estrogen plasma levels in intact and OVX female rats(Quiñones-Jenab et al., 2000b; Walker et al., 2001c). Furthermore, cocaine stimulated progesterone secretion was greater in females than OVX females, and greater in proestrous than diestrous 1 rats (Quiñones-Jenab et al., 2000b; Walker et al., 2001c). It is well known that the effects of steroids are mediated through their respective receptors which act as ligand-dependent transcription factors and

generally modulate transcription of target genes by stabilizing transcription protein complexes (Mani et al., 1997; Quinones-Jenab et al., 2001). Thus, the increases in plasma progesterone levels in turn can conceivably transform the progesterone receptors to bind to steroid response elements present in the opioid genes and modulate their gene expression (Comb et al., 1998; Min et al., 1994; Woltje et al., 2000).

Taken together, our data suggest that the female endocrinological profile influences cocaine regulation of the opioid systems. The interaction between cocaine and the ovarian hormones may further modulate the development of sensitization or tolerance to cocaine's effects in females.

Chapter 6: Temporal effects of estrogen and progesterone on behavioral and endocrinological responses to acute cocaine administration

1. Introduction

In the United State of America 36% of cocaine users are women (Substance Abuse and Mental Health Services Administration 03). Accumulating evidence suggests that there are sex differences which may reside in endocrinological differences between males and females in the behavioral response to cocaine (Bowman et al., 1999; Caihol and Morméde, 1999; Craft and Stratmann, 1996; Glick et al., 1983; Kuhn and Francis, 1997; Luine, 2001; Lynch and Carroll, 2000; Sircar and Kim, 1999; Van Haaren and Meyer, 1991). Overall, female rats display more hyperactivity and exaggerated behavioral responses to cocaine,and develop a longer lasting and more robust behavioral sensitization to cocaine than male rats (Glick et al., 1983; Kuhn and Francis, 1997; Post et al., 1981). The estrous cycle influences an animal's motivation to self-administer cocaine and its behavioral responses to cocaine. For example, rats in estrus demonstrate an increased motivation to self-administer cocaine (Roberts et al., 1989) and have higher incidences of cocaine-induced stereotypic and locomotor activity than rats during diestrus or proestrus (Quiñones-Jenab et al., 1999; Sell et al., 2000). Thus, endocrinological changes during the estrous cycle may affect cocaine reward and behavioral effects.

Many studies using single hormone replacement paradigms have determined the role of gonadal hormones in cocaine-induced behavioral effects (Cunningham, 1995; Grimm and See, 1997; Morishima et al., 1993; Perrotti et al., 2001a; Sell et al., 2000; Sell

et al., 1998; Van Etten and Anthony, 1999). For example, estrogen decreases cocaine self-administration (Grimm and See, 1997) and affects behavioral responses to acute and chronic cocaine (Cunningham, 1995; Morishima et al., 1993; Perrotti et al., 2001a; Sell et al., 2000; Van Etten and Anthony, 1999). Most behavioral studies demonstrate little or no effect of progesterone on cocaine-induced behaviors (Morishima et al., 1993; Perrotti et al., 2001a; Sell et al., 2000; Van Etten and Anthony, 1999). On the other hand, estrogen and progesterone co-administration either has no effect, inhibits or stimulates cocaine-induced behavioral alterations (Morishima et al., 1993; Perrotti et al., 2001a; Sell et al., 2000; Van Etten and Anthony, 1999).

Females have a complex endocrinological profile, where estrogen and progesterone, under the regulation of hypothalamic and pituitary hormones, fluctuate throughout the estrous cycle. Interactions between gonadal hormones, both in terms of concentrations and temporal relationships (length of time between each hormonal surge) have been postulated to be critical in the modulation of reproductive behaviors as well as neuronal activity and plasticity. For example estrogen and progesterone either synergize to facilitate lordosis or antagonize to inhibit lordosis (Morin, 1976). This temporal relationship controls different components of reproductive behaviors such as pacing, lordosis and other motor behaviors (Craft and Stratmann, 1996), as well as cellular and molecular adaptations in the central nervous system (Pfaff and Schwartz-Giblin, 1995). Due to the possible overlap of motor and reward mechanisms controlling some aspects of cocaine-induced and reproductive behaviors, cocaine-induced motor behaviors may be similarly regulated by interactions between estrogen and progesterone, according to the

length of their interactions, inhibitory or synergistic modulation of either motor components of cocaine-induced behaviors or rewarding aspects of this drug may occur. The purpose of the present study was to test this postulate by examining the effects of the temporal relationship between estrogen and progesterone on cocaine-induced behavioral and endocrinological alterations.

2. Methods

2.1 Animals

Ovariectomized (OVX) female Fischer rats purchased from Charles River (Kingston, New York) 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 AM EST. Two weeks after ovariectomy, rats were randomly assigned to experimental groups (n=8 per group). All NIH Guidelines for the Care and Use of Laboratory Animals were followed.

2.2 Hormone replacement

All rats received subcutaneous (s.c.) injections of estradiol benzoate (50 μg) dissolved in sesame oil. Progesterone (500 μg, s.c.) was administered concurrent with estrogen (0) or 1, 4, 20, 24, 30, 44, or 48 hours post estrogen administration. This timing of progesterone replacement was chosen since it has been previously shown to have a bimodal interaction with estrogen on lordosis behavior (Satou and Yamanouchi, 1996). Estrogen was given 48 hours before cocaine administration since this paradigm has been shown to affect cocaine-induced behavioral activity in previous reports from this and

other laboratories as well as have an optimal effect on modulating lordosis behaviors (Perrotti et al., 2001a; Pfaff and Schwartz-Giblin, 1995; Quiñones-Jenab et al., 2000c; Sofuoglu et al., 1999). Moreover, these doses of estrogen (50 μg) and progesterone (500 μg) have also been shown to modulate cocaine-induced neurochemical and behavioral activity and are commonly used in the study of lordosis behaviors (Perrotti et al., 2001a; Pfaff and Schwartz-Giblin, 95; Quiñones-Jenab et al., 2000c; Sofuoglu et al., 1999).

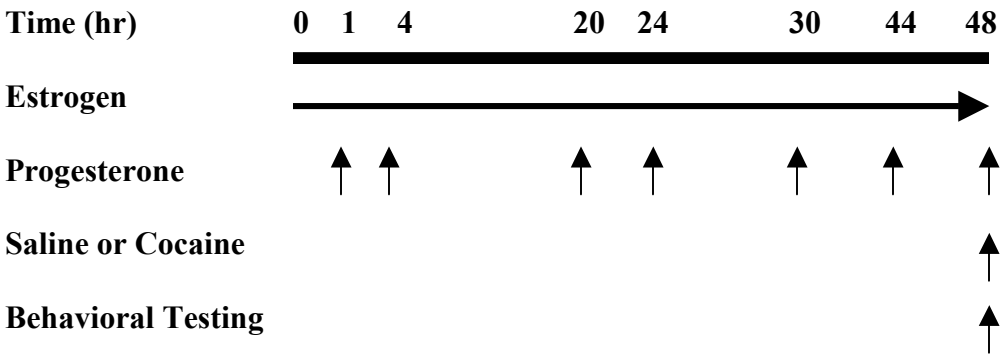


Figure 21: Hormone and cocaine administration paradigms. Temporal interactions between estrogen and progesterone. Arrows represent drug or hormone administration.

2.3 Drug administration

Throughout the study, all injections were administered in each rat's home cage. Forty-eight hours post-estrogen treatment, all animals received either a single i.p. injection of 0.9% saline or cocaine (15 mg/kg dissolved in 0.9% saline; Figure 1) 30 minutes after lights on. After behavioral testing, animals were sacrificed by decapitation following a brief exposure (30 seconds) to CO_2. Trunk blood was collected in EDTA tubes and then centrifuged at 3,000 RPM for 15 minutes at 4°C. Plasma was collected and stored at -70 °C until radioimmunoassays were run.

2.4 Behavioral assays

Behavioral measurements were performed for each rat in its home cage for 30 minutes after saline or cocaine administration. Both stereotypic and locomotor activities were analyzed for each animal. Spontaneous locomotor activity over 30 minutes after cocaine or saline treatment was monitored with a Photobeam Activity System from San Diego Instruments (CA) as previously described (Perrotti et al., 2001a). Ambulatory activity represents the number of counts produced by the interruption of two consecutive photobeam interruptions in the horizontal frame. Rearing activity represents total counts of vertical motions.

For stereotypic activity, each animal was videotaped in its home cage for 1 minute at 15 and 30 minutes after the injection of cocaine or saline. The videotapes were analyzed for behavioral stereotypy by three trained observers blind to each animal's treatment condition. The rating for cocaine-induced stereotypic behavior was based upon

a modification of the Creese and Iversen scale (Creese and Iversen, 1974). 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). A score of 10 was never observed during the course of this experiment.

2.5 BE and corticosterone radioimmunoassays

Samples were analyzed with Count-A-Coat kits for BE or Corticosterone radioimmunoassays from Diagnostic Products Corporation (CA). Intra-assay coefficients of variance were less than 10%. Results for these assays were determined using a log-logit computer program. Levels are expressed as ng/ml.

2.6 Data analysis

To examine the temporal interaction of estrogen and progesterone 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. To examine differences in the drug and hormone treatment groups' rearing or ambulatory counts , two-way repeated ANOVAs (Drug Condition X Progesterone Time) were used. To examine the effects of the length of progesterone administration on plasma levels of benzoylecgonine and corticosterone, one- and two- factor ANOVAs were used. Newman-Keuls post hoc tests were used when significant interactions were obtained. Significance in all cases was considered to be $p<0.05$.

3. Results

3.1 Temporal effects of estrogen and progesterone on behavioral responses to acute cocaine administration

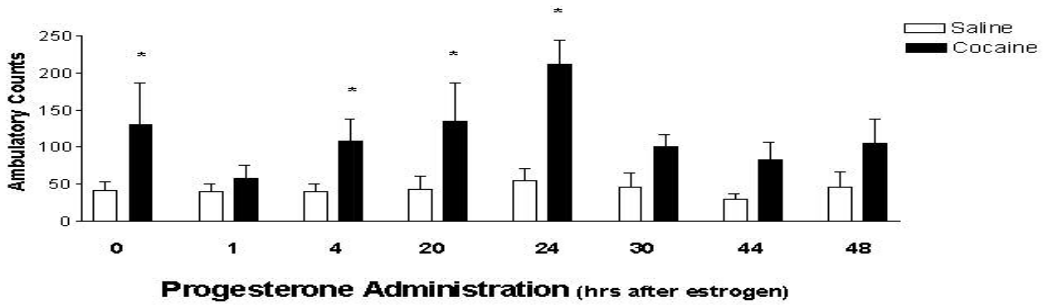
As seen in Figure 22A, cocaine significantly increased ambulatory activity when compared to saline-treated controls [$F = 153.156$, $p<0.001$]. An interaction between the drug treatment and length of progesterone administration was obtained [$F = 3.829$, $p<0.001$]. Groups receiving progesterone concurrent with estrogen, 4, 20 or 24 hours after estrogen showed significant increases in ambulatory activity after cocaine administration ([$p<0.05$ for all comparisons]; Figure 22A). Progesterone administered 24 hours post-estrogen caused cocaine-induced ambulatory activity to reach maximum levels when compared to all other treatment groups [$p<0.001$ for all comparisons]. On the other hand, a significant attenuation of typical cocaine-induced ambulatory activity was observed in rats treated with progesterone 1, 30, 44, or 48 hours following estrogen treatment. (The activity of the cocaine-treated rats in these hormone-treated groups was no different than their respective saline-treated control groups [$p>0.05$ for all comparisons]). There were no differences between saline-treated animals receiving progesterone at the different time points [$p>0.05$].

Rearing activity: Overall, cocaine caused increases in rearing activity ([$F=153.156$, $p<0.001$], Figure 22B). A significant Drug x Progesterone interaction was observed [$F= 6.005$, $p<0.001$]; cocaine-treated rats given progesterone 4, 20, 24, 30, or 44 hours after estrogen treatment had higher levels of rearing activity when compared to saline-treated rats ([$p<0.05$ for all comparisons], Figure 22B). Cocaine-treated rats that received progesterone concurrently with estrogen (0 hour group) also showed increases in rearing

activity when compared to saline-treated rats. However, this increase failed to reach significance [p=0.051]. Similar to ambulatory activity, administration of progesterone 24 hours after estrogen treatment produced the highest counts of cocaine-induced rearing activity [p<0.05 for all comparisons]. No significant differences in rearing activity between saline- and cocaine-treated rats that received progesterone 1 or 48 hours following estrogen were observed [p>0.5 for all comparisons]. This bimodal interaction between estrogen and progesterone in rearing activity was not observed in saline-treated rats [p>0.05].

Stereotypic behaviors: Overall, cocaine increased stereotypic activity ([F =73.763,p<0.01], Figure 22C). No statistically significant differences in stereotypic activity among the saline-treated rats were observed [p>0.05]. Steroid replacement neither differentially altered stereotypic activity 15 or 30 minutes post cocaine administration [F =1.300, p>0.50, data not shown], nor the overall cumulative activity (Figure 22C, p>0.50).

A



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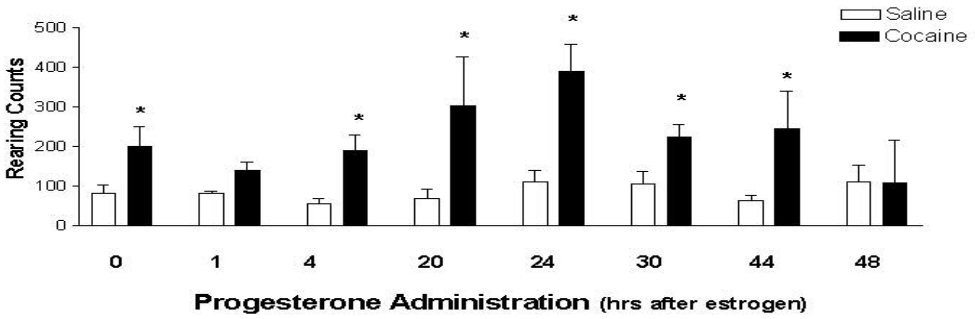

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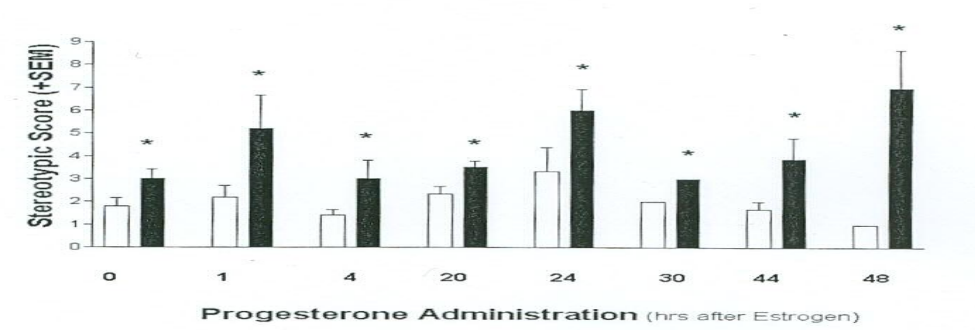

Figure 22: (A) Ambulatory, (B) Rearing, and (C) Behavioral stereotypy of cocaine- and saline-treated female rats in each of the progesterone time conditions. Graphs summarized locomotor activity following administration of saline (white bars) or cocaine (solid bars) for OVX Fischer rats pretreated with estrogen (48 hours before cocaine or saline treatment) and progesterone at 0 (concurrent), 1, 4, 20, 24, 30, 44, and 48 hours after estrogen treatment. Data are represented as cumulative counts for the 30 minutes of behavioral testing. [* indicates a significant difference from saline-treated control $p < 0.05$. # represents statistically significant differences between cocaine-treated groups].

3.2 Temporal effects of estrogen and progesterone on plasma corticosterone and BE levels:

Plasma levels of corticosterone differed significantly across each drug and progesterone treatment groups ([$F = 1.929$, $p < 0.05$], Figure 23). Cocaine-treated animals given progesterone and estrogen concurrently had significantly higher plasma levels of corticosterone than animals in any of the other drug or hormone treatment groups [$p < 0.02$ for all comparisons]. A significant difference in levels of BE among time points at which progesterone was administered ([$F = 5.587$, $p < 0.001$]; Figure 24). Cocaine-treated rats that received progesterone concurrent with, or 4, or 24 hours post estrogen treatment had significantly higher levels of BE than those treated with progesterone at the later time points (44 or 48 hours after estrogen [$p < 0.05$ for all comparisons]). BE levels were undetected in saline treated rats (data not shown).

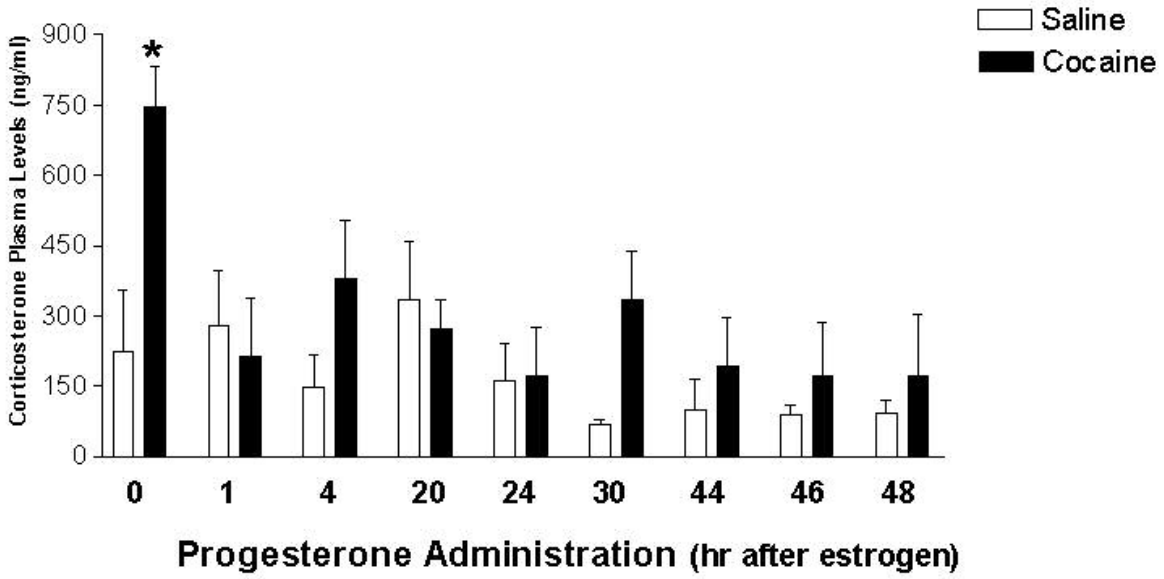

Figure 23: Plasma corticosterone levels for hormone pretreated OVX Fischer rats after cocaine or saline treatment rats Mean + SEM plasma levels of corticosterone (expressed as ng/ml) for cocaine- (black bars) and saline-treated (white bars) rats in all hormone treatment conditions. * indicates a significant difference from saline-treated control $p < 0.05$.

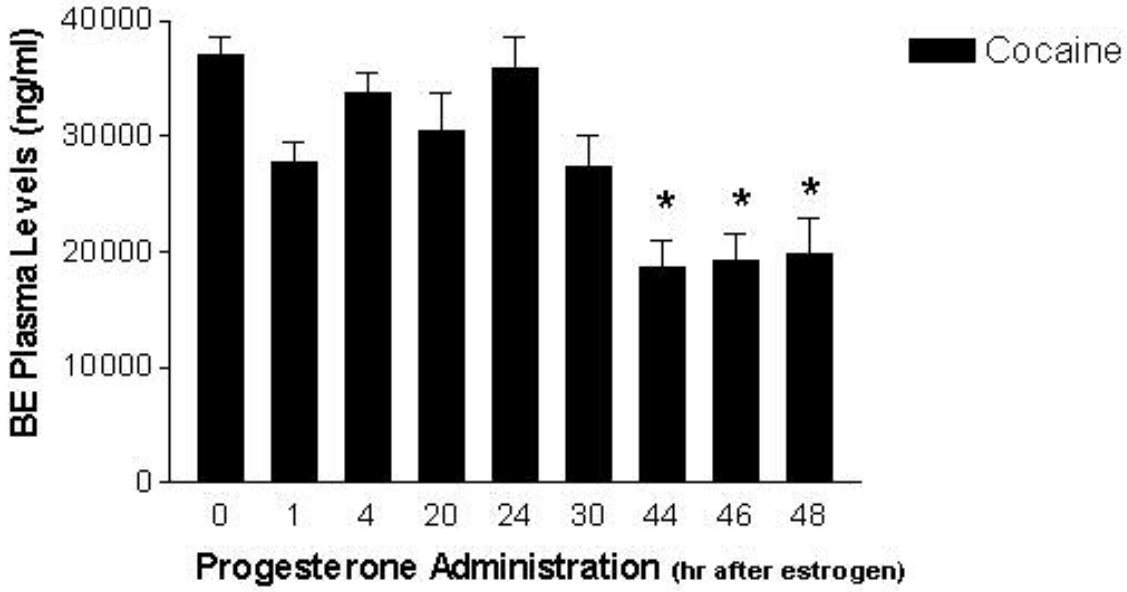

Figure 24: Plasma levels of benzoylecgonine for hormone pretreated OVX Fischer rats. Mean (+ SEM) plasma levels of benzoylecgonine for cocaine-treated animals in all hormone treatment groups. [* indicates a significant difference vs. 0 hour group, ** indicates a significant difference vs. 4 hour group, *** indicates a significant difference v. 24 hour group].

4. Discussion

Similar to previous reports (Craft and Stratmann, 1996; Kuhn and Francis, 1997; Quiñones-Jenab et al., 2000c; Quiñones-Jenab et al., 1999; Sircar and Kim, 1999), acute cocaine administration affected both stereotypic and locomotor behaviors in estrogen+progesterone treated ovariectomized rats. In reproductive behaviors, a temporal interaction between estrogen and progesterone has been previously reported; lordosis was either inhibited by estrogen and progesterone (when progesterone was administered up to 24 hours after estrogen) or facilitated (when progesterone was administered 27-48 hours after estrogen) (Satou and Yamanouchi, 1996). Similar to lordosis, we observed a bimodal/temporal interaction between estrogen and progesterone in cocaine-induced locomotor behaviors. However, the temporal aspects of this interaction differ from those previously reported for lordosis (Satou and Yamanouchi, 1996). First, cocaine-induced increases in locomotor activity (when estrogen and progesterone were co-administered) were followed by an inhibition (when progesterone was administered 1 hour after estrogen). A second longer lasting induction of cocaine-induced motor activities was observed (when progesterone was administered 4 to 30 hours post estrogen), which peaked when rats received progesterone at 24 hours following estrogen.

Cocaine-induced behavioral effects are significantly different in female rats during different stages of the reproductive cycle. The estrous cycle influences both a rat's motivation to self-administer cocaine and cocaine-induced behavioral activation (Quiñones-Jenab et al., 1999; Roberts et al., 1989). Rats in estrus self-administer more

cocaine (Roberts et al., 1989) and demonstrate more cocaine-induced locomotor behavior (Quiñones-Jenab et al., 1999). Based on the present study, it is possible that previously reported differences in the behavioral response to cocaine during the different stages of the cycle may reside in temporal interactions between estrogen and progesterone. Thus, the higher cocaine-induced activity during estrus and decreased cocaine-induced activity during diestrus may be based on progesterone+estrogen's synergistic and inhibitory effects on cocaine-induced behaviors.

Co-administration of estrogen+progesterone in ovariectomized rats has been reported to increase, inhibit, or cause no affect cocaine-induced behavioral activity (Perrotti et al., 2001a; Quiñones-Jenab et al., 2000c; Sell et al., 2000; Sircar and Kim, 1999). For example, after a single cocaine+estrogen+progesterone administration, cocaine-induced behavioral activity has been reported to be suppressed (Perrotti et al., 2001a; Quiñones-Jenab et al., 2000c). However, conversely, Sicar et al., (Sircar and Kim, 1999) found no effect on cocaine-induced behaviors using the similar hormonal replacement paradigms. After a "binge" pattern cocaine administration (3 injections, one hour apart), a bimodal interaction between estrogen+progesterone was observed where the first injection of cocaine, co-administration of estrogen and progesterone caused an inhibition of cocaine-induced locomotor activity, but after the second and third cocaine injections, an enhancement of these behaviors was observed (Quiñones-Jenab et al., 2000c). This shift from inhibition to enhancement of cocaine-induced behavioral activity during "binge" pattern cocaine administration as well as discrepancies in results after

single cocaine+estrogen+progesterone administration may reside in temporal interactions between estrogen and progesterone.

Unlike locomotor activity, no temporal interaction effects between estrogen and progesterone were observed in the modulation of stereotypic behavior. The lack of gonadal hormone effects on stereotypic behaviors supports our previous observations using single administration of estrogen, progesterone, and cocaine (where estrogen and/or progesterone administration did not modulate stereotypic behavior) (Perrotti et al., 2001a). It is possible that estrogen and progesterone modulate the frequency of components of stereotypic activities rather than the sum of these behaviors. From our observations, we can draw two conclusions: (1) temporal interactions between steroids during the estrous cycle may have profound effects on cocaine-induced behavioral outcomes and (2) not all behavioral activity will be affected in a similar manner (i.e., stereotypic behaviors were not affected after the different replacement paradigms).

Gender and estrous cycle differences as well as modulation by gonadal hormones in plasma BE levels have been reported (Bowman et al., 1999; Perrotti et al., 2001a; Quiñones-Jenab et al., 2000c). For example, estrogen+progesterone treatment decreased BE levels when compared to estrogen-, progesterone-, or vehicle-treated animals given "binge" pattern cocaine. In the present study, a temporal interaction between progesterone and estrogen on BE plasma levels was observed. Similar to behavioral activity, liver enzymes that metabolize cocaine may be regulated by estrogen and progesterone in a biphasic manner. However, a direct relationship between cocaine

metabolism and behavioral activity was not observed. For example, cocaine metabolism seemed to be reduced when progesterone was given within 10 hours of cocaine injection, yet the behavioral activity was also lower at this time. Thus, although temporal interactions between estrogen and progesterone on BE levels may partially account for estrous-cycle-related differences in cocaine metabolism, it does not completely explain cocaine-induced differences in behavioral alterations. Previous studies have also observed a lack of relationship between BE plasma levels and cocaine-induced behavioral activity (Mello et al., 1997; Quiñones-Jenab et al., 2000c; Sofuoglu et al., 1999). The direct correlation between cocaine metabolism and behavioral activities remains to be elucidated. However, it may be that early hormonal effects (i.e., 5 to 10 minutes), rather than 30 minutes after the drug administration (when BE levels were measured in this and other studies), may be critical in underlying some mechanisms in which endocrinological effects on cocaine-induced psychomotor activity may occur.

HPA activity is believed to play a major role in modulating cocaine-induced behavioral activity (Koob and LeMoal, 1997; Kuhn and Francis, 1997; Moldow and Fischman, 1987; Zhou et al., 1998). It has been previously demonstrated that cocaine administration leads to an elevation in plasma corticosterone levels in males and females (Koob and LeMoal, 1997; Kuhn and Francis, 1997; Moldow and Fischman, 1987; Zhou et al., 1998). However, female rats demonstrate an exaggerated HPA response to cocaine (Chin et al., 2001; Kuhn and Francis, 1997). Moreover, corticosterone levels vary throughout the estrous cycle of the rat (Atkinson and Waddell, 1997), suggesting that reproductive hormones may play an important role in HPA modulation of cocaine-

induced alterations. A more narrow temporal interaction between estrogen and progesterone in HPA axis activation was observed where, after only concurrent administration of estrogen+progesterone, corticosterone levels in cocaine-treated rats increased. Thus, temporal interactions between estrogen and progesterone on cocaine-induced behaviors are not completely regulated by HPA-axis activation. It is possible that other aspects of their interactions may be critical in modulating cocaine-induced neurochemical alterations and thus regulate the behavioral activity.

Gonadal regulation of CNS plasticity has been postulated to occur in stages, including an early and rapid modulation via membrane responses followed by longer genomic mediated mechanisms (Quinones-Jenab et al., 2001). The biphasic regulation by progesterone and estrogen on cocaine-induced behaviors may involve separate mechanisms of neuronal modulation. Thus, inhibitory effects observed in animals treated with 48 hours of estrogen and 1 to 4 hrs of progesterone may be mediated via rapid activation of cellular events, such as dopamine , GABA, and/or serotonin extracellular levels and/or receptor activation as well as activation of second messenger system. The gradual induction of activity observed in animals treated with estrogen and progesterone for longer time points (administered 4-40 hours) may activate other mechanisms, such as genomic alterations and/or protein synthesis (Quinones-Jenab et al., 2001). This remains to be elucidated.

Of key importance is the female addict who may use estrogen- or progesterone-based contraceptives. Based on our observations, interactions between steroids and

cocaine may ultimately affect behavioral and neurochemical responses to cocaine. Thus, women using different steroid treatments or doses as well as at different stages of their menstrual cycles may use higher doses of cocaine to achieve greater subjective effects of the drug. Moreover, interactions between cocaine and ovarian hormones may lead to overdoses and other clinical complications. This important clinical issue in females needs further investigation.

Chapter 7: Co-administration of estrogen and progesterone differentially affects behavioral responses and monoaminergic pharmacokinetics after acute cocaine administration

1. Introduction

Recent studies have suggested that there are sex differences in cocaine-induced behavioral activation (Chin et al., 2002; Russo et al., 2003b; Sell et al., 2000; Van Haaren and Meyer, 1991; Walker et al., 2001a) where females display a greater degree of locomotor and stereotypic responses and self-administer cocaine at higher rates than those of male rats (Chin et al., 2002; Chin et al., 2001; Lynch and Carroll, 2000; Walker et al., 2001a). Female rats also condition to cocaine at lower doses and after fewer pairings in comparison to males (Russo et al., 2003b). Fluctuations in ovarian hormones throughout the estrous cycle have been postulated to underlie these sex differences in behavior (Bless et al., 1997; Hu and Becker, 2003; Lynch et al., 2000; Lynch and Carroll, 2000; Perrotti et al., 2001b; Quinones-Jenab et al., 2001; Quinones-Jenab et al., 2000; Quiñones-Jenab et al., 1999; Roberts et al., 1989; Sell et al., 2002; Walker et al., 2001c; Zhou et al., 2002); i.e., cocaine-induced behavioral responses during diestrus are lower than those of rats in other stages of the cycle (Quiñones-Jenab et al., 1999; Sell et al., 2000; Walker et al., 2001c). While estrogen replacement enhances locomotor and stereotypic behaviors in response to cocaine (Chapter 2; Perrotti et al., 2001b; Sell et al., 2000), progesterone attenuates locomotor and rewarding effects of cocaine (Chapter 2; Perrotti et al., 2001b; Russo et al., 2003a; Sell et al., 2000; Sircar and Kim, 1999).

Females have a complex endocrinological profile, where estrogen and progesterone, under the regulation of hypothalamic and pituitary hormones, fluctuate throughout the estrous cycle. Interactions between gonadal hormones, both in terms of concentrations and temporal relationships (length of time between each hormonal surge) have been postulated to be critical in the modulation of reproductive behaviors as well as neuronal activity and plasticity. These interactions control different components of reproductive behaviors such as pacing, lordosis and other motor behaviors (Craft and Stratmann, 1996), as well as cellular and molecular adaptations in the central nervous system (Pfaff and Schwartz-Giblin, 1995). Due to the possible overlap in motor and reward mechanisms controlling some aspects of cocaine-induced and reproductive behaviors, cocaine-induced motor behaviors may be similarly regulated by interactions between different concentrations of estrogen and progesterone, which may inhibit or enhance motor components of cocaine-induced behaviors and/or neurochemical alterations of this drug in a synergistic manner. Most studies have used single dose and hormone replacement paradigms to examine the role of estrogen and progesterone in cocaine's effects, the few addressing this issue have shown contradictory results. Co-administration of estrogen and progesterone either no effect, inhibits, or stimulates cocaine-induced behavioral alterations and rewarding effects (Jackson et al., 2005; Morishima et al., 1993; Perrotti et al., 2001b; Russo et al., 2003a; Sell, S. L., Scalzitti, 2000; Van Etten and Anthony, 1999). Some of these discrepancies result from the different doses used, time between injections and/or type of behavior measured. Indeed, progesterone, when co-administered with estrogen, inhibits or synergize cocaine-induced activation. Moreover, we have recently demonstrated that both estrogen and progesterone

activate behavioral responses to cocaine in a dose dependent manner. To date, few studies have examined the effects of estrogen and progesterone co-administration at different ratios. The aim of the present study was to examine the postulate that the estrogen and progesterone, at varying doses, interact to differentially affect behavioral alterations and/or alterations in the monoamiergic system (the neurochemical system that controls cocaine-induced motor responses) after cocaine administration.

2. Methods

2.1 Animals

Eight-week-old OVX Fischer rats purchased from Charles River (Raleigh, NC) were individually housed in standard cages with access to food and water *ad libitum*. Rats were maintained on a 12-hour light/dark cycle with lights on at 10:30 AM. Rats were handled and weighed daily for 1 week before experimental manipulations. Experiments were conducted 2 weeks after ovariectomy. For all experimental groups, n ranged from 8 to 10. Each study consisted of at least two cohorts. All NIH guidelines for the care and use of laboratory animals were followed, and the experimental use of animals was approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Hormone Replacement

For single hormone replacements, rats received subcutaneous (s.c.) injections of either estrogen (50 μg) 48 hours, or progesterone (500 μg) 24 hours before cocaine or saline. For co-administration of hormones, rats received estrogen (10 μg or 50 μg; 48

hours; s.c.) + progesterone (100 μg or 500 μg; 24 hours; s.c.). To control for the times at which estrogen and progesterone were injected, separate control groups received vehicle (sesame oil) 24 and 48 hours before exposure to cocaine. These doses fall within the range of doses used in previously published studies that aimed to determine interactions between gonadal hormones and cocaine (Hu and Becker, 2003; Perrotti et al., 2001a; Quinones-Jenab et al., 2000; Sircar and Kim, 1999). The timing of progesterone administration was chosen on the basis of previous reports from our group showing that maximal behavioral alteration was observed when progesterone treatment was given 24 hours before cocaine administration (Perrotti et al., 2000). Furthermore, the specific doses were chosen based on a previously conducted dose response study (Chapter 2).

2.3 Drug Administration

Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intraperitoneally (15 mg/kg, i.p.) in the home cage 30 minutes after lights were turned on.

2.4 Behavioral Activity

Behavioral measurements were performed for each rat in its home cage for 30 minutes after saline or cocaine administration. Locomotive activity was monitored with a Photobeam Activity System from San Diego Instruments (CA), as previously described (Perrotti et al., 2001a). Locomotor counts represent the number of beam breaks recorded in each chamber.

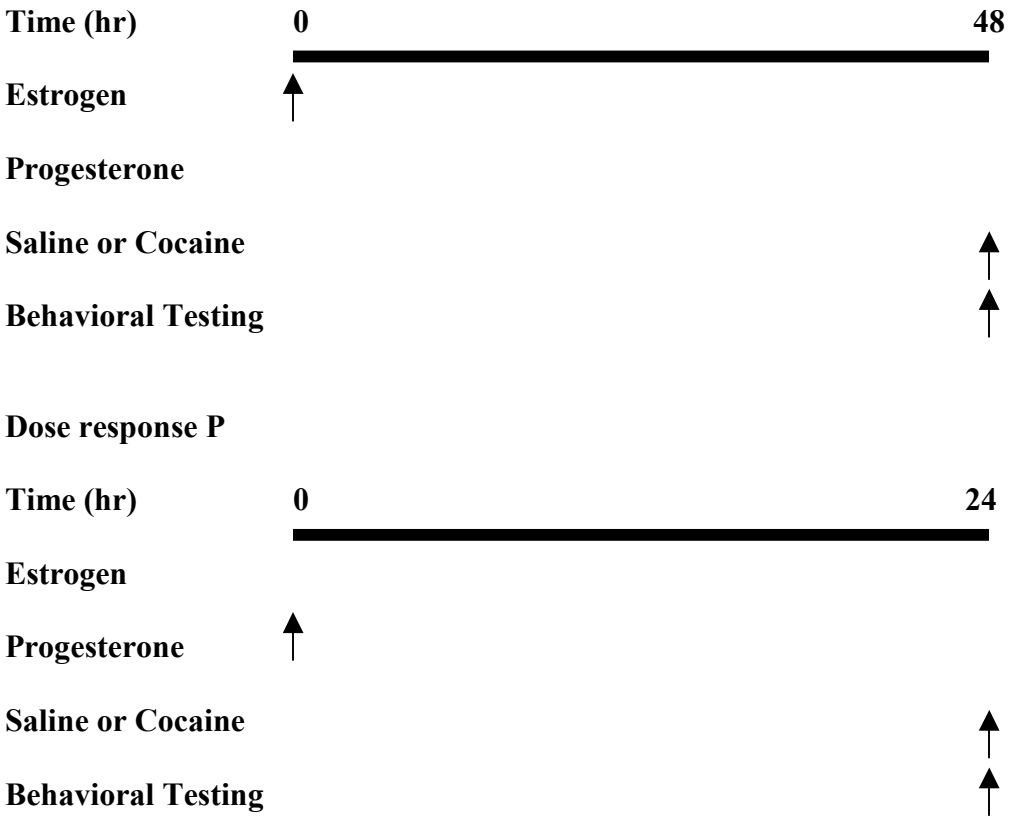

Figure 25: Administration paradigm. Interaction between gonadal hormones and monoamines after cocaine administration. Arrows represent times at which hormones and cocaine were given.

2.5 HPLC Analysis

Bilateral tissue punches (0.5 mm×0.3 mm) were taken from 300 μm sections [eight punches in the NAc shell (ranging from 1.70 mm to 1.00 mm anterior to Bregma) and four punches in the VTA (ranging from −5.20 mm to −5.80 mm posterior to Bregma)]. Tissue punches were preserved overnight with 60 μl of sodium acetate buffer+internal standard (3,4-dihydroxybenzylamine)+ascorbate oxidase. On the following day samples were freeze-thawed and centrifuged, and supernatant was collected. Tissue pellets were re-suspended in 100 μl of 1.0 M NaOH and total protein levels were measured using a Bradford assay from Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA).

As previously described (Perrotti et al. 2001), monoamine levels and their metabolites were measured in 30 μl of supernatant with a Waters Associates chromatographic system, containing a refrigerated 717 plus auto sampler and 1525 binary pump (Waters Associates, MA, USA). In line was an MD-150/RP C_{18} column with MD-TM mobile phase at pH 2.3 (ESA, MA, USA) pumped through the system. For electrochemical detection, an ESA Coulchem II EC detector with the screening electrode set at −150 mv was used (detecting electrode at +325 mv and the guard cell at +350 mv). Concentrations of DA and its metabolites [3,4-dihydroxyphenylacetic acid and homovanillic acid (HVA)] and 5-HT and its metabolite [5-hydroxyindole acetic acid (5HIAA)] were calculated with reference to standards using peak integration with Breeze software (Waters Associates).

2.6 Statistical Analysis

Total locomotor activity and HPLC data are presented as mean±standard error of the mean. To analyze locomotive activity, two-way analyses of variance were used to determine the effects of cocaine and hormone on locomotive behavior as follows: drug (saline or cocaine) X hormone (vehicle, estrogen, or progesterone). For all analyses, separate ANOVAs were performed on estrogen-, progesterone- and estrogen+progesterone-treated groups, and comparisons were made with their respective controls. When significant interactions were obtained, Fisher LSD post hoc tests were used to assess differences between cocaine groups and their respective saline controls within each hormone group. For all analyses, $p<0.05$ was considered to be significant.

3. Results

3.1 Effects of Estrogen and Progesterone on Cocaine-Induced Behaviors:

Overall, cocaine increased total locomotor activity ([$F(1,71)=64.052$, $p<0.001$], Figure 26A and [$F(1,60)=44.561$, $p<0.001$], Figure 26B, respectively). A significant interaction between hormone dose and drug treatment was observed in rats receiving estrogen and progesterone alone: administration of 10 μg E or 500 μg P decreased locomotor activity [$F(4,71)=5.325$, $p<0.001$]. As shown in Figure 26B, after co-administration of hormones, 10 μg E + 100 μg P, 10 μg E + 500 μg P and 50 μg E + 100 μg P replacements inhibited cocaine's effect [$F(4,60)=6.510$, $p<0.001$].

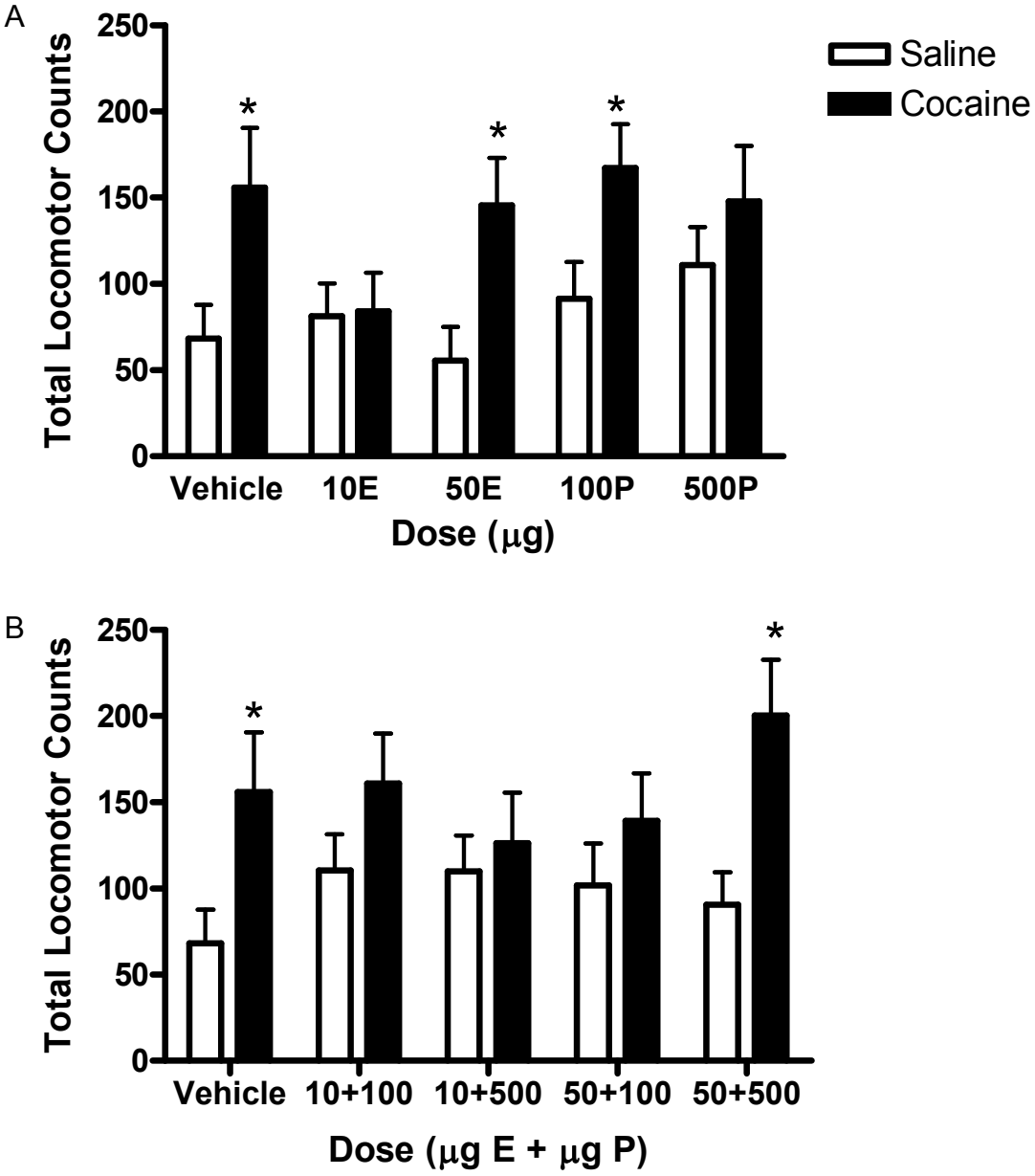

Figure 26: Total locomotor activity in response to cocaine after receiving (A) single hormone replacement and (B) estrogen+progesterone coadministration. Graphs summarize behavioral activity ± SEM after administration of saline (white bars) or cocaine (black bars) for OVX Fischer rats pretreated for 30 minutes or 24 hours with vehicle or progesterone (500 μg). Data are represented as cumulative ambulatory counts for the 30 minutes of behavioral testing. *Denotes a significant change from saline-treated controls.

Effects of Estrogen and Progesterone Administration on Levels of Monoamines

In the CPu, while administration of estrogen or progesterone alone decreased DOPAC/DA and HVA/DA ([F(4,44)=5.333, p=0.001] and [F(4,44)=20.447, p<0.001], respectively; Table 6), There was an interaction between hormone co-administration and drug in the CPu where cocaine decreased DA levels in rats that received no hormone replacement ([F(2,35)=3.368, p=0.045]; Table 9). In the NAc, both DOPAC/DA and HVA/DA were increased after hormone replacement ([F(4,47)=5.182, p=0.002], Table 7 and [F(4,44)=17.548, p<0.001], Table 7, respectively. Cocaine administration in the E+P groups decreased DA levels ([F(1,39)=9.826, p=0.003]; Table 10), and increased both DOPAC/DA ([F(1,38)=5.184, p=0.028]; Table 2) and HVA/DA ([F(1,38)=5.779, p=0.021]; Table 10). There was an interaction between hormone dose and drug in the control and 50 μg E + 500 μg P groups where levels of dopamine levels were higher in saline-treated groups in comparison to the cocaine-treated groups ([F(1,39)=3.950, p=0.027]; Table 10). In the VTA, a decrease in DA levels after 500 μg P replacement was observed regardless of cocaine administration ([F(4,45)=23.435, p<0.001]; Table 8). Furthermore, an interaction where HVA/DA was lower in the control and 50 μg E + 500 μg P groups ([F(1,39)=4.399, p=0.029]; Table 11).

Serotonin: There was main effect of drug as well as hormone dose in groups receiving single hormonal replacement where cocaine and 500 μg P decreased 5-HIAA/5-HT in the VTA ([F(1,46)=7.555, p=0.008] and [F(4,46)=19.909, p<0.001], respectively, Table 8). An increase in levels of 5-HT was also observed in rats receiving 500 μg P in comparison to the control group ([F(4,46)=13.674, p<0.001], Table 8). There was an overall increase

in 5-HT turnover in the CPu after cocaine administration in the E+P groups [F(1,35)=4.545, p=0.040]; Table 9). Additionally, there was a hormone X drug interaction where animals given 50 μg E + 500 μg P and 10 μg E + 500 μg P and cocaine had higher 5-HIAA/5-HT values([F(2,35)=3.778, p=0.032]; Table 9).

Table 8. Levels of monoamines and metabolites in the caudate putamen after E and P administration

	Dopamine		DOPAC/DA		HVA/DA		Serotonin		5-HIAA/5-HT	
Dose	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
vehicle	200.25±32.37	100.71±26.70*	0.44±0.06	0.42±0.12	0.153±0.027	0.139±0.030	4.10±1.28	3.93±1.42	2.67±0.64	2.20±0.60
10E	99.41±17.94	76.60±12.82	0.26±0.03#	0.24±0.07#	0.023±0.001#	0.019±0.001#	6.15±1.46	8.37±2.55	2.18±0.27	1.64±0.06
50E	102.52±14.86	113.90±18.78	0.21±0.02#	0.18±0.01#	0.026±0.003#	0.023±0.001#	7.50±2.55	5.90±2.71	2.63±0.22	1.90±0.32
100P	79.33±15.024	74.54±7.51	0.26±0.05#	0.17±0.01#	0.029±0.005#	0.026±0.005#	11.47±6.92	4.58±1.37	1.93±0.19	1.83±0.19
500P	181.36±63.13	113.11±27.49	0.19±0.02#	0.18±0.02#	0.023±0.002#	0.024±0.002#	23.79±10.15	11.18±5.37	2.30±0.27	1.51±0.09

Table 9. Levels of monoamines and metabolites in the nucleus accumbens after E and P administration

	Dopamine		DOPAC/DA		HVA/DA		Serotonin		5-HIAA/5-HT	
Dose	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
vehicle	137.94±24.74*	45.67±13.22	0.31±0.09	1.32±0.45	0.05±0.01	0.79±0.35	5.39±1.21	6.56±2.37	1.35±0.10	2.10±0.61
10E	10.18±2.70#	9.65±3.14#	3.92±1.12#	6.51±0.47#	0.97±0.27	1.30±0.05	16.76±8.48	7.40±2.19	2.10±0.52	2.06±0.95
50E	12.27±2.88#	14.00±5.86#	3.19±1.19#	2.10±0.61#	0.79±0.31	0.37±0.06	3.05±1.70	2.90±1.21	6.44±1.55	2.38±1.24
100P	47.00±15.24#*	27.34±7.92#	1.79±0.46#	3.57±0.72#	0.40±0.10	0.95±0.29	7.87±2.61	6.04±2.17	4.04±1.45	3.25±1.33
500P	46.09±12.92#	13.57±1.54#	3.62±1.05#	3.12±0.93#	0.45±0.05	0.59±0.21	5.77±1.38	8.47±2.60	4.92±1.13	1.44±0.37

Table 10. Levels of monoamines and metabolites in the ventral tegmental area after E and P administration

	Dopamine		DOPAC/DA		HVA/DA		Serotonin		5-HIAA/5-HT	
Dose	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
vehicle	49.32±10.69	59.23±8.53	0.26±0.02	0.21±0.02	0.16±0.03	0.13±0.02	15.07±2.06	15.88±2.45	0.52±0.06	0.51±0.06
10E	50.93±6.53	67.46±15.55	0.24±0.02	0.18±0.02	0.18±0.03	0.09±0.00	19.25±2.42	34.56±19.62	0.56±0.07	0.40±0.03
50E	80.21±11.88	69.30±10.91	0.21±0.01	0.21±0.03	0.12±0.02	0.09±0.02	17.54±2.44	12.47±1.38	0.58±0.08	0.32±0.06
100P	41.27±16.53	56.26±7.51	0.23±0.02	0.28±0.01	0.15±0.04	0.11±0.02	18.20±6.66	20.87±2.83	0.21±0.04	0.13±0.01
500P	21.37±7.85#	10.18±1.63#	0.60±0.16#	1.12±0.19#	3.60±1.50#	4.17±0.46#	40.17±15.54#	169.60±33.13#	0.06±0.02#	0.04±0.01#

Table 11. Levels of monoamines and metabolites in the caudate putamen after E and P co-administration

	Dopamine		DOPAC/DA		HVA/DA		Serotonin		5-HIAA/5-HT	
Dose	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
vehicle	200.25±32.37	100.71±26.70*	0.44±0.06	0.42±0.12	0.153±0.027	0.139±0.030	4.10±1.28	3.93±1.42	2.67±0.64	2.20±0.60
10E500P	124.86±28.08	82.43±9.51	0.54±0.13	0.48±0.08	0.196±0.048	0.169±0.025	5.70±1.84	1.31±0.30	2.08±0.54	2.80±0.60*
50E500P	120.95±27.06	213.05±82.98	0.57±0.17	0.52±0.11	0.177±0.057	0.187±0.030	4.59±1.09	3.80±1.23	1.10±0.46	6.03±2.04*

Table 12. Levels of monoamines and metabolites in the nucleus accumbens after E and P co-administration

	Dopamine		DOPAC/DA		HVA/DA		Serotonin		5-HIAA/5-HT	
Dose	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
vehicle	137.94±24.74*	45.67±13.22	0.31±0.09	1.32±0.45	0.05±0.01	0.79±0.35	5.39±1.21	6.56±2.37	1.35±0.10	2.10±0.61
10E500P	44.98±19.25	60.16±13.72	1.14±0.34	1.05±0.24	0.27±0.06	0.30±0.07	15.07±11.94	14.56±5.27	2.35±0.93	1.94±0.55
50E500P	150.51±49.55*	27.19±7.63	0.48±0.10	1.10±0.20	0.11±0.02	0.35±0.07	9.25±3.00	4.58±1.22	1.15±0.35	1.96±0.73

Table 13. Levels of monoamines and metabolites in the ventral tegmental area after E and P co-administration

	Dopamine		DOPAC/DA		HVA/DA		Serotonin		5-HIAA/5-HT	
Dose	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
vehicle	49.32±10.69	59.23±8.53	0.26±0.02	0.21±0.02	0.16±0.03*	0.04±0.01	15.07±2.06	15.88±2.45	0.52±0.06	0.51±0.06
10E500P	23.13±2.63	12.75±3.73	0.17±0.02	1.38±0.60	0.09±0.01	0.12±0.02	7.35±2.83	7.94±1.43	0.37±0.09	0.34±0.08
50E500P	16.80±2.16	19.66±4.59	0.16±0.01	0.37±0.08	0.12±0.03*	0.04±0.01	4.85±1.35	1.70±0.20	0.47±0.12	0.40±0.06

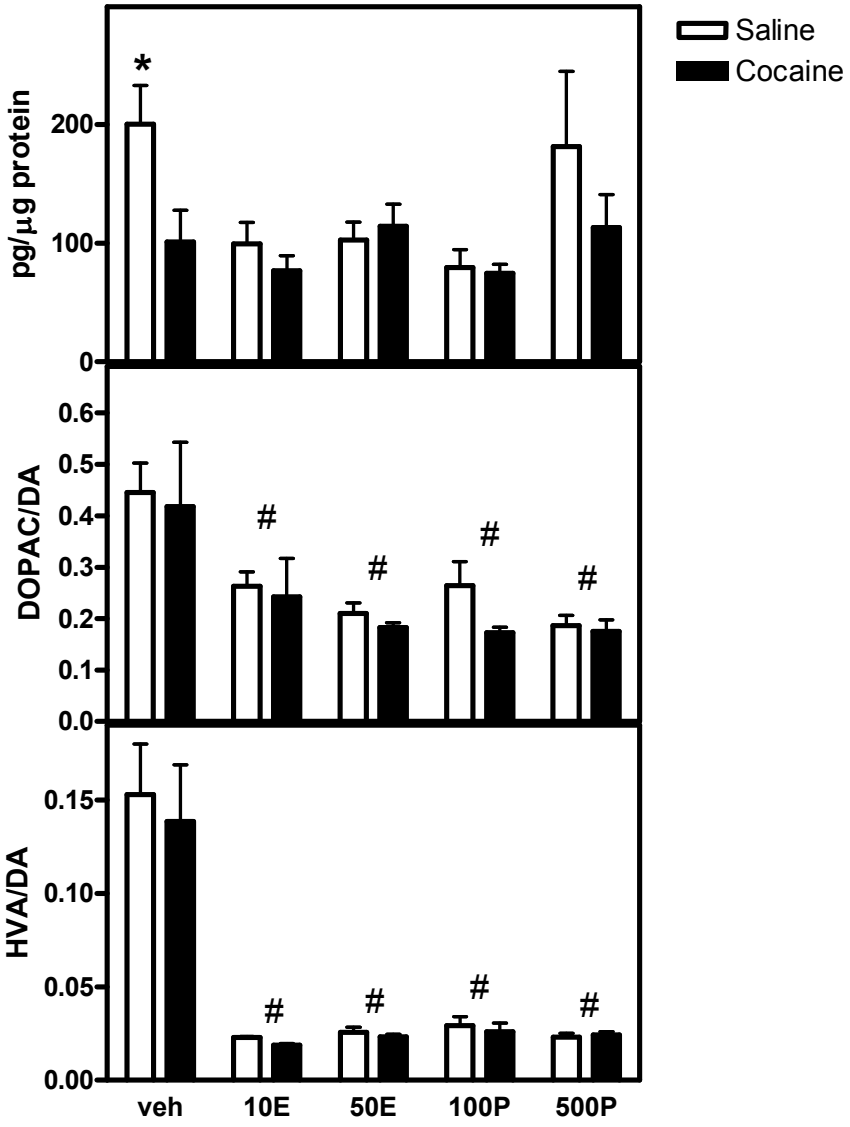

Figure 27. Levels of DA and its turnover in the CPu after hormone and drug/saline treatment. # main effect of hormone; *main effect of drug.

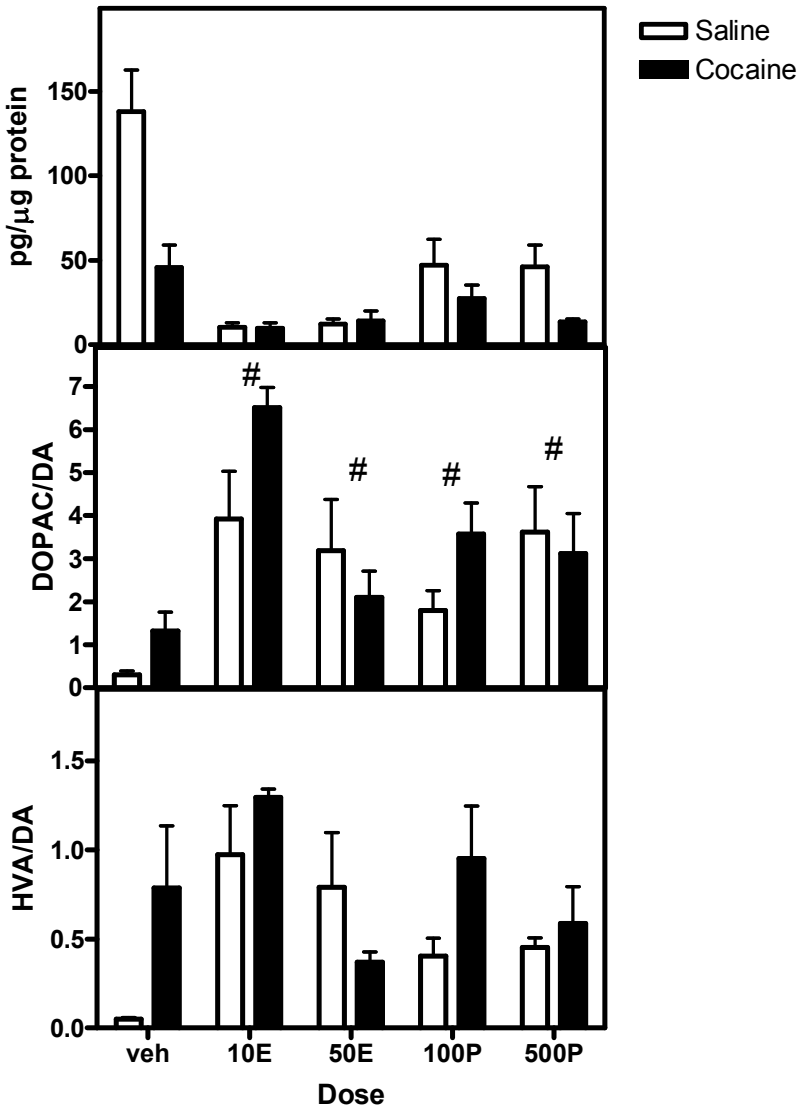

Figure 28. Levels of DA and its turnover in the NAc after hormone and drug/saline treatment. # main effect of hormone; *main effect of drug.

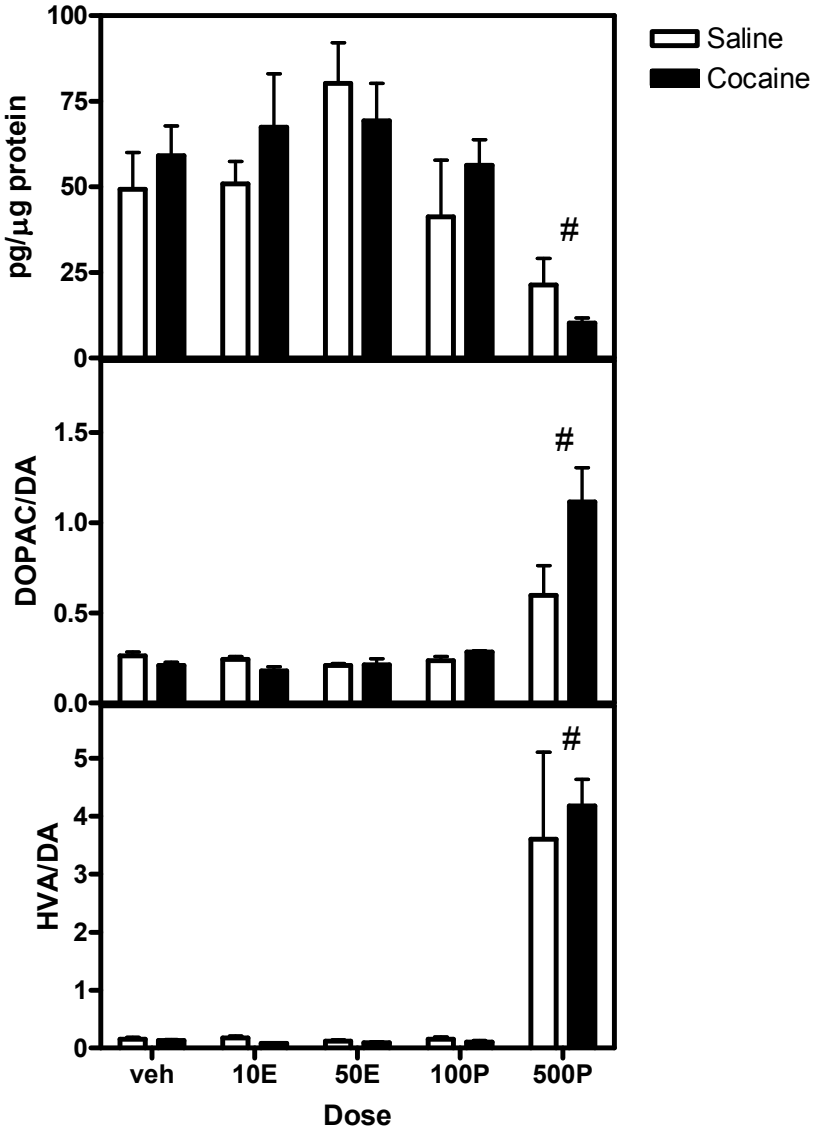

Figure 29. Levels of DA and its turnover in the VTA after hormone and drug/saline treatment. # main effect of hormone; *main effect of drug.

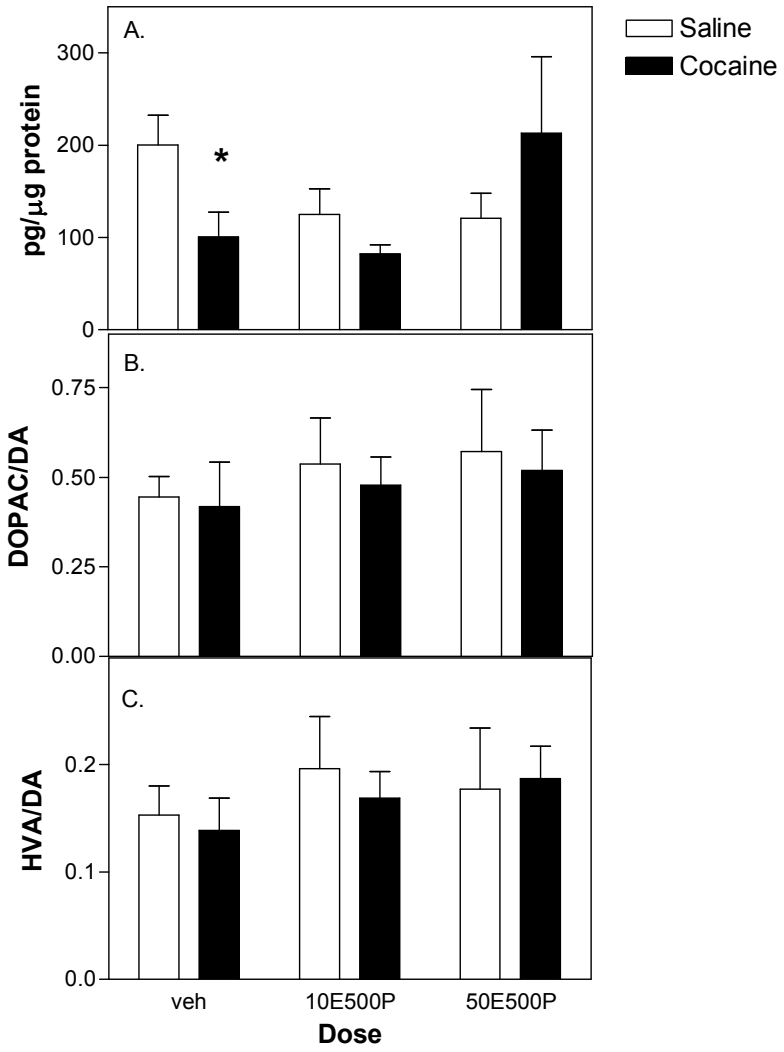

Figure 30. Levels of DA and its turnover in the CPu after hormone co-administration and drug/saline treatment. # main effect of hormone; *main effect of drug.

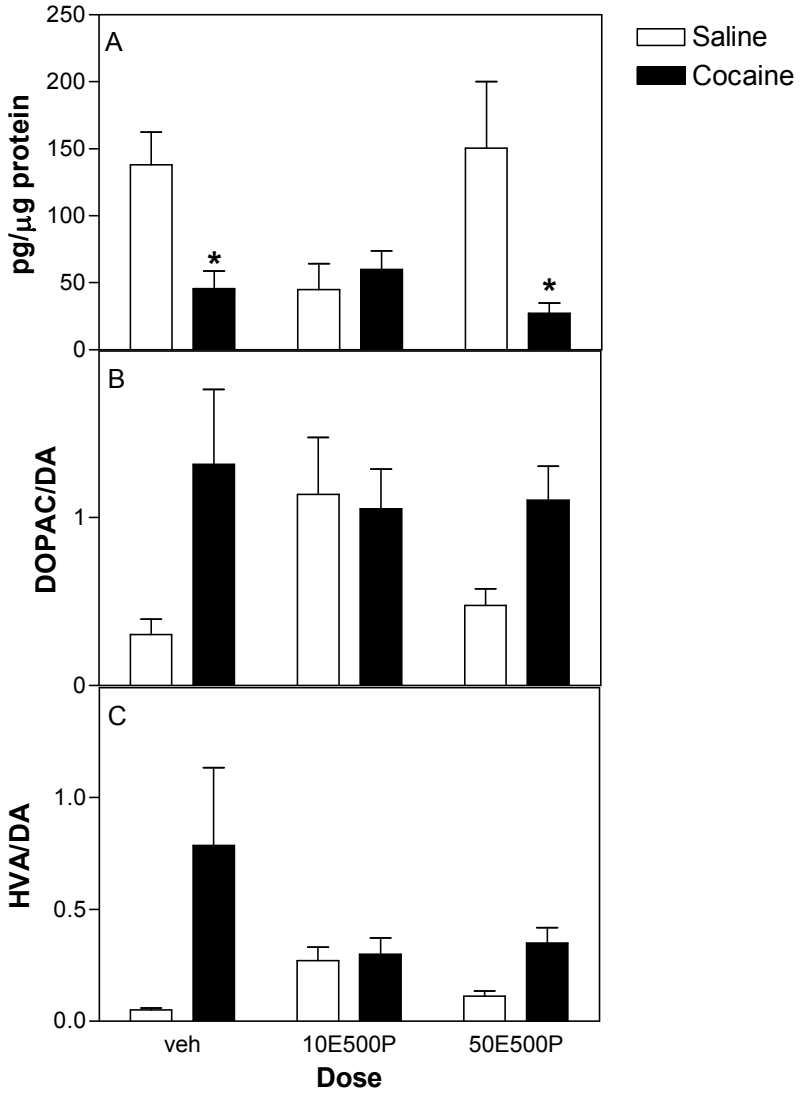

Figure 31. Levels of DA and its turnover in the NAc after hormone co-administration and drug/saline treatment. # main effect of hormone; *main effect of drug.

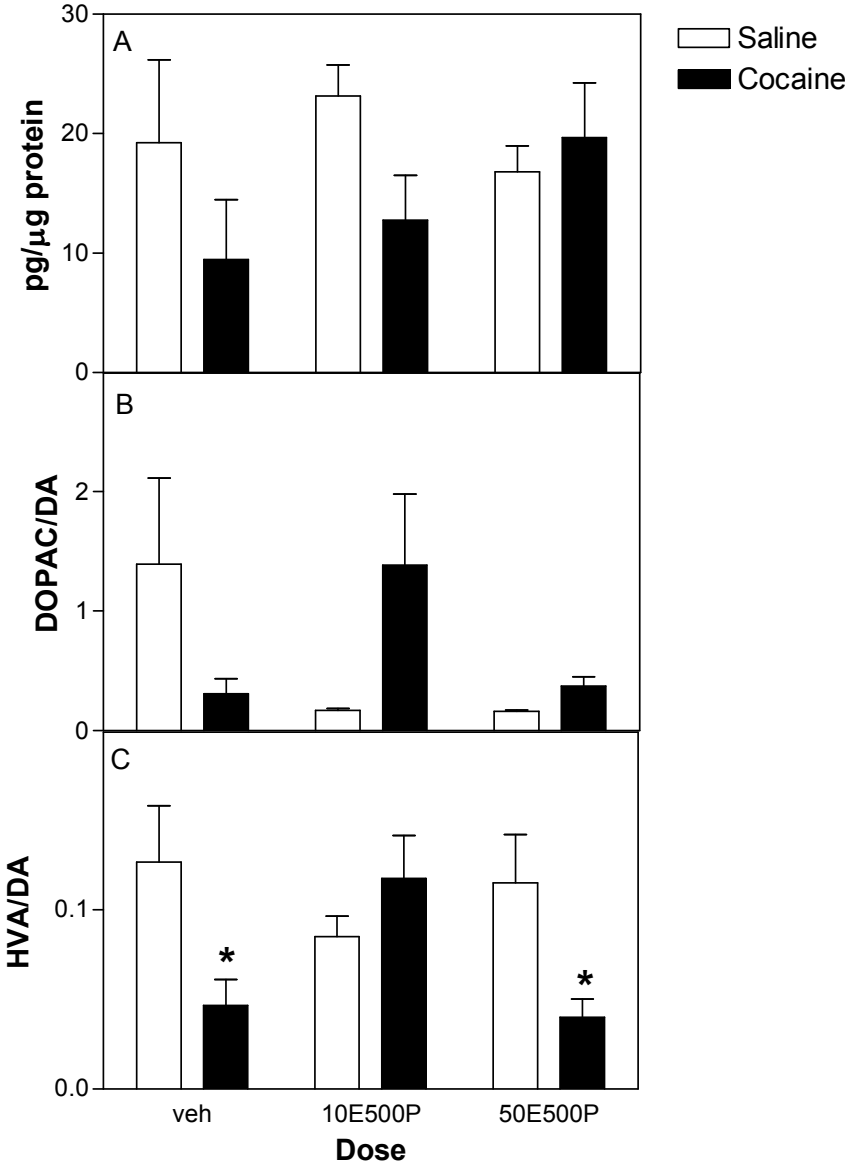

Figure 32. Levels of DA and its turnover in the VTA after hormone co-administration and drug/saline treatment. # main effect of hormone; *main effect of drug.

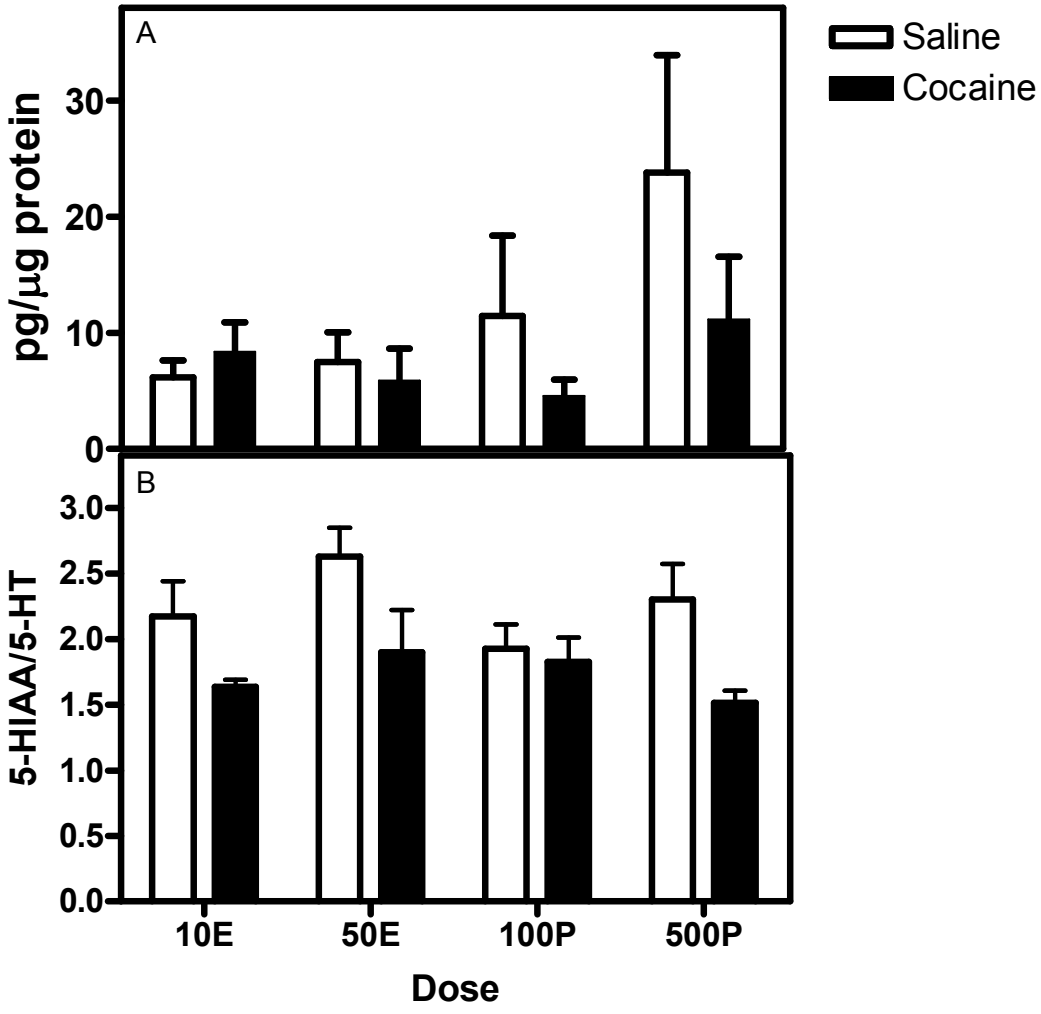

Figure 33. A. Levels of 5-HT and **B.** the ratio of 5-HIAA to 5-HT in the CPu after hormone and drug/saline treatment. # main effect of hormone; *main effect of drug.

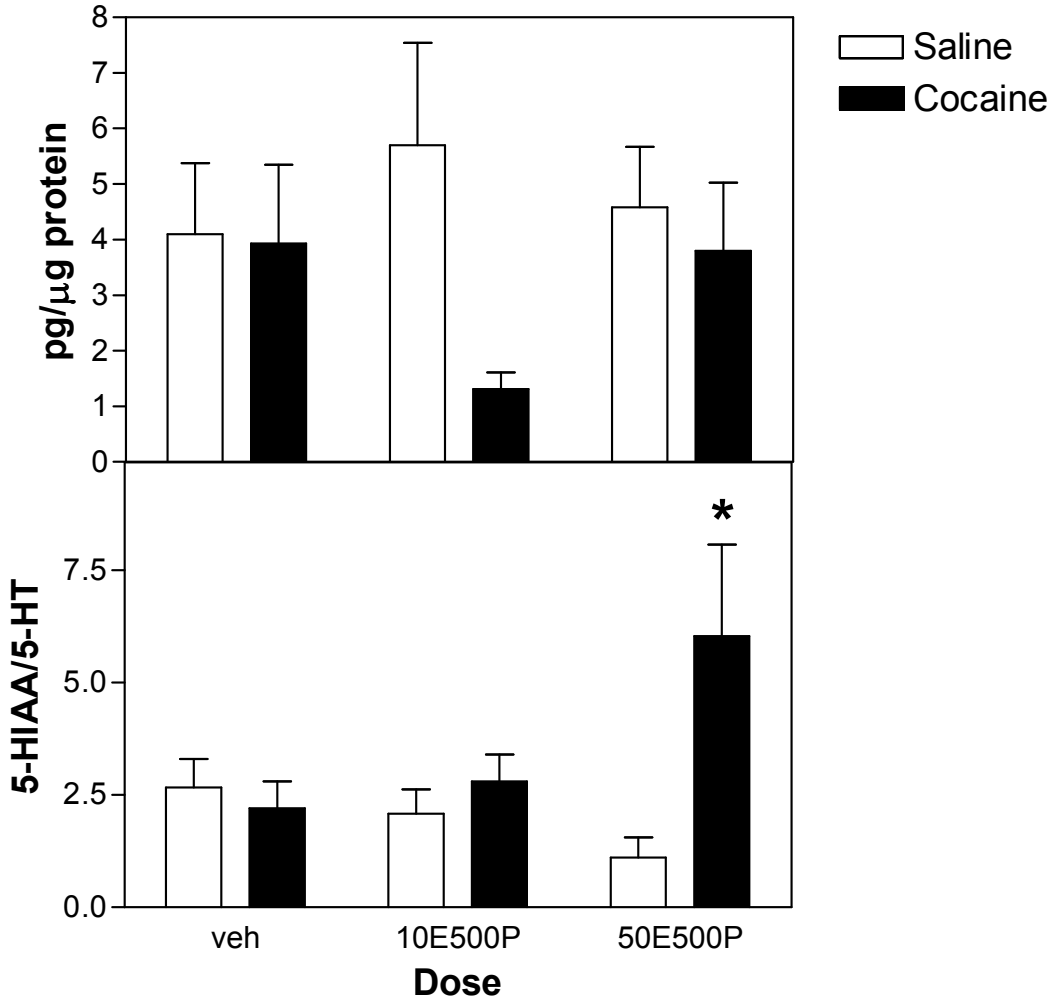

Figure 34. A. Levels of 5-HT and **B.** the ratio of 5-HIAA to 5-HT in the CPu after hormone co-administration and drug/saline treatment. # main effect of hormone; *main effect of drug.

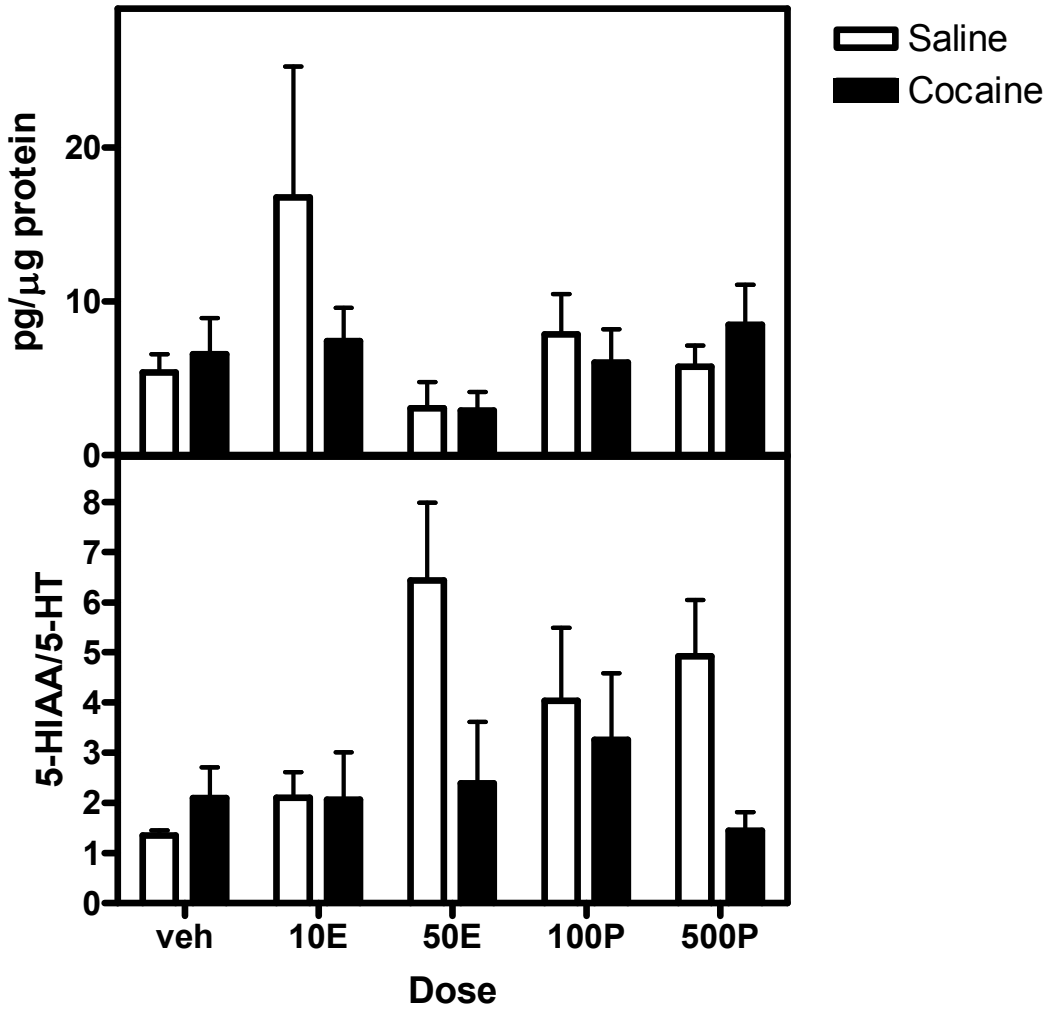

Figure 35. A. Levels of 5-HT and **B.** the ratio of 5-HIAA to 5-HT in the NAc after hormone and drug/saline treatment. # main effect of hormone; *main effect of drug.

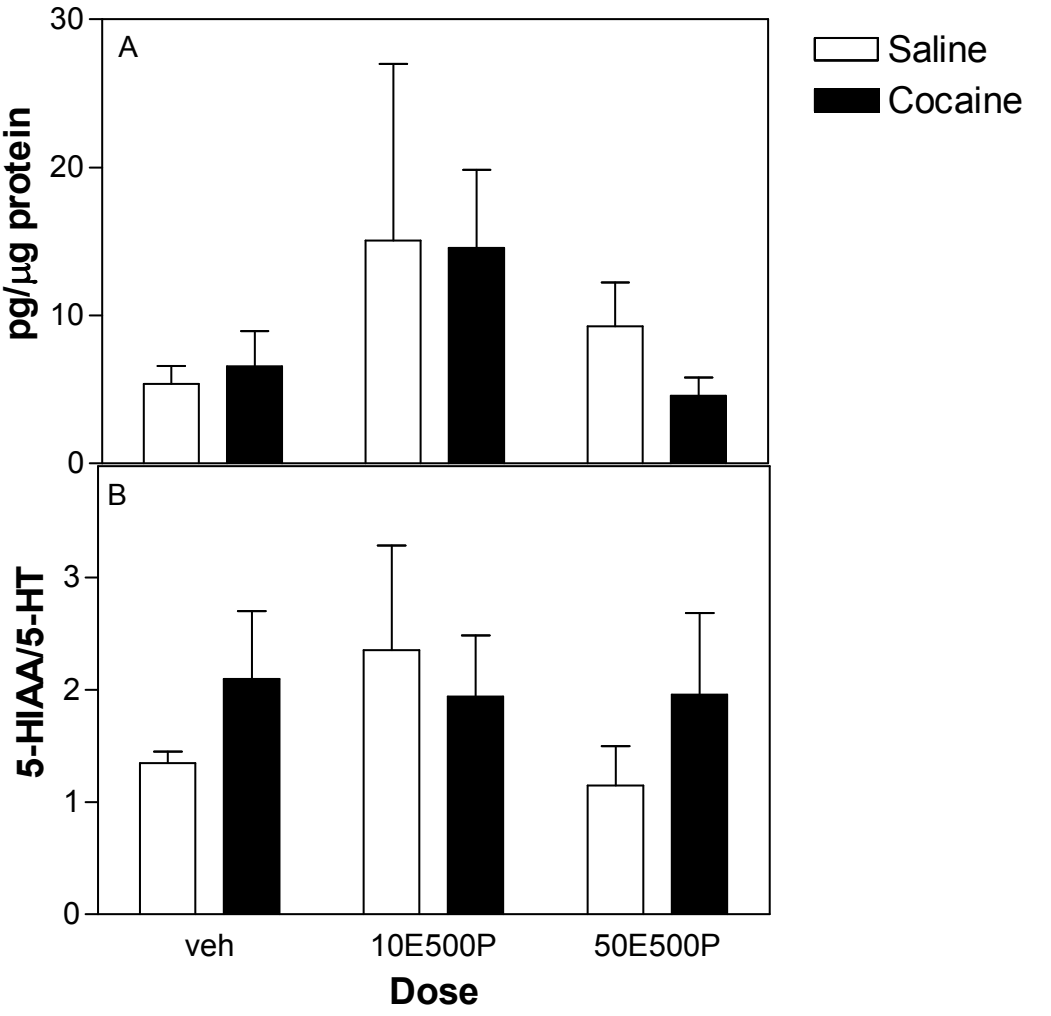

Figure 36. A. Levels of 5-HT and **B.** the ratio of 5-HIAA to 5-HT in the NAc after hormone co-administration and drug/saline treatment. # main effect of hormone; *main effect of drug.

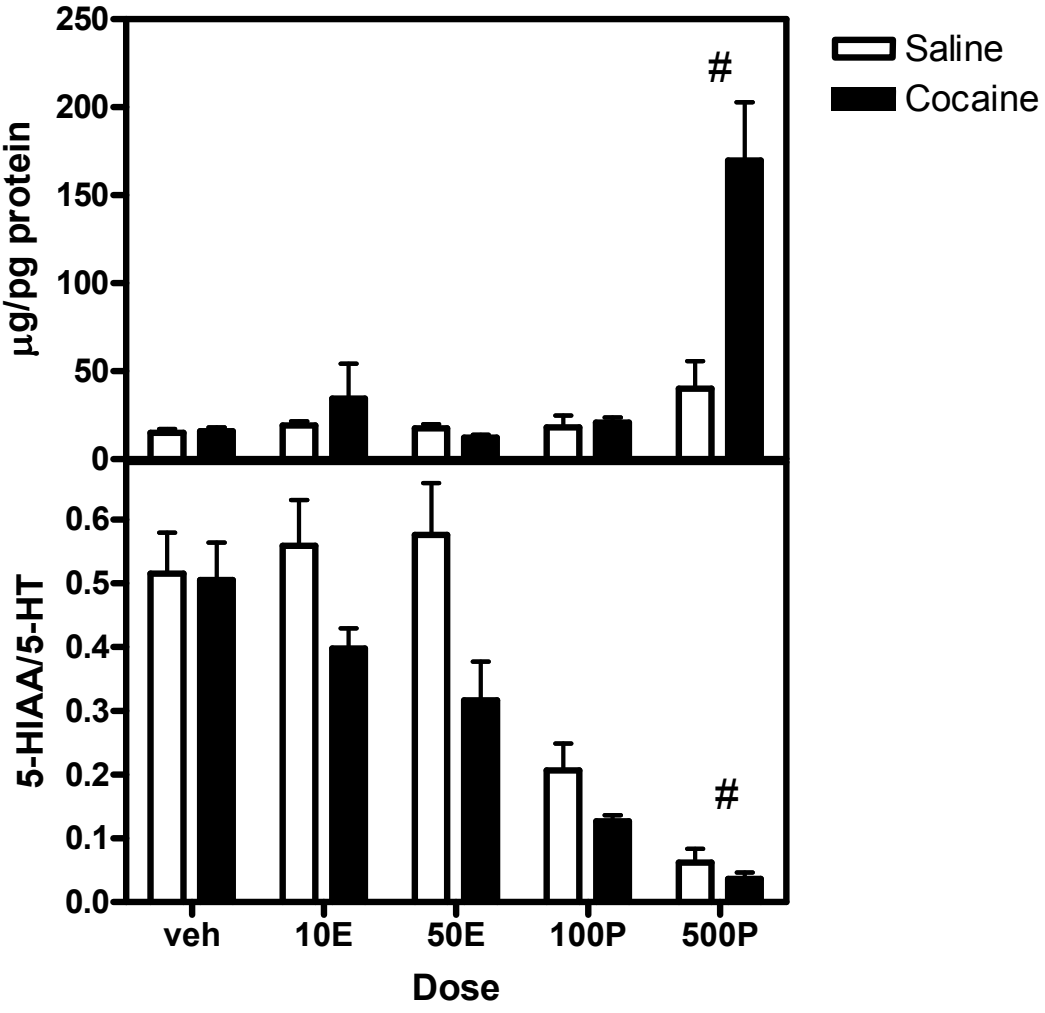

Figure 37. A. Levels of 5-HT and **B.** the ratio of 5-HIAA to 5-HT in the VTA after hormone and drug/saline treatment. # main effect of hormone; *main effect of drug.

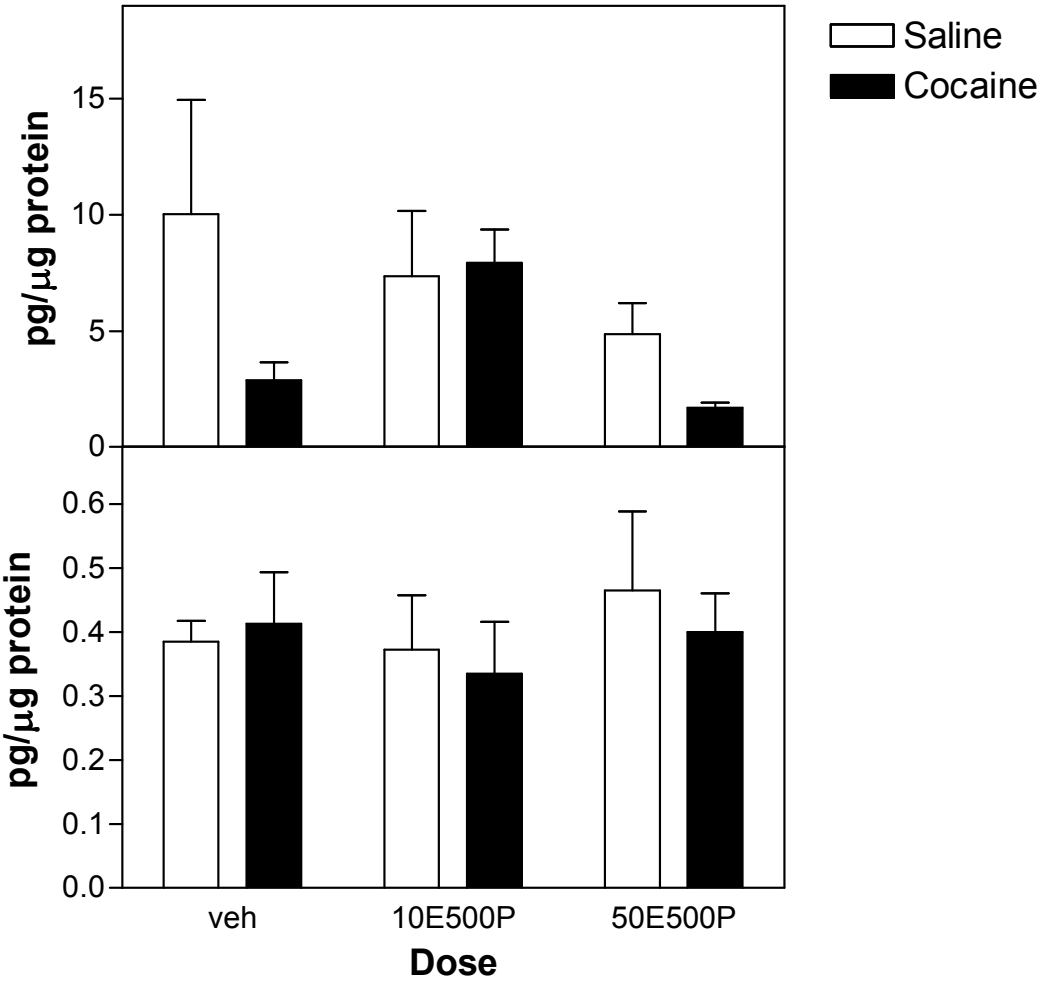

Figure 38. A. Levels of 5-HT and **B.** the ratio of 5-HIAA to 5-HT in the VTA after hormone co-administration and drug/saline treatment. # main effect of hormone; *main effect of drug.

4. Discussion

Similar to other studies from our group, a high dose of estrogen alone had no effect on locomotor activity (Chin et al., 2002; Chapter 2). Additionally, a high dose of progesterone similarly inhibited locomotor activity (Chapter 2). We recently demonstrated that estrogen and progesterone interactions interact based on the length of time between surges of each hormone. We expanded the current literature by demonstrating that estrogen and progesterone also interact differentially, based on their concentrations where ratios that simulate the late proestrus stage enhanced and the diestrus stage inhibited cocaine-induced behavioral responses. This, in part, may explain the previously reported estrous cycle-mediated responses to cocaine.

There is evidence of interactions between the monoaminergic system and ovarian hormones. DA release, number of receptors and levels of DA transporter have been shown to vary due to estrous cycle effects and sex dependent differences in the DA system (Becker and Ramirez, 1981; Castner et al., 1993; Crowley, 1982; Di Paolo et al., 1979; Di Paolo et al., 1988; Di Paolo et al., 1982; Goetz et al., 1983; Houdi et al., 1989; Hruska, 1986; Hruska and Pitman, 1982; Joyce, 1983; Robinson et al., 1982b). It has been proposed that these effects are regulated by ovarian hormones. For example, estrogen can increase striatal DA turnover (Becker, 1990; Di Paolo et al., 1979; Di Paolo et al., 1985; Hruska and Pitman, 1982; Hruska and Silbergeld, 1980) and DA release has been shown to be enhanced after estrogen administration in the nucleus accumbens (Thompson and Moss, 1994). Similarly, estrogen can stimulate DA release in the caudate putamen (Becker, 1990). In the CPu and the nucleus accumbens, we report that single

hormone replacement (regardless of dose) increases dopamine activity as indicated by turnover ratios. However, this effect was not observed when estrogen and progesterone were co-administered. This may suggest that in these areas, estrogen and progesterone can independently modulate dopamine activity after cocaine administration but the presence of both may result in an interaction that leads to the diminishing of this effect.

In estrogen-primed rats receiving progesterone, there was an increase in DA release in the striatum (Dluzen and Ramirez, 1984; Dluzen and Ramirez, 1987; Dluzen and Ramirez, 1991). Independent of estrogen effects, a physiological dose of progesterone has been shown to rapidly increase DA in this area (Petitclerc et al., 1995). However, Perrotti et al. (2000) showed that there were no changes in levels of DA, DOPAC or HVA in the prefrontal cortex. Although there were no significant effects of progesterone replacement in the CPu, we found that n the VTA, 500 μg of progesterone decreased levels of dopamine and increased turnover rates. Because excess dopamine has been shown to increase behavioral activity, the decrease in levels correspond to the inhibit in locomotor activity at this dose.

Steroid hormones have also been shown to modulate the serotonergic system and have been postulated to underlie sex differences in that males have greater activity in serotonin (5-HT) neurons compared to females (Klink et al., 2002). Estrogen is also capable of exhibiting noncompetitive inhibition of 5-HT transport (Chang and Chang, 1999). Firing rates of neurons in the dorsal raphe nucleus were correlated with plasma progesterone levels where higher progesterone corresponded to increased firing (Klink et

al., 2002). However, in the hypothalamus, when progesterone was administered following estrogen priming, levels of 5-HT were reduced (Farmer et al., 1996; Gereau et al., 1993; Maswood et al., 1999). Maswood et al. (1999) suggest that the progesterone-induced decrease in extracellular 5-HT levels may be attributed to an alteration in a change in 5-HT autoreceptor function (Maswood et al., 1999). Our laboratory has previously shown that progesterone replacement increased levels of 5-HT in the prefrontal cortex after cocaine administration (Perrotti, L. I., Beck, K. D., Luine, V. N., and Quinones-Jenab, V00). In this study, we found that in the group that received a high dose of progesterone (500 μg) there was an increase in serotonin levels and a decrease in activity in the VTA after cocaine administration, implicating progesterone-serotonin interactions in the modulation of cocaine-induced respones. Serotonin-hormone interactions have not been extensively studied but 5-HT has prominent role in drug reward and further studies of these interactions will result in an improved understanding of sex differences underlying drug effects.

After co-administration of estrogen and progesterone in the CPu, there was an increase in serotonin activity which suggests that there is an interaction between these steroid hormones and cocaine. As reviewed in Frye (2001), progesterone exhibits nongenomic effects in the VTA, possibly through interaction with the GABA-A/benzodiazepine receptor complexes. Activation of these complexes may in turn contribute to the decrease in locomotor activity. This attenuation of cocaine induced hyperactivity was also observed in the co-administration group that received the high dose of progesterone and the low dose of estrogen. However, the inhibition was abolished

when progesterone was co-administered with the high dose of estrogen suggesting that high levels of estrogen may have stimulatory effects that diminish progesterone's ability to attenuate locomotor activity after cocaine administration.

Most studies examining the role of estrogen and progesterone in the effects of cocaine have used a single hormone-replacement paradigm. However, the concentrations of these hormones fluctuate throughout the estrous and menstrual cycle. We demonstrated that changes in the ratio of estrogen and progesterone present can impact behavioral and neurochemical responses to cocaine, suggesting that in addition to interactions between individual hormones and cocaine, there are estrogen-progesterone interactions (based on the varying concentrations) that can affect cocaine-induced alterations.

Chapter 8: Conclusion

Previously, it was postulated that in females, the fluctuation of estrogen and progesterone affected cocaine-induced responses. However, the manner in which these hormones contribute to the behavioral responses has yet to be determined. In these studies we concluded that estrogen and progesterone effects on cocaine addiction are complex. The interactions between the hormones occur at multiple levels.

First, alterations on serum levels of estrogen and progesterone have a profound effect on the outcome of their effect on cocaine-induced behaviors. Using different concentrations of estrogen (5, 10, 20, or 50 μg) and progesterone (50, 100, 250, or 500 μg) we showed that the hormones can either facilitate or inhibit cocaine's effects. Specifically, although estrogen did not affect cocaine-induced ambulatory and rearing behaviors, it affected stereotypic behaviors regardless of cocaine administration (animals receiving 50 μg had higher stereotypic counts than did the OVX group). In contrast, progesterone affected rearing activity dose-dependently: 50 and 500 μg of progesterone inhibited, whereas 100 μg and 250 μg stimulated, rearing in response to cocaine. That estrogen and progesterone did not affect overall baseline behavioral activity suggests their effects are mediated in part through interactions with cocaine. Progesterone administration did not affect corticosterone levels in saline- or cocaine-treated rats. Estrogen administration, however, affected levels of corticosterone both at baseline and after cocaine treatment. After accounting for baseline differences, we found that rats receiving 5 or 10 μg of estrogen and cocaine had higher percentage-increases in serum

corticosterone levels than did the control group that did not receive estrogen. On the basis of these observations, we suggest that progesterone fluctuations during the estrous cycle impact cocaine-induced behavioral responses, whereas estrogen may affect activity in the hypothalamic-pituitary-adrenal axis. Thus, dose-dependent effects of gonadal hormones may underlie some of the reported sex differences and reproductive cycle effects of cocaine.

A second level of estrogen and progesterone interactions with cocaine in modulation of behavioral responses through activation of nuclear and membrane mechanisms. Specifically, short-term estrogen replacement decreased cocaine-induced ambulations. Short-term progesterone decreased rearing, whereas long-term progesterone decreased ambulations. Although cocaine increased levels of c-fos mRNA, none of the estrogen or progesterone replacement paradigms affected this measure. Because long-term estrogen replacement has been shown to have no effect on locomotor activity after acute cocaine administration, our observations suggest that short-term estrogen may underlie behavioral alterations. These findings suggest that after acute cocaine administration, while estrogen may activate only membrane receptors to alter behavioral responses to cocaine, progesterone activates both nuclear and membrane receptors.

Psychostimulants such as cocaine have been shown to regulate *c-fos* and opioid gene expression in male rats. Estrogen and progesterone may modulate these effects. Utilizing quantitative solution hybridization assays levels of *c-fos* and preprodynorphin mRNA after cocaine administration were measured in the caudate putamen of intact male

and female rats or ovariectomized female rats that were pretreated with vehicle, estrogen and/or progesterone. *C-fos* mRNA levels were increased in intact male and female rats after 30 min or 3 hrs of one single cocaine injection and after 14 days of single daily cocaine injections. *C-fos* mRNA levels were also increased after 30 min of a single cocaine injection in ovariectomized female rats that were treated with vehicle, estrogen and/or progesterone. The preprodynorphin mRNA levels did not change after 30 minutes, 3 hrs or 14 days in intact male or female rats. However, preprodynorphin mRNA levels were increased in the caudate putamen of ovariectomized female rats pretreated with vehicle or a combination of estrogen and progesterone but not in ovariectomized female rats that were pretreated with either estrogen or progesterone alone. These findings suggest hormonal regulation of cocaine effects on preprodynorphin mRNA levels which may modulate cocaine-induced behaviors in female rats. Although there were no changes in levels of c-mRNA in this study, there may be fluctuations in levels if a different hormone replacement paradigm were used prior to cocaine administration.

A third level by which estrogen and progesterone interact to control estrous cycle effects are temporal interactions (length of time between each hormonal surge); both estrogen and progesterone are present also play an important role in behavioral responses to cocaine. We demonstrated that estrogen and progesterone either synergize to facilitate or antagonize to inhibit cocaine-induced behaviors. When progesterone was administered 1 and 48 hours after estrogen replacement, locomotor behavior was inhibited. On the other hand, when progesterone was administered 24 hours after estrogen replacement,

behavior was enhanced. This study emphasizes the importance of temporal effects in hormonal interactions.

Interactions between estrogen and progesterone in terms of their concentrations are also an important component of cocaine-induced behavioral responses in females. E + P were co-administered at different ratios to determine whether hormonal interactions in the estrous cycle contribute to cocaine-induced alterations in behavior and in the dopamine system. Prior to an acute injection of cocaine (15 mg/kg; i.p.) or saline, ovariectomized female rats received vehicle (sesame oil), or E (10 μg or 50 μg; 48 hours; s.c.) and P (100 μg or 500 μg; 24 hours; s.c.). When animals were administered 50 μg E + 500 μg P (levels reflecting those observed during the late proestrus stage), total locomotor behavior was enhanced while levels of dopamine (DA) in the nucleus accumbens and the ratio of HVA to DA in the VTA were decreased. Administration of 10 μg E + 500 μg P (levels reflecting those observed during the diestrus stage) inhibited total locomotor activity and decreased baseline levels of DA. After co-administration of estrogen and progesterone in the CPu, there was an increase in serotonin activity which suggests that there is an interaction between these steroid hormones and cocaine. In the group that received a high dose of progesterone (500 μg) there was an increase in serotonin levels with a corresponding decrease in activity in the VTA after cocaine administration. This study suggests that alterations in monoamine activity, as a result of E and P administration, may underlie the observed changes in cocaine-induced behavior during the estrous cycle.

Finally, hormonal replacement affects cocaine pharmacokinetics. While estrogen replacement did not affect cocaine-induced locomotor activity, progesterone attenuated locomotor counts in comparison with control groups receiving only sesame oil. Estrogen, however, decreased brain levels of cocaine and norcocaine 30 minutes after cocaine administration in comparison to the group receiving vehicle at that time point. In addition, in progesterone-treated rats, levels of benzoylecgonine and ecgonine methylester were higher at 30 minutes post-administration than at 15 minutes. No changes were found in blood levels of the metabolites. These findings suggest that while progesterone has an impact on locomotor behavior, pharmacokinetic effects may have a limited role in mediating behavioral responses to cocaine.

Possible mechanisms of action:

In addition to long-term effects of estrogen, there is evidence of nongenomic activity. Beyer and Raab (1998) show that estradiol administration increases intracellular calcium levels in DA and GABA neurons. Estrogen can also rapidly decrease mu-opioid activity in the hypothalamus and inhibit episodic LH release (Condon et al., 1998; Lagrange et al., 1997). The activation of these pathways may contribute to the observed decrease in ambulations after short-term estrogen replacement and cocaine administration. Thus, besides the monoaminergic effect demonstrated herein, other neuromodulators can play a significant role on the estrogen-mediated effects of cocaine

Progesterone also exhibits long term effects as indicated by an inhibition in locomotor activity after cocaine administration. After progesterone replacement, levels of

dopamine decreased and dopamine activity was increased suggesting that the hormone modulates dopamine pharmacokinetics. The decreased availability of dopamine after progesterone treatment may account for the attenuation of locomotor activity and implicates a progesterone-dopamine interaction in the modulation of cocaine-induced behavioral responses.

Short-term progesterone replacement also affects behavioral responses after cocaine administration. Thus, a nongenomic mechanism is also involved. It is well-documented that alloprenanolone, a progesterone metabolite, interacts with the GABA-A/benzodiazepine receptor complex. Activation of the receptor and an increase in GABA activity may underlie cocaine-induced changes after short-term progesterone treatment.

When E and P are co-administered in various concentrations, the highest concentrations of both and E and P led to a potentiation of behavioral activity in response to cocaine. In contrast, there was an inhibition in these behaviors when high levels of progesterone and low levels of estrogen are present. This is consistent with previous studies demonstrating that rats, during estrus, when high levels of both estrogen and progesterone are present, show an enhancement in hyperactivity in response to psychostimulants. Furthermore, cocaine-induced activity is lowest during diestrus, when levels of progesterone are high and levels of estrogen are low. Changes in observed levels of dopamine correspond to these alterations. Levels of dopamine were lower and dopamine activity was higher in cocaine groups that displayed an inhibition of behavioral

responses suggesting that there is an interaction between different ratios of estrogen and progesterone and dopamine.

Although the administration paradigms used in the studies represent certain phases of the estrous cycle, levels of hormones are constantly fluctuating; it is therefore difficult to accurately simulate the circulating levels at a given phase. The changes that occur are the result of specific ratios of hormonal replacement and these ratios are not constant within a specific phase. Interestingly, the findings indicate that in some measures, there are similarities in the OVX and the E+P groups that were not seen when estrogen and progesterone were administered alone. For example, in those groups, there was a cocaine-induced increase in levels of c-fos in the CPu and a decrease in levels of DA in the NAc. These observations were not seen in animals receiving only estrogen and progesterone suggesting that the hormones interact in a synergistic manner in the mesolimbic pathway.

There is further evidence to support an interaction between the hormones and dopamine. It has been shown that dynorphin peptides and non peptide kappa opioid receptor agonists decrease dopamine release in the striatum and hypothesized that cocaine-induced increases in PDYN functions as a negative feedback mechanism to reduced cocaine-induced dopamine increase (DiChiara and Imperato, 1988; Manzaneres et al., 1991; Spanagel et al., 1990; Spangler et al., 1997a). Because estrogen prevents cocaine-induced increases in levels of preprodynorphin mRNA, the feedback mechanism may be less effective in regulation levels of dopamine and thus account for the reported

enhancement in behavioral responses after cocaine in rats pretreated estrogen. Thus, there are complex interactions resulting from the co-administration of estrogen and progesterone that mediate neurochemical and behavioral responses to cocaine (summary tables 14 and 15).

The findings presented in this thesis provide further insight into gender differences in cocaine abuse. Although there is considerable evidence in clinical literature of these disparities, the mechanisms underlying the differences are not well understood. We postulate that the fluctuation in levels of estrogen and progesterone throughout the menstrual cycle plays a significant role modulating the effects of cocaine on several levels; the concentrations of the hormones, time between hormonal surges, hormonally-mediated molecular and neurochemical mechanisms, and their impact on cocaine metabolism are all crucial factors contributing to cycle-related fluctuations in cocaine effects. Understanding the dynamic interactions between these factors can lead to improved approaches to developing gender-specific treatments for cocaine abuse.

Table 14. Summary table 1.

	Rearing	Ambulations	Stereotypic Behavior	HPA activity (cort levels)
E dose response	No effect	No effect	⇧ at μg 50	⇧ in %-change from saline controls at 10 and 20 μg
P dose response	⇩ at 50 and 500 μg	No effect	No effect	No effect
Short-term E ---------- Long-term E	No effect ---------- No effect	⇩ ---------- No effect	No effect ---------- No effect	No effect ---------- No effect
Short-term P ---------- Long-term P	⇩ ---------- No effect	No effect ---------- ⇩	No effect ---------- No effect	⇧ ---------- No effect
E+P	N/A	⇩ at 10E+500P, 50E+100P	N/A	No effect

Table 15. Summary table 2.

	OVX	Estrogen (50 µg)	Progesterone (500µg)	E+P (10µg+500µg)	E+P (50µg+500µg)
CPu	↓ in DA after cocaine	↓ baseline DA ↑ turnover	↓ baseline DA ↑ turnover	↓ baseline DA	↓ baseline DA
NAc	↓ in DA after cocaine	↓ DA levels regardless of drug treatment ↑ turnover regardless of drug treatment	↓ DA levels regardless of drug treatment ↑ turnover regardless of drug treatment	↓ baseline DA	No changes
VTA	No changes	No changes	↓ DA levels regardless of drug treatment ↑ turnover regardless of drug treatment	↓ turnover	No changes
Behavior	↑ after cocaine	↑ after cocaine	Inhibited by cocaine	Inhibited by cocaine	↑ after cocaine

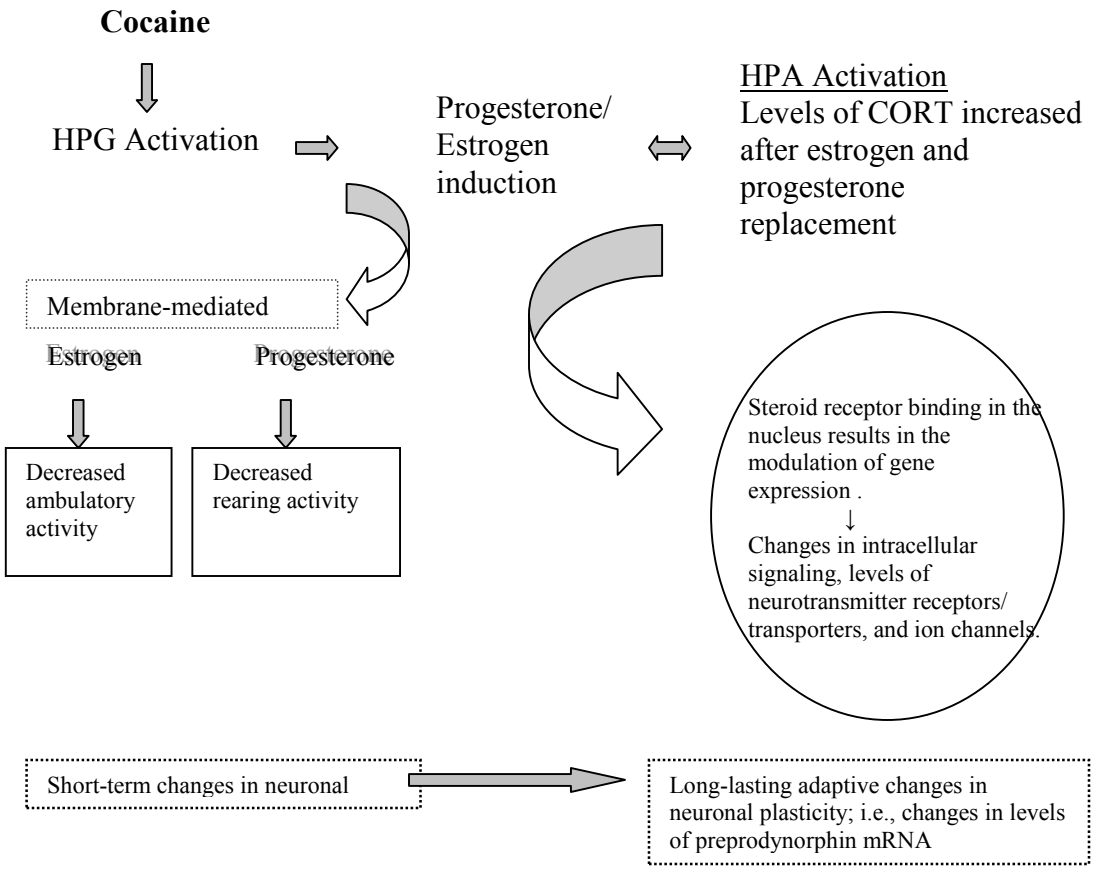

Figure 39. Proposed model of cocaine and hormonal interactions.

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