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Steroid Hormone Effects on Cocaine-Induced Alterations in Ovariectomized Fischer Rats

by

Linda Irene Perrotti

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

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5/19/00
Date

Vanya Quiñones
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THE CITY UNIVERSITY OF NEW YORK

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Linda Irene Perrotti

Advisor: Professor Vanya Quiñones

Abstract

Cocaine has a variety of pharmacological actions; however, its major effect is the inhibition of the reuptake of neuronal monoamines. Interestingly, there are sex differences and differences across the reproductive cycle of females in use and abuse of cocaine as well as in the behavioral and subjective responses to it's administration. The complex endocrinological profile of females may be involved in these sex and reproductive cycle differences. Estrogen and progesterone function in the brain to regulate neuronal activity and influence behavior. Due to the modulating effects of estrogen and progesterone on the CNS, these hormones may be an integral part of the cascade of events that are involved in cocaine's actions in the CNS. It is not clear what role(s) each of these hormones play individually or in combination in cocaine-induced subjective and physiological alterations. These investigations were conducted to understand the role(s) of these hormones on cocaine-induced alterations. This work shows that there is an interaction between ovarian hormones and cocaine on cocaine-induced behavioral, neurochemical, and molecular alterations. Modulation of the endocrine system, HPA activation and progesterone levels were observed in response to cocaine treatment. Estrogen modulated cocaine-induced alterations at the behavioral and molecular levels; chronic estrogen treatment caused

sensitization of the locomotor response to cocaine and caused increases in plasma corticosterone levels after chronic cocaine administration. Progesterone alone did not alter the locomotor or stereotypic behavioral responses to cocaine. But the time of administration of progesterone relative to estrogen administration affected the behavioral response to cocaine; progesterone administered 24 hours after estrogen enhanced cocaine-induced increases in locomotor behaviors. However, progesterone administered 43-46 hours after estrogen decreased cocaine-induced increases in locomotion. The co-administration of progesterone and cocaine increased levels of 5-HT in the medial prefrontal cortex. Additionally, estrogen and progesterone and cocaine administration affected dynorphin expression in the opioid system. These results suggest that the interactions between ovarian hormones and cocaine may be the basis of gender and reproductive cycle differences in response to cocaine. Additionally, the stage of the cycle or use of steroid-based contraceptives at the time of cocaine administration may influence the effects of cocaine on brain functioning.

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Some other faculty members I need to thank are Dirk Houben, without whom Vanya and I may have never gotten together; Peter Moller, who knew me when... and Gordon Barr for his advice along the way.

Members of the Quiñones Laboratory: Shirzad Jenab for teaching me solution hybridization and making me really nervous just after Vanya had managed to calm me down. Juliet Chin, Sosimo Fabian and Scott Russo for all of their assistance with my experiments and providing the fun and friendly atmosphere in the lab that I am going to miss immensely.

Thanks to my girlfriends, a few very important people who love me for all that I am and accept what I am not. You have all been there through some of the toughest parts; I appreciate your having stuck by me. Caroline Sprague, thank you for understanding everything, from the sublime to the ridiculous. You truly understand and really know me and, yet, you love me anyway. Adrienne Carter, you have seen me at my best and my worst and you are still by my side...thank you. And thanks for always trying to remind me to have fun. Rachel Bowman, having a friend like you for my final years in graduate school made me wonder how I made it through my first few without you. Minka Sprague, thank you for sharing with me your wisdom and for teaching me (among many other things) to always say "yes" unless there was a good reason to say "no".

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I dedicate this thesis to my parents, Anthony and Irene Perrotti. In addition to being wonderful parents you are my best and closest friends. If any two people in the world deserve a volume dedicated to them, they are you. Thank you for so much, especially for always believing in me even when I failed to believe in myself.

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CHAPTER I

History:

Cocaine is an active alkaloid found in the leaves of *Erythroxylon coca,* a tree indigenous to Peru and Bolivia. Evidence of the use of cocaine as a psychostimulant dates back between 2,500 and 5,000 years when the leaves of the coca plant were used by Columbian Indians (Platt, J. J., 1997).

A Columbian Indian legend tells of how their people sailed through the Milky Way in a canoe containing, among other "riches", the psychoactive plant *Erythroxylon coca* (McKim, W. A., 1996). When the Incas conquered Columbia, around the 10th century, coca was declared sacred and was used primarily by priests and nobility for special ceremonies (McKim, W. A., 1996). Coca use was completely banned when the Spanish first conquered the Inca's empire because they viewed its use as idolatrous and pagan. However, the Spanish later discovered that if the Indians were given coca they could work harder and longer and required less food. As a result, coca leaves were distributed to workers three to four times daily during rest periods (McKim, W. A., 1996; Platt, J. J., 1997).

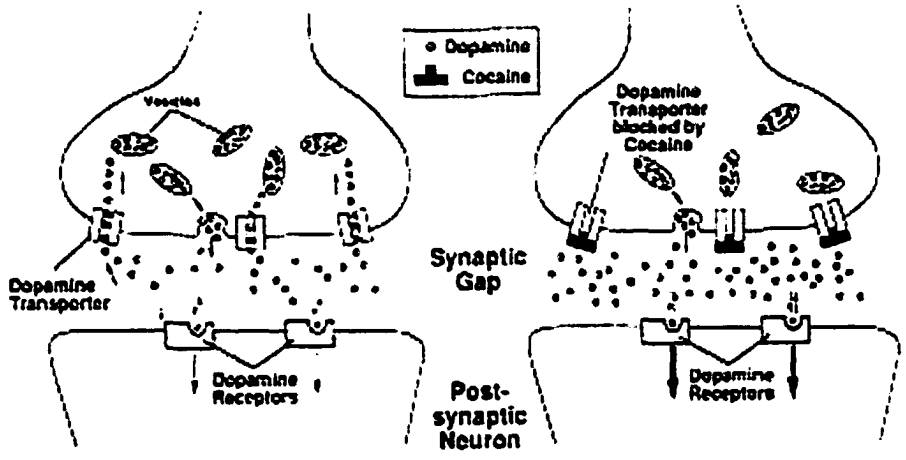
The use of cocaine remained limited to the New World until the late part of the 1800's (Platt, J. J., 1997). In 1884 Freud, who had earlier become interested in the medicinal use(s) of cocaine and had tried the drug on himself, advocated the use of cocaine as the cure to nearly all of the woes of humanity (McKim, W. A., 1996). During

this time, Karl Koller (an associate of Freud) demonstrated the only real medicinal use of cocaine, as a potent local anesthetic (McKim, W. A., 1996; Platt, J. J., 1997).

Despite earlier claims that cocaine was no more addictive than coffee or tea and could be used to treat morphine and alcohol addictions, by 1887 cocaine addiction had been reported in the literature, and by 1888 cases of toxicity and death had also been reported (Platt, J. J., 1997). In 1894 the American Medical Association made a statement expressing its concern regarding the use of cocaine (Platt, J. J., 1997). In 1914, as a result of the of the Pure Food and Drug Act of 1906, the Harrison Narcotic Act banned the use of cocaine (McKim, W. A., 1996; Platt, J. J., 1997). The Harrison Act drove cocaine underground where it has remained for decades (Platt, J. J., 1997).

In the 1960s, a wave of cocaine use began initially among middle or upper class users. Later during the late 1970's and early 1980's, cocaine became popular among the inner city poor (Platt, J. J., 1997). In 1985, cocaine use peaked where almost six million Americans were current users (Substance Abuse and Mental Health Services Administration , 1998). Since this time the number of cocaine users declined to around 1.4 million and has not changed significantly since then (Substance Abuse and Mental Health Services Administration , 1998). The 1998 National Household Survey on Drug Abuse reported that 1.8 million Americans were current cocaine users and approximately one-third of these users were women (Substance Abuse and Mental Health Services Administration , 1998).

Cocaine has a variety of pharmacological actions; however, its major effect is the inhibition of the reuptake of neuronal monoamines. Cocaine binds with dopamine, serotonin and norepinephrine transporters and prevents the reuptake of these monoamines, thus, increasing their synaptic concentrations (Figure 1) (Heikkila, R. E., Orlansky, H., & Cohen, G., 1975). These neurotransmitter systems are believed to be an integral part of the cascade of events that are involved in the psychomotor and subjective effects of cocaine in the CNS.



Reprinted (Kuhar, M. J. et al., 1995)

Figure 1. Cocaine binds to the dopamine (or other monoamine) transporter and blocks the reuptake of dopamine increasing the synaptic levels of the transmitter and, thus, the activation of the post-synaptic cell.

Effects of cocaine on monoamine systems:

The dopamine system has been postulated to have a primary role in mediating the reinforcing properties of many drugs of abuse including cocaine, amphetamine, and opiates (Koob, G. F., 1992). Cocaine's effects on the dopamine neurons of the nucleus accumbens, as well as other regions in the CNS, such as the medial prefrontal cortex (Goeders, N. E. & Smith, J. E., 1983), ventral pallidum (Hubner, C. B. & Koob, G. F.,

1990), and olfactory tubercle (Kornetsky, C., Huston-Lyons, D., & Porrino, L. J., 1991), appear to be necessary for cocaine reinforcement (Koob, G. F., 1992; Roberts, D. C. S., Koob, G. F., Klonoff, P., & Fibiger, H. C., 1980) (Figure 2).

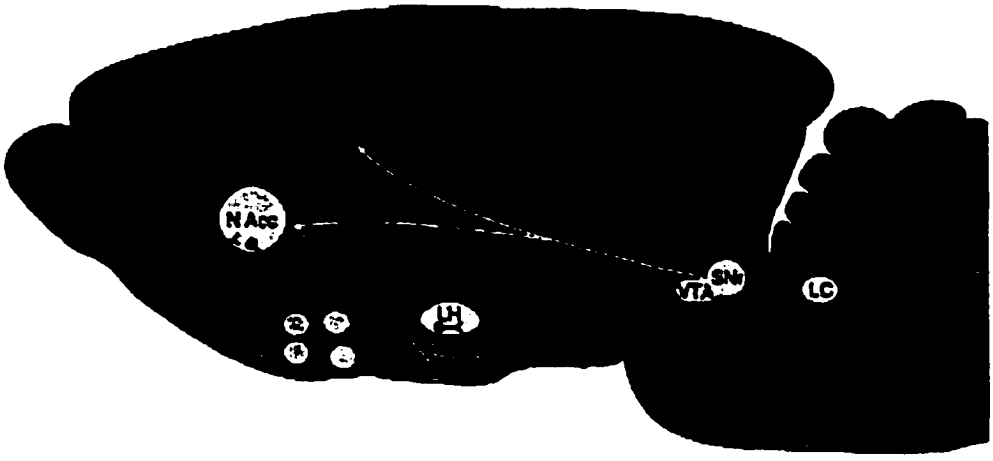


Figure 2. Postulated brain reward pathway.

Different cocaine administration paradigms have produced different effects on dopamine receptor expression, with reports of increases, decreases, and no change in the number of dopamine receptors (Roberts, D. C. S., Koob, G. F., Klonoff, P., & Fibiger, H. C., 1980; Unterwald, E. M., Ho, A., Rubenfeld, J. M, & Kreek, M. J., 1994; Zeigler, S. J., Lipton, J., Toga, A., & Ellison, G., 1991; Mayfiled, R. D., Larson, G., & Zahniser, N. R., 1992; Kleven, M. S., Perry, B. D., & Woolverton, W. L., 1999; Laurier, L. G., Corrigall, W. A., & George, S. R., 1994; Claye, L. H., Akanne, H. C., Davis, M. D., DeMattos, S., & Soliman, K. F. A., 1995; Alburges, M. E., Narang, N., & Wamsley, J. K., 1993; Goeders, N. E. & Kuhar, M. J., 1987). Similarly, different paradigms of cocaine

administration produce different effects on the dopamine transporter. No changes in the level of dopamine transporter sites have been reported after one, two, three, seven, ten, and fourteen days of cocaine administration (Izenwasser, S. & Cox, B. M., 1990; Kula, N. S. & Baldessarini, R. J., 1991; Unterwald, E. M., Rubenfeld, J. M, & Kreek, M. J., 1995). Increased dopamine transporter levels after cocaine administration were found in the striatum but not in the nucleus accumbens (Goeders, N. E. & Kuhar, M. J., 1987). Thus, most of the studies have shown no changes in dopamine transporter binding during or immediately after chronic cocaine administration.

At the mRNA level, Maggos et al., (Maggos, C., Spangler, R., Zhou, L., & Kreek, M. J., 1995; Spangler, R. et al., 1997a) reported no changes in dopamine transporter mRNA levels in the substantia nigra or ventral tegmental area following repeated cocaine administration for 3 or 14 days. However, a report by Pillote et al., (Pilotte, N. S., 1994) demonstrated by *in situ* hybridization analysis an increase in the dopamine transporter mRNA levels after withdrawal. These discrepancies may be due to differences in time of study relative to cocaine administration.

However, dopamine receptor knockout mice still self-administer cocaine, thus suggesting that dopamine is not the only mechanism responsible for the reinforcing effects of cocaine (Rocha, B. A. et al., 1998). Cocaine also binds directly to the serotonin transporter, and thus, inhibits the uptake of serotonin into presynaptic neurons. Cocaine induced alterations in the serotonin system may be important in the modulation of cocaine-induced behavioral alterations (Cunningham, K. A., Paris, J. M., & Goeders, N.

E., 1992) and in regulating cocaine self-administration (Parsons, L. H. & Justice, J. B., 2000; Richardson, N. R. & Roberts, D. C. S., 1991; Carroll, M. E., Lac, S. T., Asencio, M., & Kragh, R., 1990). Further, serotonergic lesions of the dorsal raphe did not affect cocaine-induced locomotor activity (Morrow, B. A. & Roth, R. H., 1996). Due to the role of serotonin in psychiatric symptoms of depression, anxiety, and insomnia, it has been suggested that a dysfunction of serotonin neurotransmission during cocaine abstinence could play a direct role in these cocaine withdrawal-associated symptoms (Weiss, F., Parsons, L. H., & Markou, A., 1995).

Serotonin sensitivity in the dorsal raphe neurons is enhanced after subsequent challenge with cocaine (Cunningham, K. A., Paris, J. M., & Goeders, N. E., 1992). However, knowledge of the effects of cocaine on serotonin receptors and transporters is extremely limited. Using homogenate-binding assays, no changes in serotonin binding sites were observed after cocaine treatment (Javaid, J. I., Sahni, S. K., Pandey, S. C., & Davis, J. M., 1993; Johnson, R. G., Fiorella, D., & Rabin, R. A., 1993). However, by the use of autoradiography receptor analysis Perret et al., reported an increase in serotonin receptors in the ventromedial hypothalamus and medial nucleus of the amygdala (Perret, G. et al., 1998).

Chronic cocaine administration produced significant increases in serotonin uptake sites in the dorsal raphe nucleus and in some cortical areas (Cunningham, K. A., Paris, J. M., & Goeders, N. E., 1992). However, Benmansour et al., (Benmansour, S., Tajani-Butt, S. M., Hauptman, M., & Brunswick, D. J., 1992) reported no changes in serotonin uptake

sites following cocaine administration. Currently, data demonstrate that both acute and chronic exposure to cocaine significantly alter the electrophysiological function of serotonin systems. Chronic cocaine administration causes electrophysiological effects on the amygdala neuronal sensitivity (Cunningham, K. A., 1995). However, supersensitivity to serotonin has been observed in the nucleus accumbens (White, F. J., Henry, D. J., Hu, X. T., Jeziorski, M., & Ackerman, J. M., 1991). Based on the findings of supersensitivity of serotonin receptors and the increases in density of serotonin reuptake sites, it has been suggested that the post-cocaine extracellular levels of serotonin may be decreased (Weiss, F., Parsons, L. H., & Markou, A., 1995). Consistent with the findings on supersensitivity, other reports have observed a decrease in the serotonin turnover in the medial prefrontal cortex of male rats (Carey, R. J. & Damianopoulos, E. N., 1994; Goeders, N. E. & Smith, J. E., 1993). Nothing is known about the effects of cocaine on the serotoninergic system of females. Although it is well established that cocaine also has a high affinity for the norepinephrine (NE) transporter (Heikkila, R. E., Orlansky, H., & Cohen, G., 1975), the effects of cocaine on the NE system have not been widely studied.

Effects of cocaine on the opioid system:

Both the opioid and dopaminergic systems have a role in the regulation of motivated and emotional behaviors and the control of locomotor activity (Pfaff, D. W. & Schwartz-Giblin, S., 1995). There are interactions between these two systems at the anatomical, behavioral, and neurochemical levels. These interactions have been postulated to be important in the cocaine-mediated effects in the CNS. For example, dopamine receptor antagonists attenuate cocaine-induced reward (deWit, H. & Wise, R.

A., 1977) and opioid antagonists also attenuate cocaine-induced reward (Bain, G. T. & Kornetsky, C., 1977; Houdi, A. A., Bardo, M. T., & Van Loon, G. R., 1989; Bilsky, E. J., Montegut, M. J., Delon, C. L., & Reid, L. D., 1992).

Cocaine has significant effects on several components of the endogenous opioid system. Regulation of opioid peptide precursors by cocaine has been shown after chronic, acute, and self-administration of cocaine in different areas of the mesocorticolimbic and nigrostriatal pathways (Spangler, R., Unterwald, E. M., & Kreek, M. J., 1993; Spangler, R. et al., 1997b; Daunais, J. B. & McGinty, J. F., 1995; Daunais, J. B., Roberts, J. L., & McGinty, J. F., 1999; Hurd, Y. L., Brown, E., Finlay, J. M., Fibiger, H. C., & Gerfen, C. R., 1992; Steiner, H. & Gergen, C. R., 1993). Cocaine also affects the opioid peptide levels in the CNS and plasma. Repeated and acute "binge" cocaine administration increases preprodynorphin but not preproenkephalin levels in the caudate-putamen (Spangler, R., Unterwald, E. M., & Kreek, M. J., 1993; Spangler, R. et al., 1996; Spangler, R. et al., 1997b). Additionally, mRNA levels of preprodynorphin in the caudate-putamen were shown to be elevated acutely after one and two days of "binge" cocaine administration (Spangler, R. et al., 1997b). Preproenkephalin mRNA is elevated following some subacute "binge" cocaine administration paradigms (Spangler, R., Unterwald, E. M., & Kreek, M. J., 1993; Spangler, R. et al., 1997a), but not after 14 days of "binge" cocaine administration (Branch, A. D., Unterwald, E. M., Lee, S. E., & Kreek, M. J., 1996). Cocaine increases levels of dynorphin immunoreactivity in the striatum, substantia nigra and nucleus accumbens, but not in the hippocampus (Sivam, S. P., 1989; Smiley, P. L., Johnson, M., Bush, L., Gibb, J. W., & Hanson, G. R., 1990).

Cocaine administration also affects the number (Hubner, C. B. & Koob, G. F., 1990; Koob, G. F., Vaccarino, R. J., Amalric, M., & Swerdlow, N. R., 1987; Hammer, R. P., 1989; Unterwald, E. M., Rubenfeld, J. M, & Kreek, M. J., 1994; Unterwald, E. M., 1995; Unterwald, E. M., Horne-king, M. J., & Kreek, M. J., 1992) and mRNA levels (Spangler, R. et al., 1997b; Buzas, R., Rosenberg, J., & Cox, B. M., 1996; Azaryan, A. V., Coughlin, L. J., Buzas, B., Clock, B. J., & Cox, B. M., 1996) of different subtypes of opioid receptors in the mesolimbic and nigrostriatal neurons, including κ- and μ-opioid receptors (Azaryan, A. V., Coughlin, L. J., Buzas, B., Clock, B. J., & Cox, B. M., 1996) (Yuferov, V. et al., 1999). Thus, the endogenous opioid system could be involved in mediating some effects of cocaine. The relationship between the opioid system and the reinforcing properties of cocaine is not clearly understood. However it has been postulated that dopamine released from terminals of neurons in the substantia nigra which project to the caudate-putamen modulate postsynaptic cell activity (Spangler, R. et al., 1997b). Cocaine acts to elevate dopamine levels at release sites by blocking reuptake from the synaptic cleft, this effect results in the alteration of gene expression via modulating opioid mRNA (Spangler, R. et al., 1997b). This regulatory loop between dopamine cells and dynorphinergic cells may provide a feedback mechanism by which levels of opioid peptides inhibit the release of dopamine (Spangler, R. et al., 1997b). It has been postulated that interactions between both systems play a pivotal role in the control of behavioral and subjective effects of cocaine.

Sex differences in the behavioral response to cocaine:

Recent studies suggest that male and female humans and animals respond differently to different psychomotor stimulants (Robinson, T. E., Camp, D. M., Jacknow, D. S., & Becker, J. B., 1982; Robinson, T. E., Becker, J. B., & Presty, S. K., 1982; Craft, R. M. & Stratmann, J. A., 1996; Kuhn, C. & Francis, M. S., 1997; Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996; Sofuoglu, M., Duidish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K, 1999). Sex differences in response to acute cocaine administration in humans have been reported. Using smoked cocaine and intranasal cocaine administration, men achieved faster and higher peak plasma cocaine levels and reported episodes of euphoria or dysphoira faster and with more intensity than women (Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996; Sofuoglu, M., Duidish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K, 1999). However, a recent report by Mendelson et al., (Mendelson, J. H. et al., 1999), in which cocaine was administered intravenously, reported no gender differences in reports of the subjective effects of cocaine or peak plasma cocaine levels. This discrepancy in results is most likely due to the route of administration of cocaine or differences between subject pools (occasional users versus addicts). Both smoked and intranasal cocaine need to penetrate mucus membranes the thickness of which varies in women during the course of the menstrual cycle (personal communication, Scott Lukas, 2000).

Rodents also show sex differences in response to cocaine. The toxic effects of cocaine are sexually dimorphic; male rats developed a cardiovascular toxic reaction to cocaine at lower plasma concentrations of the drug than females (Morishima, H. O. et al., 1993). Female rats show an exaggerated behavioral response to both acute and chronic

cocaine administration (Van Haaren, F. & Meyer, M. E., 1991; Caihol, S. & Morméde, P., 1999; Sircar, R. & Kim, D., 1999; Chin, J. et al., 2000a). Female rats also displayed increased behavioral responses to acute cocaine and show a more robust and longer lasting sensitization in response to repeated cocaine administration than males (Van Haaren, F. & Meyer, M. E., 1991; Caihol, S. & Morméde, P., 1999; Sircar, R. & Kim, D., 1999; Chin, J. et al., 2000a). Furthermore, female rats acquire cocaine discrimination at a faster rate than males (Craft, R. M. & Stratmann, J. A., 1996) and also display an increased motivation to self-administer cocaine on a progressive ratio reinforcement schedule than males (Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989). Additionally, reinstatement of extinguished cocaine-reinforced responding was greater in female rats than in males and this effect occurred after a lower priming dose of cocaine in females (Lynch, W. J. & Carroll, M. E., 2000). Thus, overall, female rats have either an augmented or exaggerated response to cocaine-induced behavioral activation.

Sex differences in the metabolism of cocaine metabolism:

Cocaine is metabolized into two major metabolites, benzoylecgonine and ecgonine methyl ester, by plasma and liver cholinesterases (Johanson, C. E. & Fischman, M. W., 1989). Ecgonine methyl ester is formed by the action of serum and tissue esterases (Warner, A. & Norman, A. B., 2000). Benzoylecgonine is formed both nonenzymatically as well as through the action of esterases (Warner, A. & Norman, A. B., 2000) (Figure 3).

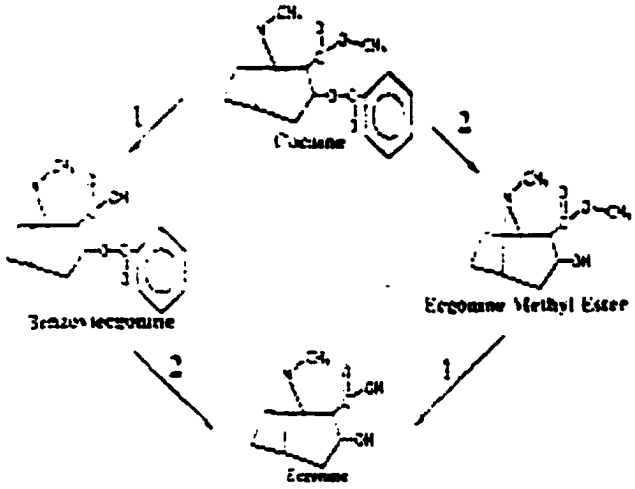


Figure 3. Schematic representation of the metabolism of cocaine.

Cholinesterase activity is affected by age, sex, and oral contraceptive use (Sidell, F. R. & Kaminskis, A., 1975). Cholinesterase activity is greater in men than women under the age of 60 and lower in females taking oral contraceptives (Sidell, F. R. & Kaminskis, A., 1975). Thus, it has been postulated that male and female rats (Zhang, J., Dean, R. A., Brzezinski, M. R., & Bosron, W. F., 1996) and humans (Sidell, F. R. & Kaminskis, A., 1975) also metabolize cocaine differently. In rats, Bowman et al., (Bowman, B. et al., 1999) reported that in response to acute cocaine administration, female rats had higher brain and plasma levels of the metabolite ecgonine methyl esterase, while males had higher levels of benzoylecgonine. However, although in our lab we also observed gender differences in benzoylecgonine levels after acute cocaine administration, female rats had higher levels of benzoylecgonine than males (Chin, J. et al., 2000a). These sex differences in levels of cocaine metabolites may be related to differences in the content and activity of the liver enzymes, cocaine methyl esterase and

ethyl transferase, in male versus female rats (Zhang, J., Dean, R. A., Brzezinski, M. R., & Bosron, W. F., 1996).

Sex differences in the HPA response to cocaine:

Hypothalamic-pituitary-adrenal (HPA) axis activation regulates the stress response. Briefly, the release of corticotropin releasing hormone from the hypothalamus causes the release of ACTH from the pituitary, which in turn causes the adrenals to secrete corticosterone (Figure 4). Corticosterone serves as a feedback mechanism in the hypothalamus and other brain areas.

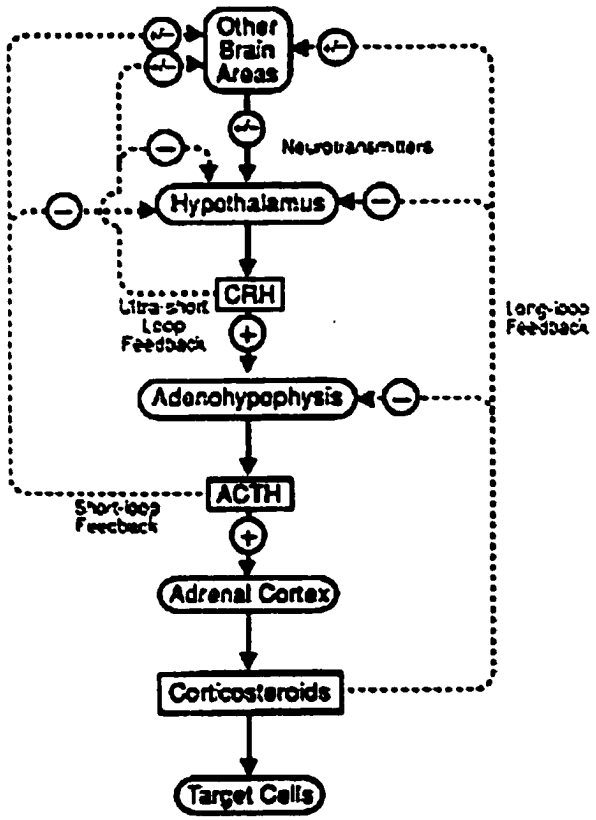


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Figure 4. The hypothalamic-pituitary-adrenal axis.

Cocaine causes activation of the hypothalamic-pituitary-adrenal (HPA) axis, which is characterized by increases in adrenocorticotropic releasing hormone (ACTH) and corticosterone plasma levels (Moldow, R. L. & Fischman, A. J., 1987). Increases in corticosterone levels have been observed in male rats after acute and chronic "binge" pattern cocaine administration (Spangler, R., Zhou, Y., Schlussman, S. D., Ho, A., & Kreek, M. J., 1997; Romualdi, P., Donatini, A., Izenwasser, S., Cox, B. M., & Ferri, S., 1996; Sarnyai, Z., Dhabhar, F. S., McEwen, B. S., & Kreek, M. J, 1998).

Recently, sex differences have been observed in cocaine-induced activation of the HPA axis. Kuhn and Francis (Kuhn, C. & Francis, M. S., 1997) reported that female rats have a greater ACTH response to acute cocaine than males. Similarly, we observed that female rats had higher cocaine-induced corticosterone plasma levels in response to both a single dose and challenge dose of cocaine when compared to male rats (Chin, J. et al., 2000a). Ovariectomy decreased the cocaine-induced ACTH response in females, while castration had no effect on the male ACTH response to cocaine (Kuhn, C. & Francis, M. S., 1997); suggesting that the endocrinological profile of the female may affect the neurotransmitter systems responsible for initiating the HPA response to cocaine. It has been postulated that this exaggerated HPA response in females contributes to the gender differences in response to cocaine and is probably due, in part, to the differences in the endocrine profiles between both sexes. Additionally, stage of the estrous cycle has also been shown to influence cocaine-stimulated ACTH and corticosterone secretion in female rats (Atkinson, H. C. & Waddell, B. J., 1997). Cocaine-induced secretion of corticosterone and ACTH was greatest in rats on proestrus when compared to those on

metestrus and diestrus (Falk, J. L., Fang, M. A., & Lau, C. E, 1991). Taken together, these results suggest that both the higher level of HPA activation in females as well as their endocrinological profile at the time of cocaine administration are important in the resulting behavioral response to the drug.

Hormonal regulation of the female reproductive cycle:

Females have a complex reproductive cycle (Figures 5 and 6). This may underlie gender differences in response to cocaine. The female hypothalamic-pituitary-gonadal system is responsible for regulation of events that occur through the cycle (Figure 4). Hypothalamic gonadotropin releasing hormone (GnRH), which is regulated by a plethora of neurotransmitters and neuropeptides, modulates tonic or basal secretion of luteinizing and follicle stimulating hormones (Brown, R. E., 1994). In the female rat, GnRH cells of the anterior hypothalamic area stimulate the preovulatory surge of luteinizing hormone (LH) in response to positive feedback from ovarian estrogen (Brown, R. E., 1994). LH stimulates ovulation and the formation of progesterone-secreting cells (corpora luteal) (Brown, R. E., 1994). While follicle-stimulating hormone (FSH) stimulates the growth of the follicle it also promotes the development of the ova and secretion of estrogen (Brown, R. E., 1994).

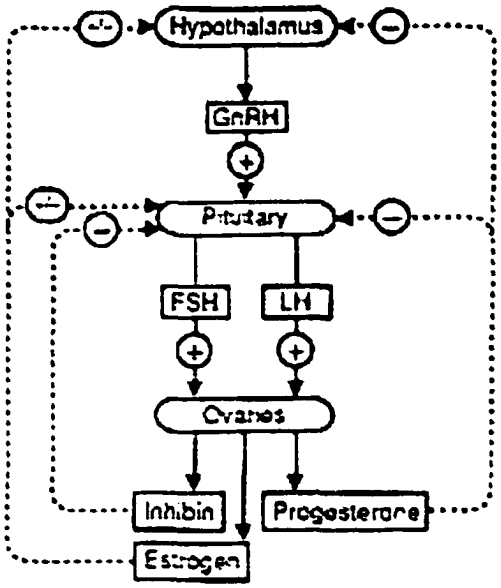

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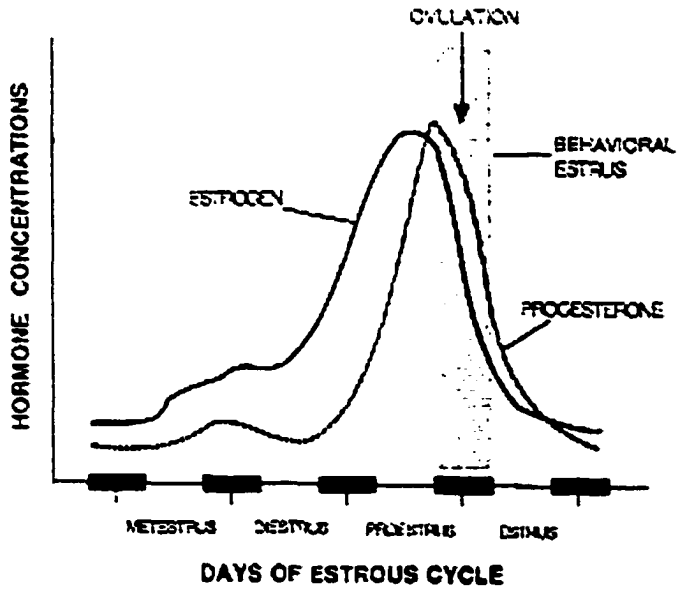
Figure 5. The female hypothalamic-pituitary-gonadal system.

As the follicle matures and develops, pituitary LH and FSH induce the ovary to begin gametogenesis (Carter, C. S., 1993). The maturing follicle nurtures the egg and produces estrogen, and it is during this stage that estradiol levels rise steadily reaching a peak during the late follicular phase (Carter, C. S., 1993). During this phase, progesterone levels are low. During the periovulatory phase, the egg increases its rate of estrogen secretion (Carter, C. S., 1993). It is during the ovulatory phase that levels of estrogen are highest and levels of progesterone are beginning to increase (Figure 6). The increase in estrogen as a result of secretion from the egg induces the hypothalamus to release GnRH causing the pituitary to release LH (Carter, C. S., 1993). This pulse of LH causes the follicle to rupture causing ovulation and a surge of progesterone from ovaries (Carter, C. S., 1993). During the luteal phase the corpus luteum secretes progesterone (and some estrogen), which aids in implantation of the egg in uterine wall (Carter, C. S., 1993).

In humans, the menstrual cycle ranges from 28-30 days. The rat four-day estrous cycle is characterized by four phases (Figure 6). Although the cycle is shorter than in humans, it closely resembles the endocrinological changes seen in humans. The first phase, metestrus, is a day of reduced hormonal and behavioral activity (Carter, C. S., 1993). Diestrus is characterized by the onset of follicular activity and estrogen secretion (Carter, C. S., 1993). During this phase estrogen levels begin to rise while progesterone levels remain low. In proestrus, estrogen and progesterone levels peak while GnRH and LH surges occur that trigger ovulation (Carter, C. S., 1993). Levels of estrogen and progesterone fall during the estrus phase of the cycle (Carter, C. S., 1993).



Reprinted (Carter, C. S., 1993)

Figure 6. Relative concentrations of ovarian hormones in serum during the estrous cycle of the female rat.

Effects of cocaine on gonadal hormones:

In rats, cocaine administration increased progesterone plasma levels in intact and pregnant female (Quiñones-Jenab, V et al., 2000; Quiñones-Jenab, V, Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 2000) and male rats (Quiñones-Jenab, V, Zhou, Y., Jenab, S., Ho, A., & Kreek, M. J, 2000; Kuhn, C. M., Francis, R. S., & Walker, Q. D., 1999). However, cocaine treatment in ovariectomized animals given progesterone replacement did not alter progesterone levels (Quiñones-Jenab, V et al., 2000). Thus, increases in progesterone plasma levels in intact rats were probably due to an increase in secretion rates of progesterone rather than an acceleration of its biotransformation. Very little is known about the effects of cocaine on estrogen levels. However, a recent study reported that acute cocaine administration increased plasma estradiol levels in rhesus monkeys in the follicular phase of their menstrual cycle (Mello, N. K., Mendelson, J. H., Kelly, M., & Bowen, C. A., 2000).

Because estrogen and progesterone levels fluctuate during the estrous cycle, it is possible that ovarian hormones modulate cocaine's effects in the CNS. This modulation may both underlie differences during the estrous cycle and play a role in the gender differences observed in neurobiological effects of cocaine.

Cocaine effects on luteinizing and follicle stimulating hormones:

Cocaine caused increases in lutenizing hormone (LH) and decreases in prolactin levels in monkeys during the midluteal phase of the cycle (Mello, N. K., Sarnyai, Z., Mendelson, J. H., Drieze, J. M., & Kelly, M., 1993). Interestingly, in monkeys studied

during the follicular phase of the menstrual cycle, cocaine also decreased prolactin, but did not affect LH levels (Mello, N. K., Mendelson, J. H., Drieze, J. M., & Kelly, M., 1990). Further, administration of a synthetic luteinizing hormone releasing factor prior to cocaine administration caused increases in LH and decreases in peak plasma cocaine levels (Mendelson, J. H., Mello, N. K., & Negus, S. S., 1999). Additionally, midfollicular phase Rhesus monkeys had increases in estradiol but not progesterone levels in response to acute cocaine administration (Mello, N. K., Mendelson, J. H., Kelly, M., & Bowen, C. A., 2000). Little is known about the effects of cocaine on LH and FSH levels in rats.

Estrous/menstrual cycle differences in the response to cocaine:

Cocaine-induced physiological and behavioral effects are significantly different in females during different stages of their reproductive cycles. Human females self-administering (smoked or intranasal) cocaine in the follicular phase, exhibit higher peak plasma cocaine levels than women during the luteal phase of the cycle (Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996; Sofuoglu, M., Duidish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K, 1999). Cocaine also affects duration and phases of the menstrual cycle, possibly through its interactions with fluctuating ovarian hormones or through direct action on hypothalamic transmitters regulating LH secretion. Rhesus monkeys self-administering cocaine exhibited menstrual cycles of abnormal duration and some animals exhibited anovulatory menstrual cycles with low mid-luteal progesterone levels (Mello, N. K., Mendelson, J. H., Kelly, M., Diaz-Migoyo, N., & Sholar, J. W., 1997). Similar to its effects on human and non-human primates, cocaine has been reported to affect the estrous cycle of rodents. King et al.,

(King, T. S., Schenken, R. S., Kang, I. S., Javors, M. A., & Riehl, R. M., 1990) found that within seven days of cocaine treatment female rats exhibited estrous cycle irregularities, characterized by repetitive days of estrus, absence of proestrus and prolonged periods of diestrus, as well as decreased ovulation rates during estrus (King, T. S., Schenken, R. S., Kang, I. S., Javors, M. A., & Riehl, R. M., 1990). Further, this chronic cocaine treatment decreased luteinizing hormone secretion in rats on proestrus (King, T. S., Schenken, R. S., Kang, I. S., Javors, M. A., & Riehl, R. M., 1990). However, a recent report demonstrates that chronic cocaine administration did not interfere with normal estrous cyclicity (Booze, R. M., Wood, M. L., Welch, M. A., Berry, S., & Mactutus, C. F., 1999). This issue of disruption of reproductive cycle activity is in need of much further investigation as cocaine-abusing human females can and do often become pregnant (thus, have the ability to ovulate).

In rats, the estrous cycle also influences an animal's motivation to self-administer cocaine. Rats in estrus demonstrate an increased motivation to self-administer cocaine compared to rats at other stages of the cycle (Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989), indicating that endocrinological changes occurring during estrous affect cocaine reinforcement.

The estrous cycle also affects both cocaine-induced stereotypic and locomotor behavioral activities. Rats in estrus demonstrate higher incidences of cocaine-induced stereotypic (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) and locomotor activity (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J.,

& Kreek, M. J., 1999; Sell, S. L., Scalzitti, J. M., Thomas, M. L., & Cunningham, K. A., 2000) than rats during diestrus. After "binge" pattern cocaine administration animals in estrus exhibited more stereotypic behaviors than animals in metestrus/diestrus following the first injection, but not the second injection (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). Interestingly, plasma levels of the cocaine metabolite benzoylecgonine during metestrus/diestrus were significantly higher than during estrus and proestrus, probably reflecting a more rapid biotransformation of cocaine at the metestrus/diestrus stage of the cycle.

Gonadectomy and steroid replacement effects on cocaine-induced alterations:

In order to avoid the hormonal fluctuations of the estrous cycle, ovariectomized female rats provide a model system to investigate gonadal influences on cocaine-induced alterations. Estrogen-treated ovariectomized rats demonstrated enhanced behavioral sensitization to cocaine (Peris, J., Decambre, N, Coleman-Hardee, M. L., & Simpkins, J. W., 1991). In contrast, Grimm & See (Grimm, J. W. & See, R. E., 1997) reported that estrogen-replacement treatment in ovariectomized rats decreased cocaine self-administration. Furthermore, Sell et. al, (Sell, S. L., Thomas, M. L., Clarke, C. H., & Cunningham, K. A., 1998) demonstrated that estrogen receptor antisense reduced locomotor hyperactivity in intact female rats in response to cocaine.

Progesterone has also been implicated in modulating cocaine-induced behavioral and neuroendocrinological alterations. It has been demonstrated that RU486, a progesterone antagonist, decreases cocaine toxicity in rats (Sharma, A., Plessinger, M.

A., Miller, R. K., & Woods, J. R., 1993; Glantz, J. C. & Woods, J. R., 1994). Furthermore, cocaine induces steroid-dependent reproductive behaviors in rats, which are blocked by RU486 (Apostolakis, M. E., Garai, J., Clark, J. H., & O'Malley, B. W., 1996). However, the direct effects of progesterone in CNS response to cocaine are unclear.

The co-administration of estrogen+progesterone augmented locomotor behavioral sensitization to repeated cocaine administration (Sircar, R. & Kim, D., 1999). Interestingly, this same hormone treatment paradigm caused suppression of cocaine-induced increases in response to the first injection of "binge" pattern cocaine administration and an enhancement of total locomotor activity after the second and third injections (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). Thus, the administration of estrogen, progesterone or estrogen+progesterone may regulate cocaine-induced alterations differently.

Effects of ovarian steroids and estrous cycle variations on monoamine systems:

The basis of the differential modulation of ovarian hormones in cocaine-induced behavioral activation may reside in the regulation of the monoamine systems by these gonadal hormones. Ovarian hormones modulate dopamine system activity (Di Paolo, T., Poyet, P., & Labrie, F., 1982; Di Paolo, T., Carmichael, R., Labrie, F., & Raymond, J. P., 1979; Hruska, R. E., 1986; Beigon, A., Fischette, C. T., Rainbow, T. C., & McEwen, B. S., 1983; Crowley, W. R., 1982; Di Paolo, T., Poyet, P., & Labrie, F., 1981; Hruska, R. E. & Pitman, K. T., 1982; Goetz, C. et al., 1983; Jori, A. & Cecchetti, G., 1973), which has been shown to vary throughout the estrous cycle (Morissette, M. & Di Paolo, T.,

1993; Jori, A. & Cecchetti, G., 1973; Cabrera, R., Diaz, A, Pinter, A., & Belmar, J., 1993; Crowley, W. R., O'Donohue, T. L., & Jacobowith, D. M., 1978; Fludder, J. M. & Tonge, S. R., 1975; Carr, L. A. & Voogt, J. L., 1980; Davis, C. F., Davis, B. F., & Halaris, A. E., 1977). Dopamine is postulated to be one of the major neurotransmitters responsible for proceptive motor behaviors exhibited by female rats on estrus such as ear wiggling, hopping, and darting (Becker, J. B. & Beer, M. E., 1986). In addition, dopaminergic neurotransmission and the frequency of the occurrence of dopamine-dependent behaviors vary throughout the estrous cycle (Becker, J. B. & Ramirez, V. D., 1981; Hruska, R. E., 1986; Kazandjian, A., Spyraki, C., Sfikakis, A, & Varonos, D. D., 1987; Castner, S. A., Xiao, L., & Becker, J. B., 1993; Jori, A. & Cecchetti, G., 1973; Crowley, W. R., O'Donohue, T. L., & Jacobowith, D. M., 1978). Animals during estrus show greater locomotor activity and greater intensity of stereotyped behaviors than rats at other stages of the cycle (Becker, J. B. & Cha, J, 1989). Estrous cycle variations also occur in striatal dopamine metabolite, DOPAC, concentration (Jori, A. & Cecchetti, G., 1973) and striatal dopamine concentration (Crowley, W. R., O'Donohue, T. L., & Jacobowith, D. M., 1978). Dopamine binding sites fluctuate during the estrous cycle; i.e. striatal postsynaptic dopamine receptors increase and presynaptic activity decreases during diestrus. Furthermore, Morissett & Di Paolo (Morissette, M. & Di Paolo, T., 1993) showed that striatal dopamine levels were higher for rats during the morning of proestrus, when estrogen levels are at their highest, compared to rats at other stages of the cycle. Rats on late proestrus or early estrus exhibit a greater behavioral response to drugs that stimulate the dopamine system (Becker, J. B. & Cha, J, 1989). It has also been suggested that dopamine release in the striatum is controlled by progesterone (Cabrera, R., Diaz, A,

Pinter, A., & Belmar, J., 1993). However, the modulation by progesterone seems to be bimodal (Cabrera, R., Diaz, A, Pinter, A., & Belmar, J., 1993).

Ovarian hormones have been shown to modulate both the striatal and mesolimbic dopaminergic systems in rats. Ovariectomy decreases dopamine activated drug-induced rotational behavior and decreases presynaptic dopamine release (Becker, J. B. & Beer, M. E., 1986; Becker, J. B., 1990a). It has been suggested that estrogen acts directly on the striatum to affect dopamine release (Becker, J. B., 1990b), as estrogen treatment results in increased striatal dopamine turnover rates (Becker, J. B., 1990a; Hruska, R. E. & Pitman, K. T., 1982; Hruska, R. E. & Silbergeld, E. K., 1980). Estrogen treatment of ovariectomized animals enhances amphetamine-induced striatal dopamine turnover and increases dopamine release (Becker, J. B., 1990b; Di Paolo, T., Rouillard, G., & Bedard, P., 1985; Becker, J. B. & Beer, M. E., 1986; Peris, J., Decambre, N, Coleman-Hardee, M. L., & Simpkins, J. W., 1991).

The possible regulation of the dopaminergic system by ovarian steroids has important implications for the sex differences seen in response to cocaine and other drugs of abuse. It is possible that the dopamine system, which has been shown to be important for cocaine-rewarding effects, may also be affected differentially during the different stages of the estrous cycle. Overall, these studies suggest that gonadal hormones could influence the activity of psychoactive drugs through monoamine systems. Due to the modulation of dopamine and serotonin levels by estrogen and progesterone, cocaine-

induced behavioral activity may be augmented or attenuated in the presence of ovarian hormones.

Serotonin levels have also been shown to fluctuate in various brain regions throughout the cycle (Luine, V. N., 1993). Further striatal serotonin levels peak during the morning of proestrus (Morissette, M. & Di Paolo, T., 1993). One investigation reported that male rats had higher striatal serotonin levels than females; this difference was abolished following amphetamine administration (Camp, D. M. & Robinson, T. E., 1988). Little is known about the interactions between cocaine and sex and/or ovarian hormones on the striatal serotonergic system.

Estrogen and progesterone modulation on the opioid system:

As previously described, the cocaine also affects components of the endogenous opioid system. There are male/female differences in the distribution of and levels of opioid peptides and receptors (Romano, G. J., Mobbs, C. V., Lauber, A. H., Howells, R. D., & Pfaff, D. W., 1990; Hammer, R. P., 1990; McCabe, J. T. & Pfaff, D. W., 1989). Similar to the dopaminergic system, the opioid system has reported to be modulated by ovarian steroids. For example, opioid receptors have been shown to be sensitive to estrogen treatment. Increased opioid binding has been reported in the hypothalamic homogenate of ovariectomized rats exposed chronically to physiological levels of estrogen compared to non-hormone treated ovariectomized animals (McCabe, J. T. & Pfaff, D. W., 1989; Wilkinson, M., Bhanot, R., Wilkinson, D. A., & Brawer, J. R., 1983; Wilkinson, M., Brawer, J. R., & Wilkinson, D. A., 1985). However, short-term exposure

to estrogen and progesterone has been shown to result in either decreased preoptic area opiate binding (Weiland, N. G. & Wise, P. M., 1990) or an increased number of preoptic area μ-opioid receptors (Mateo, A. R., Hijazi, M., & Hammer, R. P., 1992). In mice, there is a region-specific loss of μ-opioid receptor labeling following long-term ovariectomy (Mobbs, C. V., Harlan, R. E., Burrous, M. R., & Pfaff, D. W., 1988). Thus, it has been hypothesized that estrogen directly or indirectly influences the density of μ-opioid receptors in the rostral forebrain of female mice and rats (Weiland, N. G. & Wise, P. M., 1990; Mateo, A. R., Hijazi, M., & Hammer, R. P., 1992; Mobbs, C. V., Harlan, R. E., Burrous, M. R., & Pfaff, D. W., 1988). The mechanism of regulation of μ-opioid receptors by estrogen is not yet completely understood. Quiñones-Jenab et. al (Quiñones-Jenab, V, Jenab, S., Ogawa, S., Inturrisi, C., & Pfaff, D. W., 1996; Quiñones-Jenab, V, Jenab, S., Ogawa, S., Inturrisi, C., & Pfaff, D. W., 1997) demonstrated that estrogen affects the μ- and δ-, but not κ-opioid receptors mRNA levels in areas implicated in the regulation of reproductive process of rodents.

Previous studies have shown an induction of preproenkephalin mRNA in female mice (Quiñones-Jenab, V, Ogawa, S., Jenab, S., & Pfaff, D. W., 1997) and rats (Romano, G. J., Mobbs, C. V., & Pfaff, D. W., 1989; Harlan, R. E., Shivers, B. D., Romano, G. J., Howells, R. D., & Pfaff, D. W., 1987; Romano, G. J., Harlan, R. E., Shivers, B. D., Howells, R. D., & Pfaff, D. W., 1988) by estrogen in areas of the mesolimbic system. Proopiomelanocortin levels are also induced in the medial preoptic area of the hypothalamus after estrogen replacement in ovariectomized animals (Hammer, R. P., Zhou, L., & Cheung, S., 1994). In contrast, preprodynorphin levels have been shown to

decrease after estrogen replacement in ovariectomized rats (Spaminato, S., Canossa, M., Campana, G., Carboni, L., & Bachetti, T., 1995; Wagner, E. J., Manzaneres, J., Moore, K. E., & Lookingland, K. J., 1994). Furthermore, mRNA levels of opioid peptides also change during the different stages of the estrous cycle (Funabashi, T., Brooks, P. J., & Pfaff, D. W., 1996). Thus, estrogen and progesterone have the potential to alter genomic transcription or mRNA stability of the opioid system. The interactions between progesterone, estrogen, and cocaine in the opioid system remain to be elucidated. Due to the tonic inhibition of the opioid system on the dopamine system, modulation of the opioid system by estrogen and progesterone may down- or up-regulate dopamine levels and thus affect dopamine availability.

Hypothesis and specific aims of the thesis:

The working hypothesis of this thesis is that there is an interaction between ovarian hormones and cocaine on the development of cocaine-induced behaviors and cocaine-induced genomic alterations in the opioid system. This interaction can affect the behavioral outcome in one of two ways; via rapid cellular alteration (i.e. neurotransmitter binding or release or second messenger activation) or by altering gene expression in the opioid system (see Figure 7). The goal of this research is to elucidate whether estrogen and progesterone affect cocaine-induced genomic alterations in the opioid system, which may consequently influence locomotor and stereotypic behaviors in female rats. The specific aims of the research presented here are:

Specific Aim 1: To determine the interaction(s) between steroids and cocaine on the development of cocaine-induced behaviors in ovariectomized female rats.

Specific Aim 2: To determine the interaction(s) between steroids and cocaine on cocaine-induced genomic alterations in the dynorphin system in ovariectomized female rats.

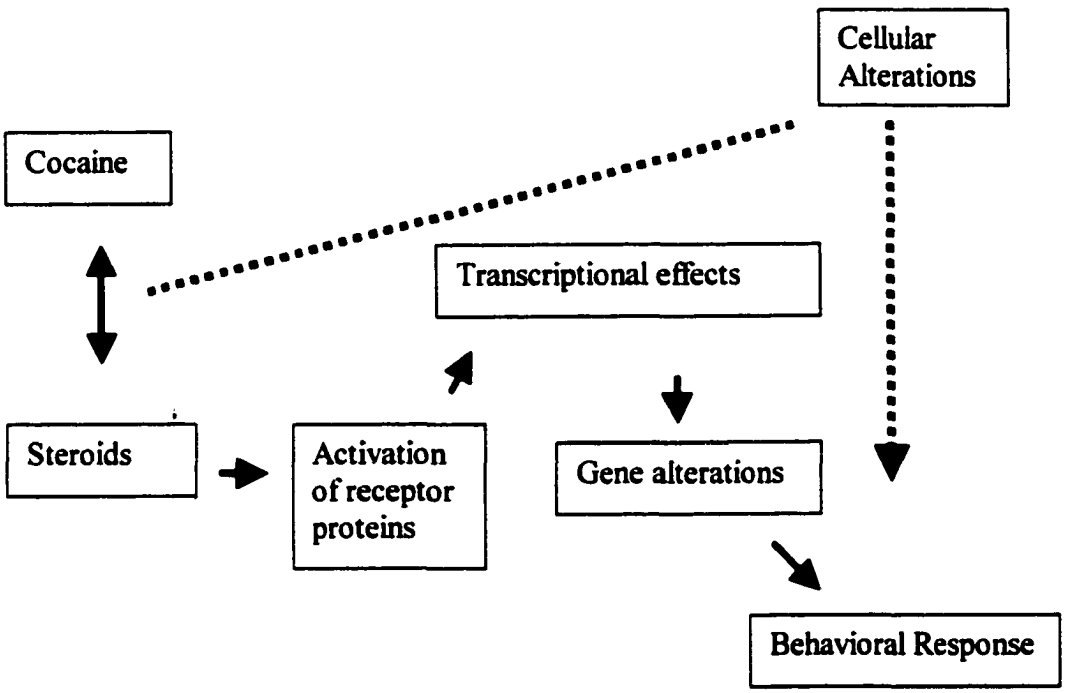


Figure 7. A model of the working hypothesis of this thesis. Cocaine and steroids interact to affect behaviors via either rapid cellular alterations (i.e. neurotransmitter release and/or binding) or through alterations at the level of the gene, which eventually affect protein levels. Both mechanisms have the ability to modulate dopamine and other neurotransmitters and further affect the behavioral response to cocaine.

CHAPTER 2: SEX DIFFERENCES IN COCAINE-INDUCED LOCOMOTOR AND STEREOTYPIC BEHAVIORS IN FISCHER RATS.

Evidence is accumulating which suggests that there are sex differences in the cocaine-induced alterations in humans and animals. For example, Kuhn and Francis (Kuhn, C. & Francis, M. S., 1997) reported sex differences in cocaine-induced HPA-axis activation in rats, where female rats showed exaggerated HPA responses when compared to male rats. Female rats also displayed greater hyperactivity (Post, R. M., Lockfeld, A., Squillace, K. M., & Contel, N. R., 1981), less toxicity to cocaine (Craft, R. M. & Stratmann, J. A., 1996), more intense cocaine-induced locomotor activity (Glick, S. D., Hinds, P. A., & Shapiro, R. M, 1983; Van Haaren, F. & Meyer, M. E., 1991), and markedly enhanced stereotypic behaviors (Walker, Q. D., Li, S., & Kuhn, C. M., 1997) when compared to male rats.

Behavioral sensitization to cocaine has been defined as a progressive increase in motor stimulation after repeated cocaine administration (Kalivas, P. W. & Stewart, J., 1991). Two different sensitization paradigms have been used in rats: a continued-chronic administration and a challenge dose of cocaine given following a period of withdrawal from chronic cocaine administration. In male rats, both protocols have been shown to produce sensitization (Robinson, T. E., Becker, J. B., & Presty, S. K., 1982; Kalivas, P. W. & Stewart, J., 1991). Although, female rats demonstrated higher levels of sensitization to repeated cocaine administration (Glick, S. D., Hinds, P. A., & Shapiro, R. M, 1983) and were sensitized with a lower dose of cocaine than male rats (Post, R. M., Lockfeld, A., Squillace, K. M., & Contel, N. R., 1981), it is not clear if there are sex

differences in psychomotor sensitization to cocaine using both sensitization protocols. To extend our understanding of sex differences in cocaine-induced alterations, this study was designed to determine how gender influences different components of locomotor activity (rearing, ambulations, total locomotion, and stereotypic behaviors) after acute, sub-chronic, chronic and challenge dose administration of cocaine.

Methods

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

Cocaine administration: Rats received daily i.p. injections of cocaine (15 mg/kg; dissolved in 0.9% saline) or saline for 1 day (acute; n=6), 14 days (chronic; n=12), or a challenge dose of cocaine [(n=6); after 14 days of saline or cocaine administration and 6 days of no handling rats received a single cocaine or saline injection]. All injections were administered in each rat's home cage.

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

Locomotor activity: The spontaneous locomotor activity of each rat was monitored with a Photobeam Activity System from San Diego Instruments (C.A.). The monitor consists of two frames in which the rat's home cage was placed. The lower frame recorded horizontal activity and the upper frame vertical activity. For total locomotor activity, the sum of all horizontal counts was used. Ambulatory activity was determined by counts produced by the interruptions of two consecutive photobeams on the lower frame. Rearing activity was determined by counts in interruptions of the upper frame. All locomotor activities were then subdivided into 5, six-minute time bins.

Stereotypic activity: On days 1, 7, 14, or after the challenge dose of cocaine or saline administration, rats were videotaped for 40 seconds at 15 and 30 minutes post-injection. The videotapes were later analyzed for behavioral stereotypy by three trained observers blind to each animal's treatment group. The rating for cocaine-induced stereotypic behaviors was based upon a modification (Daunais, J. B. & McGinty, J. F., 1995) of the Creese and Iversen (Creese, I. & Iversen, D., 1974) scale. This scale (summarized in Table 1) consists of 10 scores, ranging from a score of 1 (given to an animal that was asleep or inactive) to 10 (given to an animal that exhibited splayed hind limbs). A score of 10 was never observed during the course of this experiment.

Table 1. 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

Plasma levels of cocaine metabolite and corticosterone: Trunk blood was allowed to clot and then centrifuged 3,000 RPM for 15 minutes at 4° C. Plasma was collected and stored at -70 C until used. Samples were analyzed for benzoylecgonine and corticosterone with Coat-A-Count Radioimmunoassay (RIA) kits (Diagnostic Product Corporation, CA). Intra-assay coefficient of variance averaged less than 10%. Results were determined using a log-logit computer program.

Data analysis:

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

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

Plasma levels of cocaine metabolite and corticosterone: To examine the effects of acute (1 day), chronic (14 days), and challenge dose administration on plasma levels of benzoylecgonine and corticosterone, t-tests were used.

Results

Cocaine-induced total locomotor activity: There was significantly greater total locomotor activity in cocaine-treated rats than saline-treated control groups [$F(1,54) = 88.380$, $p<0.001$]. Furthermore, cocaine-treated female rats displayed higher total locomotor activity than male rats [$F(1,54) = 9.362$, $p<0.005$]. Across the length of drug treatment, there was a significant interaction between drug and day of cocaine administration [$F(3,162) = 2.775$, $p<0.05$]; cocaine-treated male rats displayed higher counts of total locomotor activity on Days 14 and 21 after the cocaine challenge when compared to Days 1 and 7 [Day 14: $p<0.05$; $p<0.005$; Day 21: $p<0.05$;$p<0.02$; respectively]. However, cocaine-treated female rats had significantly higher total locomotor activity only after cocaine challenge when compared to Days 1, 7 and 14 [$p<0.02$; $p<0.05$; $p<0.05$; respectively]. No differences were observed in the locomotor activity of female or male saline-treated rats ([$F(3,87) = 1.33$, $p>0.1$]; [$F(3,87) = 1.85$, $p>0.1$], respectively Figure 8).

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

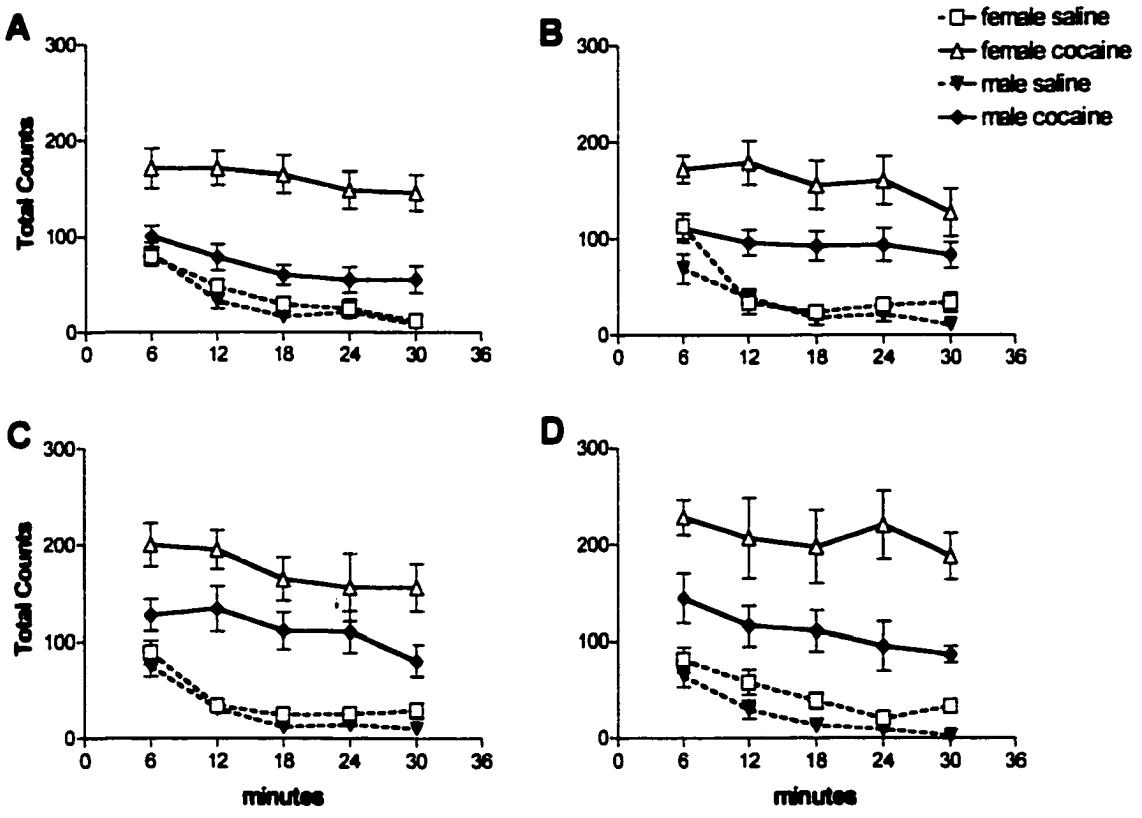


Figure 8. Total locomotor activity of male and female rats after (A) Day 1, (B) Day 7, (C) Day 14. And after a challenge dose of cocaine on (D) Day 21. n = 6 per group, except for Day 21 (n=3). All data represent a mean ± SEM of five six-minute time bins.

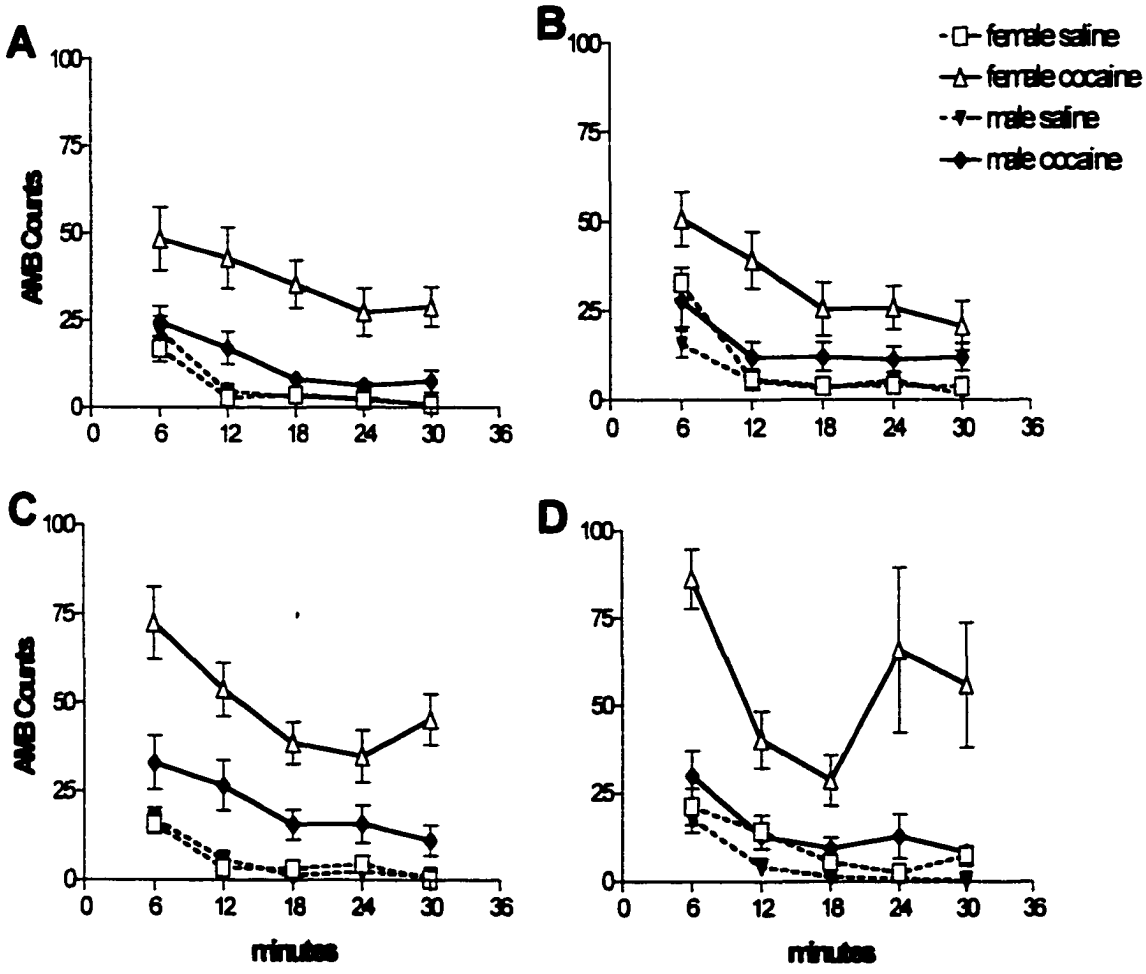


Figure 9. Ambulatory activity of male and female rats after (A) Day 1, (B) Day 7, (C) Day 14. And after a challenge dose of cocaine on (D) Day 21. n = 6 per group, except for Day 21 (n=3). All data represent a mean ± SEM of five six-minute time bins..

ambulatory activity during the first six minutes of cocaine administration after chronic and challenge dose of cocaine [$p<0.05$ for all analysis] when compared to 18 to 30 minutes of post injection (Figure 9).

Rearing Activity: Overall, cocaine-treated rats displayed more rearing activity than saline-treated rats [$F(1,106)=66.251$, $p<0.001$](Figure 10). Although cocaine-treated female rats reared more than male rats [$F(1,106)=4.327$, $p<0.05$], there were no statistically significant changes in rearing activity over time in both male and female rats [$F(3,318)=1.988$, $p>0.1$]. In female rats, similar to ambulatory activity rearing activity was significantly higher the first six minutes after the challenge dose of cocaine when compared to the rearing activity 18 to 30 minutes after cocaine administration [$p<0.05$].

Cocaine induction of stereotypic behaviors: On Day 1, saline-treated control female rats had statistically significant differences between 15 and 30 minutes scores of stereotypic behavior [Friedman ANOVA: [$H(1,N=46)=5.444$; $p<0.02$]. This could be due to stress-induced stereotypic activity after the first time of handling. Since no statistically significant changes were observed in any of the other treatment groups or any of the other treatment days, the sum of scores after 15 and 30 minutes was used for all groups.

Cocaine-treated rats had higher scores of stereotypic behavior than saline-treated controls [Day 1: H (1, N=48)=25.979, $p<0.001$; Day 7: H (1, N=48)= 24.968, $p<0.001$; Day 14: H (1, N=48)=24.913, $p<0.001$; Challenge dose: H (1, N=24)= 14.175, $p<0.001$]. When compared to male rats, cocaine-treated female rats had significantly higher

stereotypic scores after 1, 7 and 14 days of cocaine administration [Day 1: H (1, N=24)=6.857, p<0.01; Day 7: H (1, N=24)=4.133, p<0.05; Day 14: H (1, N=24)=8.767, p<0.005]. No statistically significant differences between male and female rats were observed in stereotypic activity after a cocaine challenge [H (1, N=12)=2.600, p>0.1]. Furthermore, across the length of cocaine administration, no differences in the stereotypic response to cocaine were observed in either female or male rats ([p>0.1]; Figure 11).

Gender differences in benzoylecgonine and corticosterone plasma levels: When compared to cocaine-treated male rats, female rats had higher levels of benzoylecgonine after acute cocaine administration [t=2.66, p<0.02], but not after chronic and challenge cocaine administration ([Day 14: t=0.122, p>0.2; Challenge dose: t=0.53, p>0.2, respectively]; Figure 12).

Overall, female rats had higher plasma levels of corticosterone than male rats [F(1,17) 6.689, p<0.001]. Cocaine-treated female rats had higher corticosterone plasma levels after acute and challenge cocaine treatments when compared to their respective saline-treated controls [Day 1: p<0.05 and Challenge dose: p<0.02]. No significant differences in corticosterone plasma levels were observed in male rats throughout the different cocaine administration paradigms ([p<0.1]; Figure 13).

Discussion

Similar to previous observations (Craft, R. M. & Stratmann, J. A., 1996; Glick, S. D., Hinds, P. A., & Shapiro, R. M, 1983; Kuhn, C. & Francis, M. S., 1997; Sircar, R. &

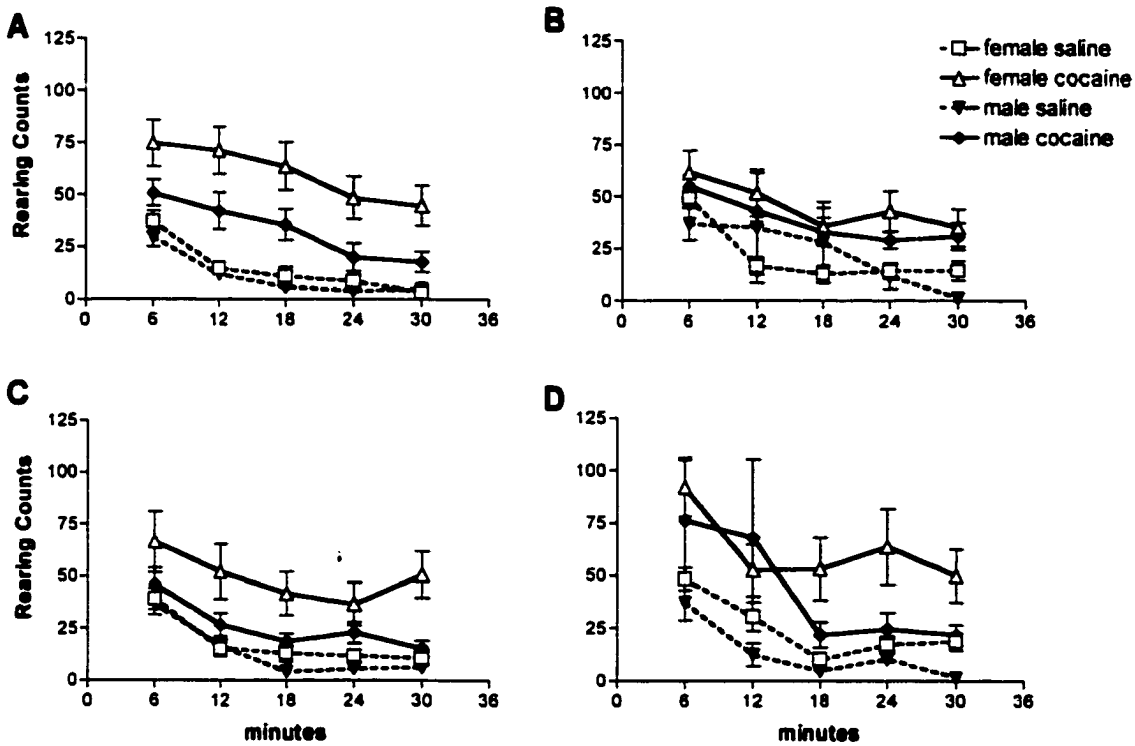


Figure 10. Rearing activity of male and female rats after (A) Day 1, (B) Day 7, (C) Day 14 and after a challenge dose of cocaine on (D) Day 21. n = 6 per group, except for Day 21 (n=3). All data represent a cumulative mean ± SEM of five six-minute time bins.

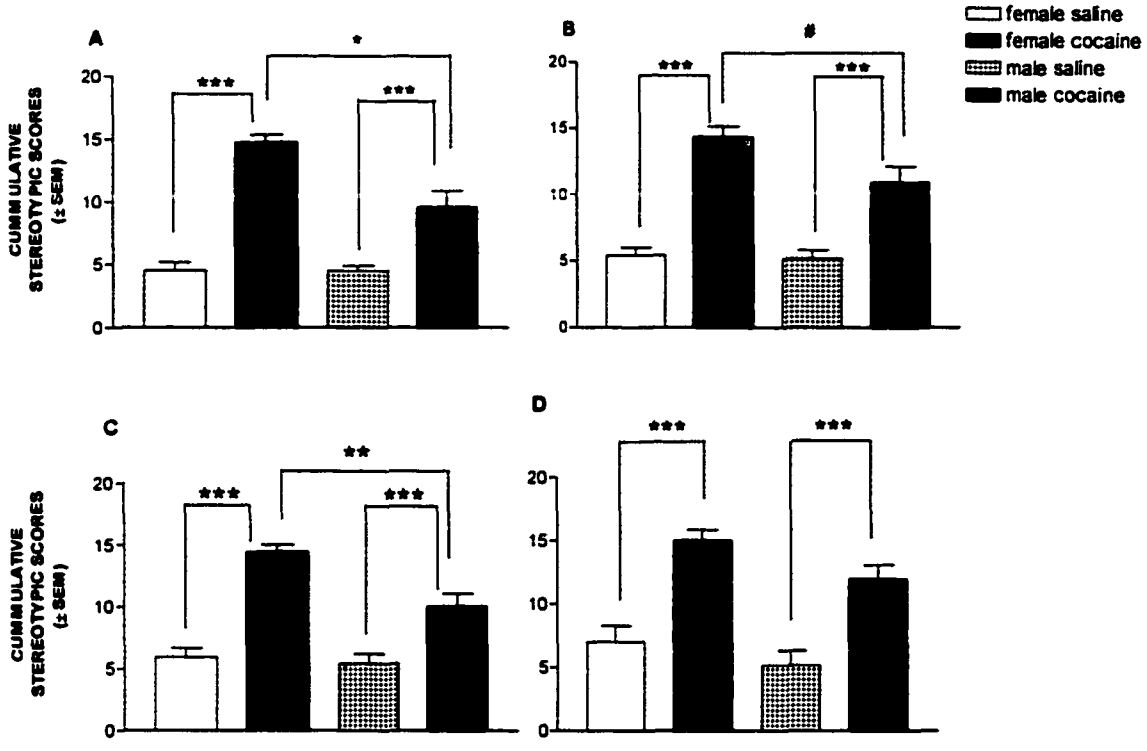


Figure 11. Cumulative scores of stereotypic behaviors for male and female rats after Day 1(A), Day 7 (B), Day 14 (C), and after a challenge dose of cocaine on Day 21 (D). N = 6 per group except for Day 21 (n=3). All data represent the cumulative of 15 and 30 minute scores + SEM. (# $p<0.05$; *$p<0.01$; ** $p<0.005$; $p<0.001$)

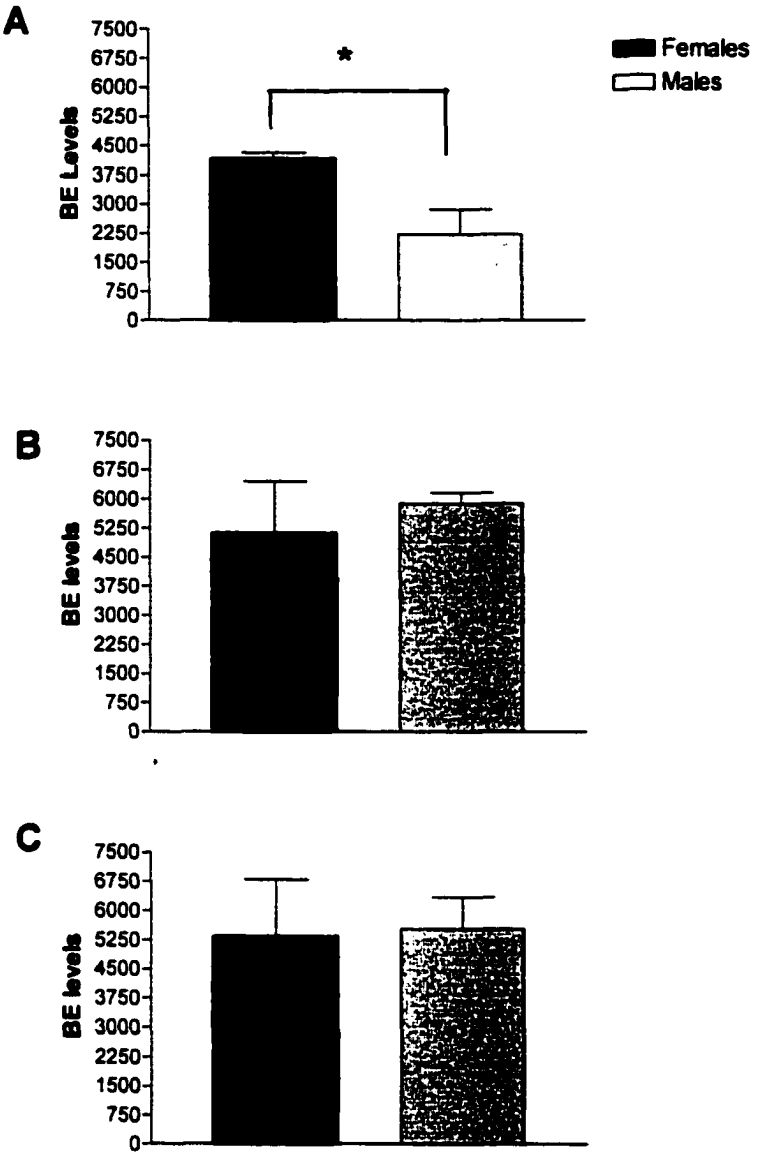


Figure 12. Mean (+SEM) levels of benzoylecgonine measured in ng/ml for cocaine-treated female and male rats after (A) acute or (B) chronic cocaine administration or (C) after a challenge dose of cocaine. *$p<0.05$.

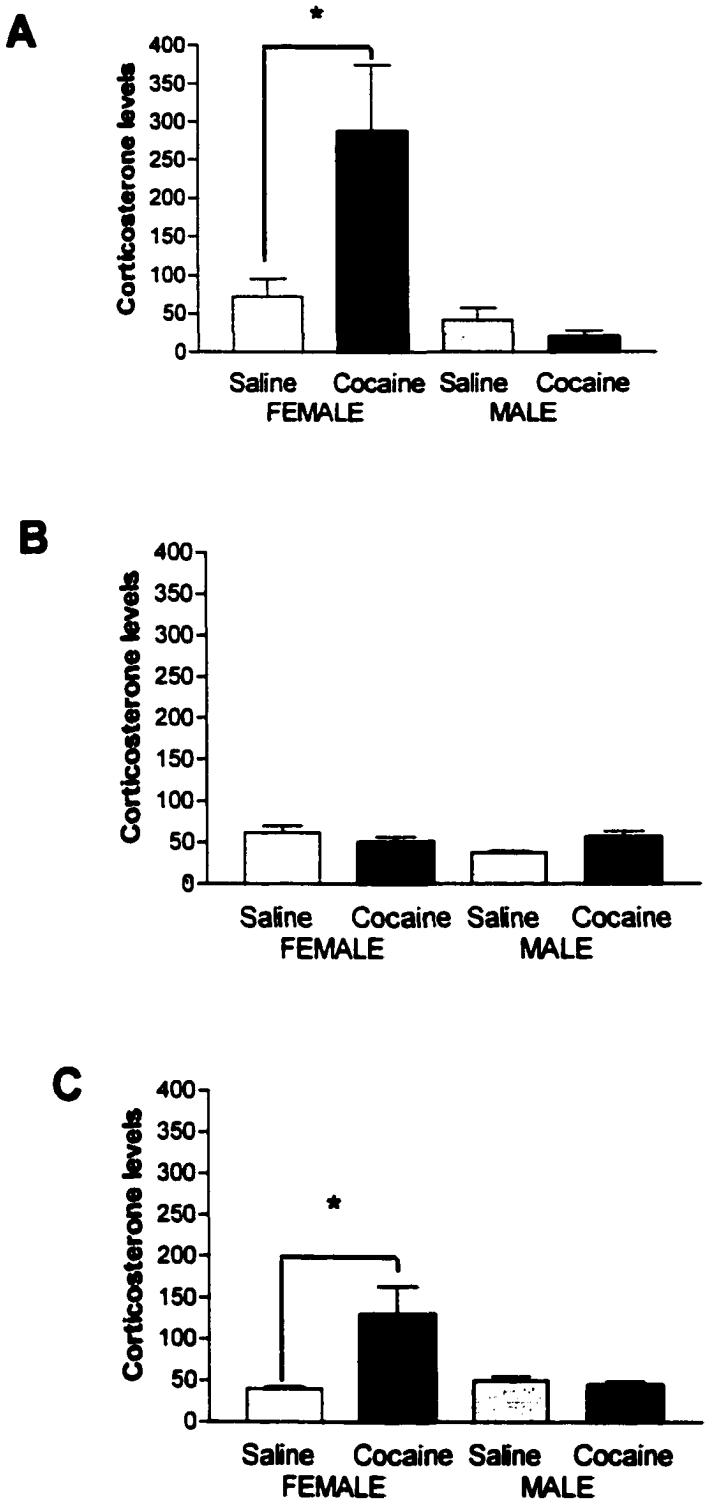


Figure 13. Mean (+ SEM) levels of corticosterone measured in ng/ml for female and male rats after (A) acute or (B) chronic cocaine administration, or (C) following a challenge dose of cocaine. *$p<0.05$

Kim, D., 1999), male and female rats had different psychomotor responses to cocaine. Moreover, female rats had significantly higher ambulatory and total counts after acute cocaine administration than male rats. Female rats also demonstrated higher rearing activity after acute cocaine administration than male rats. Thus, overall, after acute cocaine administration, female rats demonstrated higher hyperactivity in all the elements of the locomotor activity measured when compared to cocaine-treated males.

It has been previously demonstrated that there are sex differences in the development of sensitization. In this report, we observed the development of sensitization to cocaine-induced ambulatory and total locomotor activities after chronic cocaine administration in both male and female rats. Using a different cocaine administration paradigm to produce sensitization (chronic administration followed by a period of withdrawal and a challenge dose of cocaine), female rats demonstrated sensitization in ambulatory and total locomotor activity, while male rats demonstrated sensitization only in total locomotor activity. Furthermore, although overall rearing activity was not affected by repeated injections or a challenge dose of cocaine administration in both male and female rats, female rats reared more during the first 5 minutes after the challenge dose of cocaine on day 21. Taken together, this study suggests that there are sex differences in cocaine-induced sensitization on these different psychomotor responses. This may highlight differences in the pattern of chronic cocaine abuse between males and females.

Similar to Walker et al., (Walker, Q. D., Li, S., & Kuhn, C. M., 1997), we observed that female rats have markedly enhanced stereotypic behaviors in response to the first dose of cocaine than males. This study extends these observations by demonstrating that although, female rats' stereotypic response to acute chronic and sub-chronic cocaine administration was higher than male rats, no sensitization to cocaine-induced stereotypic activity was observed in either male or female rats.

The hypothalamic-pituitary-adrenal (HPA) axis activation has been postulated to be essential for the control of behavioral and neurochemical alterations by cocaine. For example, the manipulation of corticosterone levels influences locomotor responses to cocaine (Marinelli, M. et al., 1994; Marinelli, M. et al., 2000) as well as the development of sensitized responses following cocaine administration (Rough-Pont, F., Marinelli, M., Le Moal, M., Simon, H., & Piazza, P. V., 1995). Similar to observations by Kuhn and Francis (Kuhn, C. & Francis, M. S., 1997), female rats exhibit greater HPA activation than male rats after acute cocaine administration. Chronic cocaine administration did not alter corticosterone plasma levels in female rats, indicating the possible development of tolerance of HPA activity. However, a challenge dose of cocaine in sensitized female rats caused an increase in corticosterone levels; suggesting a desensitization of HPA activity after withdrawal of cocaine or a return to a hypersensitive HPA activity. Although, augmented responses in corticosterone plasma levels have been previously shown after challenge with cocaine in sensitized male rats (Marinelli, M. et al., 2000), we did not observe alterations in corticosterone levels in male rats over time. Discrepancies between both reports may reside in the strain of rat or cocaine administration paradigm

used. This remains to be elucidated. It is provocative to postulate that differences in HPA activity might underlie some mechanisms in which sex differences to cocaine induced psychomotor activity may occur.

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

lower BE serum levels than male rats. However, van Haaren et al., (Van Haaren, F. & Meyer, M. E., 1991), used a different dose and length of cocaine administration (22 days continuous, i.p., 10 mg/kg). Overall, sex differences in BE do not completely explain the exaggerated locomotor and stereotypic responses after the different cocaine administration paradigms, nor the difference in sensitization to the behavioral effects of cocaine of female rats. This may support the idea that differences in the endocrinological profiles of females versus males or HPA activity are more likely to underlie sex differences to cocaine-induced alterations than cocaine metabolism.

CHAPTER 3: COCAINE AFFECTS PROGESTERONE PLASMA LEVELS IN FEMALE RATS.

Cocaine, a psychostimulant, is one of the most widely abused drugs in Western countries. Based on the 1998 National Household Survey on Drug Abuse, approximately 36 percent of an estimated 1.75 million Americans who used cocaine in a month were women. Females have a complex endocrinological profile. Female hormones alter a variety of reproductive (Arnold, A. P. & Breedlove, S. M., 1985; Pfaff, D. W. & Schwartz-Giblin, S., 1995) and non-reproductive behaviors (Priest, C. A. & Pfaff, D. W., 1995) probably through their actions on the dopamine, serotonin, and opioid systems (Funabashi, T., Brooks, P. J., & Pfaff, D. W., 1996; Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996; Pfaus, J. & Pfaff, D. W., 1992; Di Paolo, T., Carmichael, R., Labrie, F., & Raymond, J. P., 1979). It is well established that estrogen and progesterone hormones function in the brain to regulate neuronal activity and influence behavior in females. Because these gonadal hormones have profound effects on brain function, the female's hormonal state at the time of cocaine administration may influence cocaine-induced behavioral and molecular alterations in brain function.

Several studies suggest that gonadal hormones influence the activities of psychoactive drugs on neuronal dopamine systems. For example, significant differences have been shown in females during the different stages of their reproductive cycle in response to cocaine and amphetamine administration. Women in the follicular phase of their menstrual cycle, given a single challenge dose of cocaine by nasal route of administration, have been reported to have higher peak plasma cocaine levels than during the luteal phase (Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996). However, Mendelson et al., (Mendelson, J. H. et al., 1999) reported that there are no gender or menstrual cycle differences in cocaine levels (area under the plasma concentration curve) of half-life in humans after intravenous administration. In rats, the

estrous cycle influences an animal's motivation to self-administer cocaine (Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989), the intensity of cocaine-induced stereotypic and locomotor activity (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) and dopamine release in the striatum (Becker, J. B. & Cha, J, 1989). Rats in estrus exhibit significantly higher behavioral responses to amphetamine than at other stages of the estrous cycle. Sensitivity to amphetamine has been reported to be augmented during estrus (Becker, J. B. & Cha, J, 1989; Quiñones-Jenab, V, Ogawa, S., Jenab, S., & Pfaff, D. W., 1997; Diaz-Veliz, G., Baeza, R., Benavente, F., Dussaubat, N., & Mora, S., 1994; Kazandjian, A., Spyraki, C., Sfikakis, A, & Varonos, D. D., 1987). Estrous cycle variations are also found in levels of striatal dopamine and its metabolite DOPAC (Jori, A. & Cecchetti, G., 1973; Crowley, W. R., O'Donohue, T. L., & Jacobowith, D. M., 1978) and in amphetamine-stimulated dopamine release (Becker, J. B. & Ramirez, V. D., 1981). Since estrogen and progesterone levels fluctuate during the estrous cycle, it is possible that ovarian hormones modulate cocaine's effects in the CNS. This modulation may both underlie differences during the estrous cycle, and play a role in the gender differences observed in neurobiological effects of cocaine.

Little is known of the possible interaction of progesterone with cocaine or other drugs of abuse. It has been demonstrated that RU486, a progesterone antagonist, decreases cocaine toxicity in rats (Sharma, A., Plessinger, M. A., Miller, R. K., & Woods, J. R., 1993; Glantz, J. C. & Woods, J. R., 1994). We have previously observed that cocaine increases progesterone plasma levels in pregnant rats (Quiñones-Jenab, V, Krey, L. C., Schlussman, S. D., Ho, A., & Kreek, M. J, 2000). Furthermore, cocaine induces lordotic behaviors in rats, which can be blocked by RU486 (Apostolakis, M. E., Garai, J., Clark, J. H., & O'Malley, B. W., 1996). Progesterone plasma levels are also increased after amphetamine treatment in male rats (Budziszewska, B., Jaworska-Feil, L., & Lason, W., 1996). Progesterone administration in OVX rats also affects stimulation of locomotor

response to amphetamine (Naik, R. S., Kelkar, M. R., & Sheth, U. K., 1978). Thus, the progesterone system may be an important component in the cascade of events following administration of cocaine or other psychostimulants.

This study was designed to determine the effects of cocaine on progesterone plasma levels in female rats. Elucidating the response to cocaine across the reproductive cycle of female rats may have implications for the prevention and treatment of substance abuse in humans. This study extends our understanding of differences between gender and across the estrous cycle in the CNS response to cocaine.

Methods

Animals:

Eight-week-old female Fischer rats were purchased from Charles River and were single-housed in a standard cage with free access to food and water, and maintained on a 12-hour light/dark cycle (lights on at 9:30 A.M.) in one room dedicated for this study of a suite of rooms of this laboratory, with access only by the experimenters.

Estrous cycle determinations:

For estrous cycle studies, animals were acclimated for 10 days before the start of the experiment with daily handling for approximately five minutes. Vaginal lavage (30 minutes after lights on) of each rat for 10 consecutive days was used to determine its stage of the estrous cycle, as previously described (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). Two separate cohorts (each with 24 animals) were studied. In the first cohort, rats were randomly assigned to either cocaine or saline treatment groups. In order to insure that there were enough animals in each stage of the estrous cycle, animals from the second cohort were assigned to cocaine or saline groups according to the smear from the previous day. Some animals (four in the cocaine-

treated group and six in the saline-treated group) did not exhibit a clear metestrus phase during the course of the experiment. In order to include these rats in the final data analysis, all the subjects in metestrus and diestrus were grouped together (metestrus/diestrus) for each drug treatment group. A total of three animals (one in the saline-treated group and two in the cocaine-treated group) could not be reliably staged. These animals were not included in any data analysis of the estrous cycle effects. The locomotor and stereotypic activity of animals used for estrous cycle determinations have been reported previously (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999).

For five days before cocaine or saline treatment, animals received three i.p. injections of 0.9% saline at one-hour intervals (starting 30 minutes after lights on) immediately following lavage. On the sixth day, animals received three hourly i.p. injections of cocaine (15 mg/kg of body weight dissolved in 0.9% saline at a concentration of 15 mg/ml) or 0.9% saline (1 ml/kg of body weight). This "binge" dosing schedule was chosen to mimic the manner in which cocaine is often self-administered by humans both in terms of temporal pattern and in relation to circadian rhythm and has been use in many studies (Branch, A. D., Unterwald, E. M., Lee, S. E., & Kreek, M. J., 1996; Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999; Quiñones-Jenab, V, Krey, L. C., Schlussman, S. D., Ho, A., & Kreek, M. J, 2000). Throughout the study all injections were give in the animal's home cage. Neither necrosis nor convulsions were expected or observed with this treatment of cocaine or saline injections.

Single injections in intact females:

Intact 8-week-old female rats were pre-treated for five days with "binge" pattern saline administration, one week after arrival in our animal facility. On the sixth day,

animals received a single injection of cocaine (15 mg/kg of body weight dissolved in 0.9% saline at a concentration of 15 mg/ml) or saline 30 minutes after lights on (n=5 per experimental group). This dose was the equivalent to one injection of the "binge" administration paradigm. Animals were sacrificed either 30 minutes or three hours after this single cocaine or saline administration.

OVX females:

Fourteen day post-OVX rats were pre-treated for five days with "binge" pattern saline administration. Forty-eight hours before cocaine administration, animals received either vehicle (sesame oil) or estrogen benzoate (50 μg, s.c.). Forty-four hours after estrogen administration, animals received 500 μg of progesterone or vehicle (sesame oil). Four hours after progesterone (48 hours after estrogen), animals received three i.p. injections of cocaine (15 mg/kg of body weight dissolved in 0.9% saline at a concentration of 15 mg/ml) one hour apart or 0.9% saline (1 ml/kg of body weight). These doses and the injection paradigm were chosen because they have been shown to induce reproductive behaviors in OVX rats (Lauber, A. H., Romano, G. J., Mobbs, C. V., Howells, R. D., & Pfaff, D. W., 1990). Thirty minutes after the last injection, animals were sacrificed and trunk blood was collected.

Plasma levels of progesterone:

Blood was allowed to clot and centrifuged at 3,000 RPM for 15 minutes at 40°C. Plasma was collected and stored at -40°C until analyzed by radioimmunoassay (RIA) for progesterone. Samples (100 μl) were analyzed with a RIA Kit (National Diagnostic; San Diego, CA) with internal standards containing known amounts of progesterone run to correct for extraction losses. Intra-assay coefficients of variation averaged 7.0± 1.0%. Results for these assays were determined using a log-logit computer program. Progesterone levels are expressed as ng/ml.

Data analysis:

To determine whether plasma levels were significantly different between cocaine or saline treatment groups in each hormonal replacement condition, t-tests were used. To examine the effects of the phase of the estrous cycle and steroid replacement on plasma levels of progesterone, an analysis of variance (ANOVA) was used, followed by Newman-Keuls *post hoc* tests. Significance in all cases was considered to be $p<0.05$.

Results

Progesterone plasma levels were significantly higher in intact female rats after acute "binge" pattern cocaine administration than saline-treated animals (t=6.49, df=42, p=.003, Figure 14).

In order to examine whether cocaine effects on progesterone plasma levels were due to a cumulative effect of cocaine after "binge" pattern administration, we examined cocaine effects on progesterone plasma levels after a single dose of cocaine, both 30 minutes and three hours after injection. Similar to what was found 30 minutes after "binge" pattern cocaine administration, 30 minutes after an acute single dose of cocaine (15 mg/kg), progesterone levels were significantly higher in cocaine-treated animals than controls (t=4.20, df=10, p=.002; Figure 15). However, 3 hours after a single cocaine administration, plasma progesterone levels had returned to levels comparable to those of the saline treated group (t=1.5, df=10, p=0.082; Figure 15). There were no differences between groups after saline injections.

When progesterone levels were analyzed according to estrous cycle stage, plasma levels of progesterone after cocaine administration varied significantly during the estrous

cycle ($F_{2,16}$=4.19, p=0.034). Progesterone plasma levels were significantly higher in cocaine-treated animals during proestrus than during estrus (p=0.028) and during proestrus just failed to be significantly higher than during metestrus/diestrus (p=0.054; Figure 16).

Administration of "binge" pattern cocaine did not alter progesterone plasma levels in OVX rats treated with progesterone or estrogen+ progesterone ($F_{1,20}$=0.067, p=0.680; Figure 17).

Discussion

We observed that acute-repetitive or single dose cocaine administration significantly increased plasma levels of progesterone in intact female rats. Cocaine modulation of progesterone plasma levels, therefore, does not depend on cumulative cocaine levels after "binge" pattern administration, since a single dose of cocaine significantly increased progesterone plasma levels in intact females. Three hours after an acute-single dose of cocaine, progesterone plasma levels returned to control levels. These results suggest that cocaine has an acute, short-lived effect on progesterone plasma levels.

We have previously shown that chronic "binge" cocaine administration doubled progesterone levels in pregnant rats (Quiñones-Jenab, V, Krey, L. C., Schlussman, S. D., Ho, A., & Kreek, M. J, 2000). The magnitude of the increase in progesterone levels in this study after acute and "binge" pattern cocaine administration is comparable to that seen in pregnant rats. Amphetamine administration has been shown to increase progesterone levels in male rats (Budziszewska, B., Jaworska-Feil, L., & Lason, W., 1996). Other reports have implicated progesterone in the modulation of the CNS response to drug exposure (Budziszewska, B., Jaworska-Feil, L., & Lason, W., 1996;

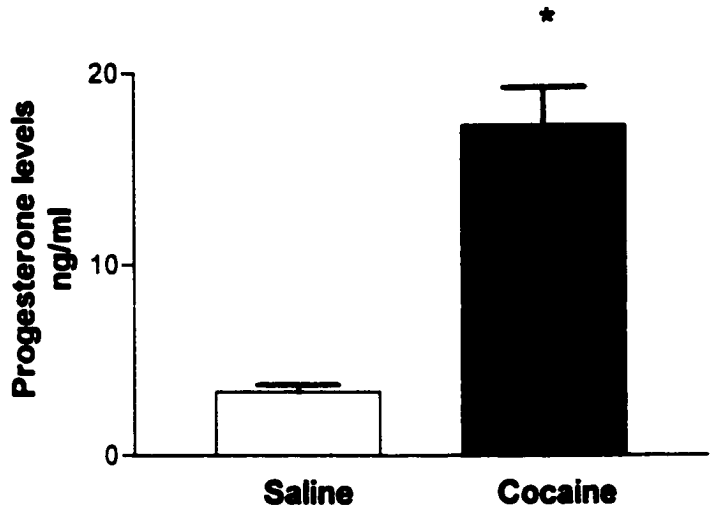


Figure 14: Mean (+ S.E.M.) Progesterone levels in female rats after "binge" pattern cocaine or saline administration. All animals (n=24/group) received 5 days of saline pretreatment, followed by one day of cocaine or saline administration (3 x 15 mg/kg/ 1 hour apart), p=.003.

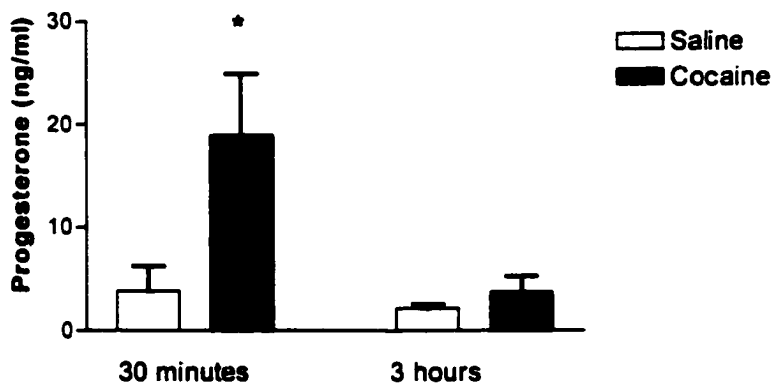


Figure 15: Effects on progesterone plasma levels of an acute administration of 15 mg/kg cocaine or saline in female rats. Animals receive a single i.p. injection and were sacrificed 30 minutes or three hours later (n=5/group); p<0.001.

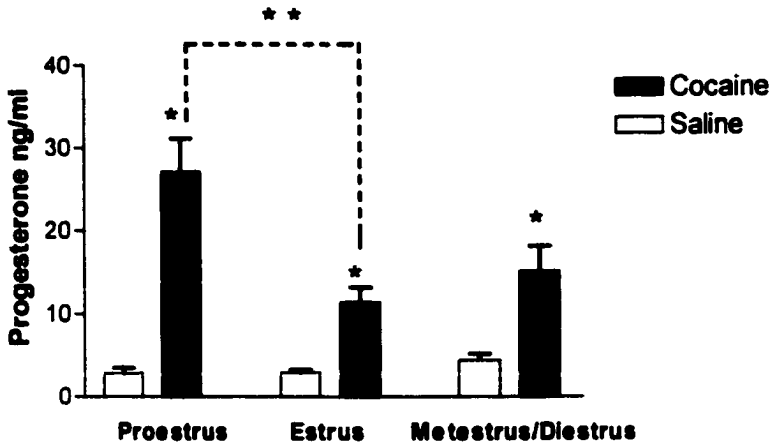


Figure 16: Progesterone levels in female rats after "binge" pattern cocaine or saline administration in different stages of the estrous cycle. The values represent mean + S.E.M. of the same females as Figure 14; *= cocaine vs. saline, $p<0.05$; **= Proestrus vs. Estrus; $p<0.03$ (n=6/group).

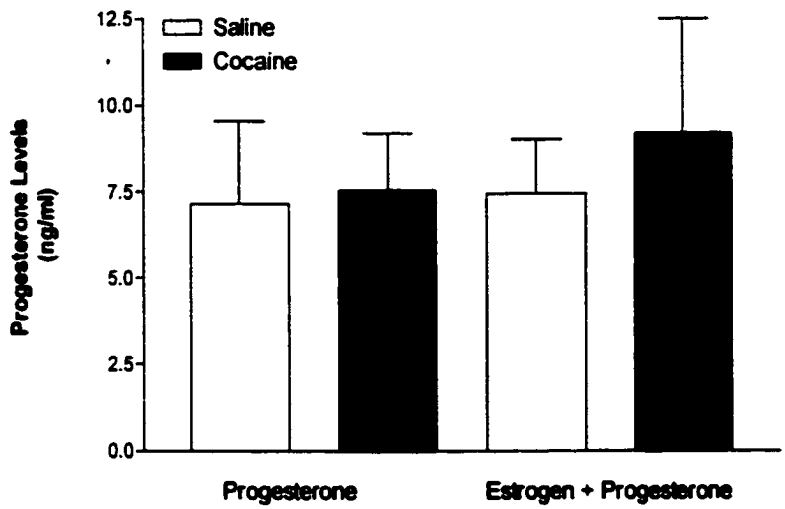


Figure 17: Mean (+S.E.M.) progesterone levels resulting from exogenous administration of steroids in female rats after binge pattern cocaine or saline administration with different steroid replacement treatments (n=6/group).

Glantz, J. C. & Woods, J. R., 1994; Quiñones-Jenab, V, Krey, L. C., Schlussman, S. D., Ho, A., & Kreek, M. J, 2000; Apostolakis, M. E., Garai, J., Clark, J. H., & O'Malley, B. W., 1996; Naik, R. S., Kelkar, M. R., & Sheth, U. K., 1978). Progesterone plays a major role in female reproductive functioning, including the control, reward and locomotor aspects of reproduction. It has been reported that cocaine interrupts the menstrual/estrous cycle in humans, rabbits, monkeys, and rats (Chen, C. & Vandenbergh, J. G., 1994; King, T. S., Schenken, R. S., Kang, I. S., Javors, M. A., & Riehl, R. M., 1990; Mello, N. K., Mendelson, J. H., Kelly, M., Diaz-Migoyo, N., & Sholar, J. W., 1997). Furthermore, cocaine can interrupt the progress of pregnancy and development of maternal behaviors in humans and animal models (154,64}. The modulation of progesterone plasma levels by cocaine may explain the profound effect of cocaine on different aspects of the female reproductive cycle, including effects on the menstrual cycle and pregnancy.

When progesterone levels were analyzed according to the stage of the estrous cycle, we observed cocaine-induced increases of progesterone levels in each stage of the rat's cycle. However, cocaine induction on progesterone plasma levels were even higher at proestrus (when progesterone levels are normally the lowest). Thus, the effect of cocaine on progesterone levels may be affected by the endocrinological profile of the female rat. The interruption or cessation of the menstrual/estrous cycle by cocaine in monkeys, rabbits, and rodents may be due to this disregulation of the plasma levels of progesterone (Chen, C. & Vandenbergh, J. G., 1994). The endocrinological profile of female rats also affects other aspects of cocaine responses, including cocaine-induced alterations in locomotor and stereotypic behaviors, and the levels of cocaine metabolites in the female rat (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999).

There are two major mechanisms whereby cocaine modulation of progesterone levels may occur. Cocaine may either stimulate progesterone secretion or it may affect the metabolism or biotransformation of this steroid. Since we did not observe higher levels of progesterone in OVX rats after co-administration of cocaine and "replacement" steroids, it is likely that the effect of cocaine is due to the secretion of progesterone rather than to its biotransformation and clearance. However, the mechanisms underlying these effects remain to be elucidated.

Results presented here suggest that the progesterone system is an important component in the cascade of events following the administration of cocaine or other psychostimulants in females. The modulation of progesterone plasma levels by cocaine may play an important role in the effects of cocaine on different aspects of the reproductive cycle. Of further clinical importance, cocaine may affect the progesterone levels of women utilizing progesterone based contraception or steroid replacement treatment after menopause. These important issues affecting women's health need further study.

CHAPTER 4: OVARIAN HORMONES AFFECT COCAINE-INDUCED BEHAVIORAL ACTIVITY IN OVX FEMALE RATS:

Cocaine is one of the most widely abused drugs in Western countries. Based on the 1998 National Household Survey on Drug Abuse, an estimated 1.75 million Americans used cocaine in a month's time, 36% of those users were female. Recent studies suggest that male and female humans and animals respond differently to different psychomotor stimulants (Robinson, T. E., Camp, D. M., Jacknow, D. S., & Becker, J. B., 1982; Robinson, T. E., Becker, J. B., & Presty, S. K., 1982; Craft, R. M. & Stratmann, J. A., 1996; Kuhn, C. & Francis, M. S., 1997). For example, Lukas, et al., (Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996) reported significant sex differences in response to acute cocaine administration in humans, with male participants achieving a faster and higher peak of plasma cocaine levels than females. Furthermore, men reported having experienced more episodes of euphoria than women (Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996). However, Mendelson et al., (Mendelson, J. H. et al., 1999) reported no gender or menstrual cycle differences in peak plasma cocaine levels. Rodents show behavioral sex differences in response to psychostimulants. Female rats display more intense behavioral responses to drugs of abuse than males (Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989), and have markedly enhanced stereotypic behaviors to their first cocaine dose and significantly higher locomotor activity than males (Craft, R. M. & Stratmann, J. A., 1996). Females also show less toxicity to cocaine than male rats (Craft, R. M. & Stratmann, J. A., 1996). Cocaine-induced stereotypic and locomotive behaviors have been shown to vary across the estrous cycle of the rat (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., &

Kreek, M. J., 1999). The fluctuating steroid hormone levels during the estrous cycle may play an important role in modulating the behavioral effects of cocaine. Furthermore, there may be an interaction between ovarian hormones and the behavioral effects of cocaine. These hormonal influences may contribute to the sex differences in the neurobiological effects of cocaine.

It is well established that estrogen and progesterone function in the brain to regulate neuronal activity and influence behavior. These hormones alter a variety of reproductive (Arnold, A. P. & Breedlove, S. M., 1985) and non-reproductive behaviors (Priest, C. A. & Pfaff, D. W., 1995) possibly through their actions on the dopamine, serotonin, and opioid systems (Funabashi, T., Brooks, P. J., & Pfaff, D. W., 1996; Lauber, A. H., Romano, G. J., Mobbs, C. V., Howells, R. D., & Pfaff, D. W., 1990; Romano, G. J., Mobbs, C. V., & Pfaff, D. W., 1989; Pfaus, J. & Pfaff, D. W., 1992; Di Paolo, T., Poyet, P., & Labrie, F., 1982; Di Paolo, T., Carmichael, R., Labrie, F., & Raymond, J. P., 1979). Due the modulating effects of estrogen and progesterone on the CNS, these hormones may be an integral part of the cascade of events that are involved in cocaine's actions in the CNS. However, it is not clear what role(s) each of these hormones play individually or in combination in cocaine-induced subjective and physiological alterations. The present study was conducted to understand how estrogen and progesterone affect cocaine-induced stereotypic and locomotor activities. We hypothesize that due to the profound effect of ovarian hormones in the CNS, these steroids may affect cocaine-induced alterations in behavior. The aim of this study is to test this postulate. Results from this study may help to explain previous observations of

gender and estrous cycle differences in response to cocaine (Craft, R. M. & Stratmann, J. A., 1996; Kuhn, C. & Francis, M. S., 1997; Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999).

Methods

Animals: Two cohorts (each with 24 animals) of ovariectomized (OVX) female Fischer rats purchased from Charles River were individually housed in standard cages, in a stress-minimized facility with free access to food and water, and maintained on a 12-hour light/dark cycle with lights on at 10:30 AM EST. All NIH Guidelines for the Care and Use of Laboratory Animals were followed.

Drug and hormone treatment: All chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Two weeks after ovariectomy, rats were randomly assigned to either cocaine- or saline-treatment groups, and then, further divided into one of four hormone pretreatment conditions: vehicle control (vehicle), estrogen, progesterone, or estrogen+progesterone (n=6 animals per group). Forty-eight hours prior to the start of drug treatment, animals in the estrogen and estrogen+progesterone groups received subcutaneous (s.c.) injections of estrogen benzoate (50μg) dissolved in sesame oil, and control groups (vehicle and progesterone) received vehicle injections. Four hours prior to cocaine treatment (44 hours post estrogen treatment) animals in the progesterone and estrogen+progestrone groups received s.c. injections of progesterone (500μg) dissolved in sesame oil. Vehicle and estrogen animals received injections of sesame oil. This administration paradigm and the doses of steroids used have been shown to induce lordosis behavior in ovariectomized rats (Pfaff, D. W. & Schwartz-Giblin, S., 1995).

Four hours after progesterone or vehicle administration (11:00 AM EST), animals received the first of three interperitoneal (i.p.) injections, administered one hour apart, of 0.9% saline (1 ml/kg) or cocaine (15 mg/kg dissolved in 0.9% saline at a concentration of 15 mg/ml). This "binge" dosing schedule was chosen to mimic the manner in which cocaine is often self-administered by humans both in terms of temporal pattern and in relation to circadian rhythm (Branch, A. D., Unterwald, E. M., Lee, S. E., & Kreek, M. J., 1996). Throughout the study all injections were administered in each rat's home cage.

Locomotor activity: The spontaneous locomotor activity of each animal was monitored electronically. The monitor consists of a frame in which the standard cage is placed; three channels of digital information record interruptions of red light beams that traverse the cage (Unterwald, E. M., Ho, A., Rubenfeld, J. M, & Kreek, M. J., 1994). The sum of counts on all three channels for each animal in six-minute time bins was used as a measure of spontaneous locomotor activity. With this technique, there is no change in the animal's environment during behavioral recording and it permits the simultaneous videotaping for later stereotypy scoring.

Stereotypic behaviors: Each animal was videotaped in its home cage for 25 seconds at 15, 30, and 45 minutes after the first two injections of cocaine or saline (no filming was done after the third injection in order to prepare to sacrifice the animals 30 min after the last injection of cocaine). The videotapes were analyzed for behavioral stereotypy by a trained observer blind to each animal's treatment condition. The rating for cocaine-

induced stereotypic behavior was based upon a modification (Daunais, J. B. & McGinty, J. F., 1995) of the Creese and Iversen scale (Creese, I. & Iversen, D., 1974) (see Table 1). A score of 10 was never observed during the course of this experiment.

Plasma levels of cocaine metabolite: Thirty minutes after the third injection, animals were sacrificed by decapitation, following brief (10-15 seconds) exposure to CO_2. This procedure is in compliance with NIH Guidelines for the Care and Use of Laboratory Animals and AVMA standards. Trunk blood was collected, allowed to clot, and then centrifuged at 3,000 RPM for 15 minutes at 4°C. Plasma was collected and stored at -40°C until radioimmunoassay for the cocaine metabolite benzoylecgonine. Internal standards containing known amounts were run to correct for extraction losses. Samples (diluted 1:100; 25µl) and standards (0 to 5,400 ng/ml) were analyzed with Count-A-Coat Cocaine Metabolite radioimmunoassay kit from Diagnostic Products Corporation (CA). The intra-assay coefficient of variation was less than 3%. Results for assays were determined using a log-logit computer program.

Data analysis:

Stereotypic behaviors: Since the distribution of the cumulative scores of stereotypy does not depart from normality, we examined the effects of hormone treatment on cumulative scores of stereotypic behavior using one and two-way repeated measures ANOVAs. Due to differences in the scores of behavioral stereotypy between hormone treatment groups in the saline baseline condition, examination of the effect of cocaine was made using

difference scores: the mean saline score of the same hormone treatment group was subtracted from each cocaine-treated animal's score.

Locomotor activity: To examine the response to "binge" pattern cocaine administration for different hormone conditions, a three-way ANOVA of total locomotor counts was used: CONDITION (Saline vs. Cocaine) × HORMONE-TREATMENT (Vehicle, Estrogen, Progesterone or Estrogen+Progesterone) × INJECTION (first 30 minutes of behavior after each injection) with repeated measures on the last factor, followed by Newman-Keuls *post hoc* tests.

Results

Locomotor activity: Overall, cocaine treatment significantly increased locomotor activity [F (1,40) 29.61, $p < 0.000005$]. In addition, there was a significant increase in locomotor activity of the cocaine-treated animals over time [F(2,80) 13.924, $p < 0.00001$]. Interestingly, cocaine-treated animals had significantly higher locomotor activity after the third injection compared to the second and third injections [$p < 0.0002$; $p < 0.005$, respectively, Figure 18]. When data for cocaine-treated animals were analyzed by hormone group, estrogen+progesterone pretreatment appeared to suppress cocaine-induced locomotor activity after the first injection. *Post hoc* tests revealed that this effect was significant when compared to estrogen-pretreated animals [Newman-Keuls, $p< 0.05$].

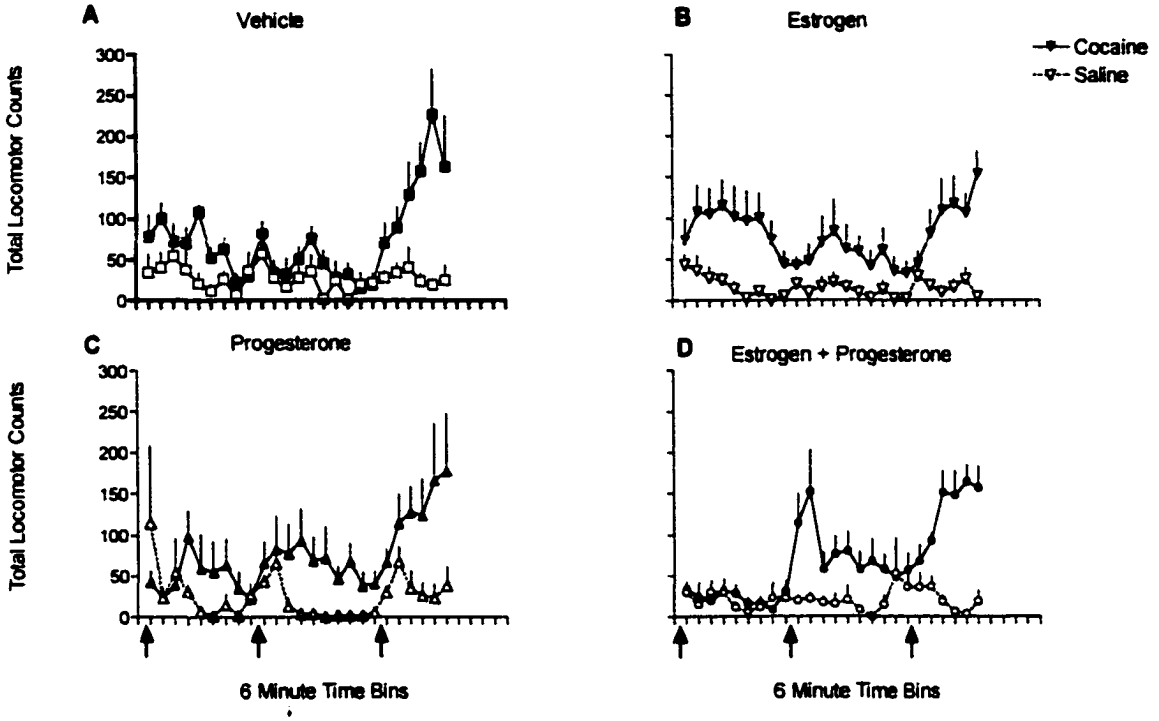


Figure 18: *Locomotor behavior of cocaine- and saline- treated animals in each of the four hormone pretreatment groups.* Mean (+ SEM) total counts of locomotor behavior in the home cage after administration of cocaine or saline to OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen+progesterone.

Stereotypic behaviors: After the first injection, saline-treated animals in the estrogen-pretreatment group had higher stereotypic activity scores than those in the other three hormone groups [F (3,20) 4.81, $p < 0.02$; Figure 19].

Due to these differences in the saline baseline data, the scores of stereotypy from each cocaine-treated animal were expressed as the difference from saline-treated control mean (delta values; Figure 20). A significant HORMONE * INJECTION interaction was found [F (3,17) 3.261, $p < 0.05$]; the estrogen+progesterone group displayed significantly more stereotypic behavior after the second cocaine injection, but not after the first [$p < 0.02$].

Benzoylecgonine levels: Pretreatment of ovariectomized rats with estrogen+progesterone resulted in differences in cocaine-induced behaviors. Interestingly, this hormone pretreatment group also differed from the other groups in plasma levels of the cocaine metabolite, benzoylecgonine (Table 2). Benzoylecgonine plasma levels differed significantly across the different hormone groups [F (3,18) 4.589, $p < 0.05$]. Animals in the estrogen+progesterone group had significantly lower levels of cocaine metabolite than animals in the estrogen- or progesterone-pretreatment groups [Newman-Keuls, $p < 0.05$; $p < 0.02$, respectively].

Discussion

Cocaine increases both stereotypic and locomotor behaviors in ovariectomized rats pretreated with different steroid replacement treatments. This finding confirms and

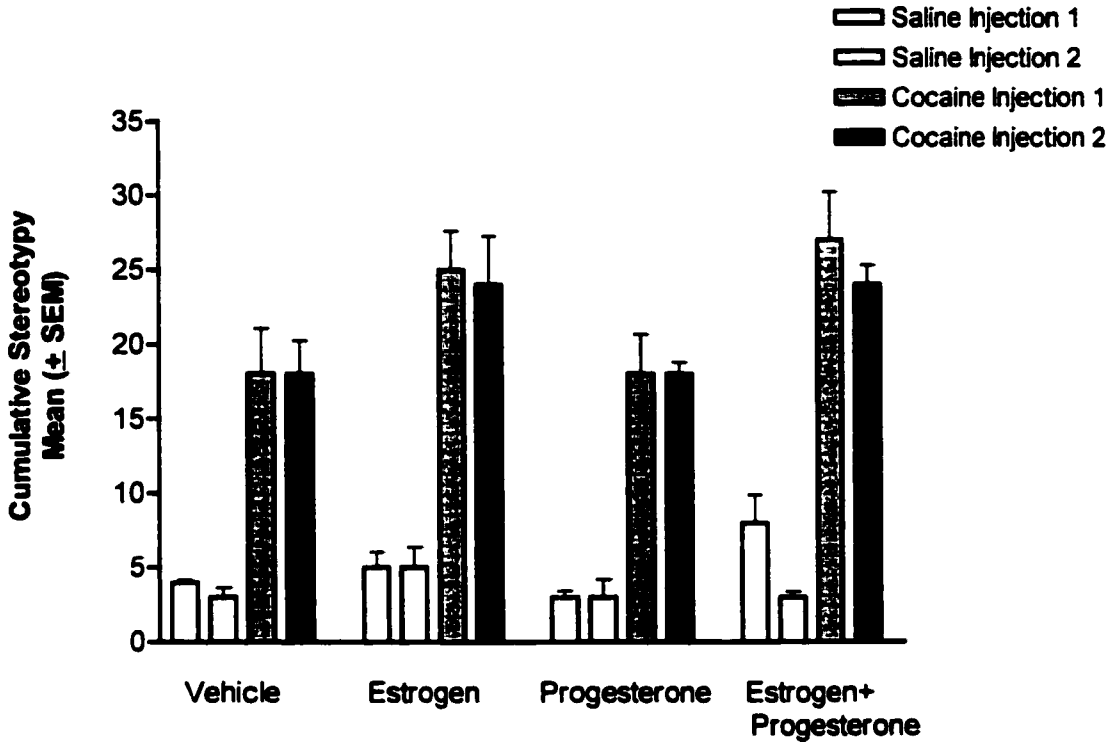


Figure 19: *Behavioral stereotypy of cocaine- and saline-treated female rats in each of the four hormone-treated conditions.* Mean (+ SEM) scores of stereotypic behavior in the home cage after administration of cocaine or saline to OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen+progesterone.

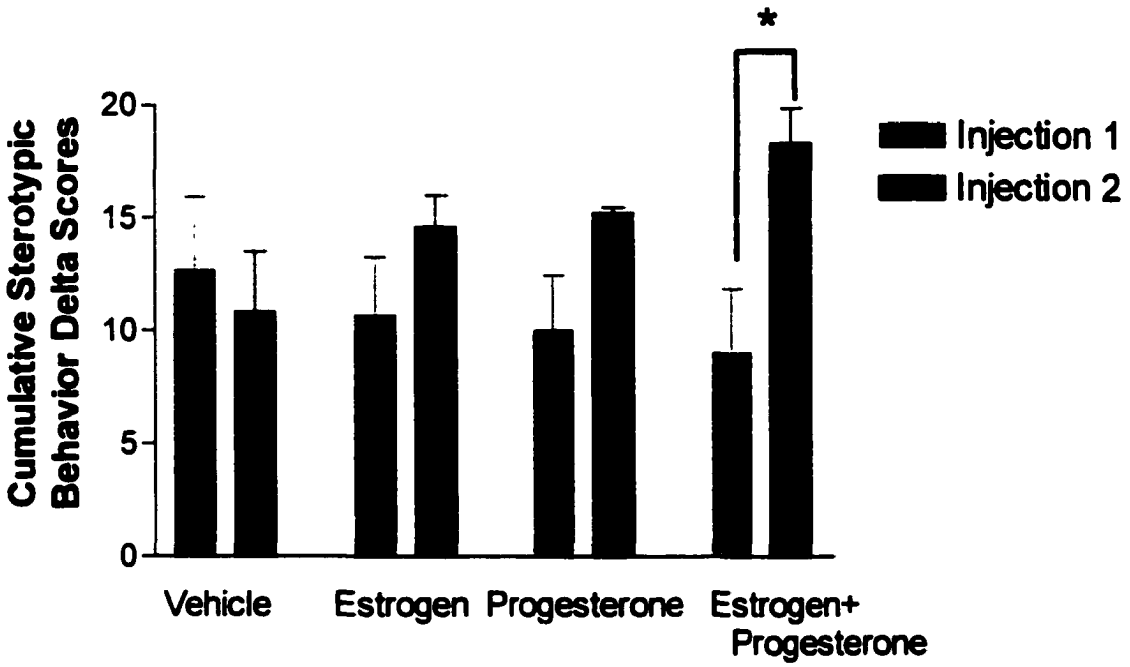


Figure 20: *Cocaine-induced difference scores of stereotypic behavior.* Cumulative behavior scores after injection 1 (gray bars) and two (black bars) with data expressed as difference scores (each cocaine-treated animal's score minus the mean of saline-treated animal's in the same hormonal condition. Values shown are mean + SEM.

Table 2. The mean (± SEM) plasma levels of benzoylecgonine for all cocaine-treated rats.

Hormone-Treatment Group	*n*	Benzoylecgonine Level (ng/ml)
Vehicle control	6	400 ± 81
Estrogen	6	582 ± 61
Progesterone	6	644 ± 79
Estrogen+Progesterone	5	349 ± 53*

**** differs from all other hormone groups*** **p** ***<0.05***

extends previous research in intact male and female rats (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999; Unterwald, E. M., Ho, A., Rubenfeld, J. M, & Kreek, M. J., 1994). Male Fischer rats, treated with chronic "binge" pattern cocaine administration for 14 days displayed increases in spontaneous locomotor activity for approximately 30 minutes following each injection (Unterwald, E. M., Ho, A., Rubenfeld, J. M, & Kreek, M. J., 1994). Interestingly, Quiñones et al., (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) reported that the locomotor activity of cycling female rats in response to acute "binge" pattern cocaine administration was similar to the pattern of the male response. However, the overall activity for females was higher than that for males, suggesting that females may be more "sensitive" to cocaine than males (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). In the present study, ovariectomized rats had a different pattern of locomotor activity in response to cocaine than that reported for male and intact female rats; after the third cocaine injection, locomotor activity was higher than after the first two injections. With the exception of the ovariectomized control rats, the locomotor response of ovariectomized female rats to acute "binge" pattern cocaine administration and estrogen, progesterone, or estrogen+progesterone pretreatment appears to be similar to that of intact females. Overall, the level of locomotor activity for OVX females in this study was higher than that previously induced by acute cocaine administration in male rats.

No differences in cocaine-induced activity after estrogen or progesterone pretreatment were observed. Interestingly, cocaine-induced locomotion after the first

injection appeared to be suppressed in the estrogen+progesterone replacement group. However, after the second and third injections locomotor behavior levels for estrogen+progesterone-pretreated animals reached levels comparable to the other three groups' (control, estrogen, or progesterone). In lordosis behavior, the interaction between estrogen and progesterone is bimodal. Progesterone can first act in synergy with estrogen facilitating reproductive behavior and than act to inhibit sexual receptivity (Morin, L. P, 1976). This temporal relationship is important in the control of lordotic behaviors. The regulation of cocaine-induced behaviors and female reproductive behaviors may overlap in similar CNS mechanisms; progesterone may exert similar modulation on either the motor components of cocaine-induced behavior or rewarding aspects of the drug stimulation. The possible temporal relationship between estrogen and progesterone in the control of cocaine-induced locomotor activity remains to be elucidated.

Cocaine administration has been shown to increase stereotypic behaviors in both intact male and female Fischer rats (Spangler, R., Zhou, Y., Schlussman, S. D., Ho, A., & Kreek, M. J., 1997) (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). We have previously demonstrated estrous cycle effects on cocaine-induced stereotypic behaviors after acute "binge" cocaine administration (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) finding that after the second injection of cocaine, rats during estrus (when both estrogen and progesterone are present) had higher stereotypic scores than those in the other phases of the cycle. In the present study, there were higher levels of stereotypic behaviors in the estrogen+progesterone group in the saline baseline condition. This effect may be related to the increase in motor

activity associated with the facilitation of reproductive behaviors by ovarian hormones. When stereotypic behavior scores for cocaine-treated animals were expressed as difference from saline control groups, there were increases in scores of stereotypy in response to the second injection of cocaine when compared to the first injection in the estrogen+progesterone group. This finding suggests an interaction between hormones and cocaine-induced stereotypic behaviors and further, suggests a possible temporal interaction between estrogen and progesterone in the modulation of cocaine-induced behaviors.

Estrous cycle (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) and gender differences (Bowman, B. et al., 1999) in benzoylecgonine levels have been observed. It has been postulated the ovarian hormones modulate cocaine metabolism. Although it has been reported that cocaine can be spontaneously hydrolyzed to benzoylecgonine in solution (Isenschmid, D. S., Levine, B. S., & Caplan, Y. H., 1989), when levels of benzoylecgonine were measured in a cocaine-saline solution (pH 7.4, 25°, 15mg/ml) as used in our study, no detectable levels of benzoylecgonine were found (data not shown). The cocaine methyl esterase and ethyl transferase activities in the liver are significantly greater in male than in female rats (Romano, G. J., Mobbs, C. V., & Pfaff, D. W., 1989). It is possible that steroid regulation of these enzymes may underlie gender (Bowman, B. et al., 1999) and estrous cycle (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) differences in pharmacokinetics of cocaine metabolism (Sharma, A., Plessinger, M. A., Miller, R. K., & Woods, J. R., 1993). In the present study, plasma levels of benzoylecgonine were affected by estrogen+progesterone

administration. Thus, it is possible that these esterase mechanisms of degradation may be sensitive to estrogen and progesterone concentrations in the plasma, as is the case of the hepatic P450 related enzymes. Interestingly, the estrogen+progesterone pretreatment group also differed from the other groups in cocaine-induced behaviors. This suggests that differences cocaine metabolism may contribute to the observed differences in cocaine-induced behaviors.

Based on these observations, we hypothesize that cocaine-induced behavioral alterations are affected by the animal's endocrine profile. Because gonadal hormones have profound effects on brain functioning, the female's hormonal state at the time of cocaine administration may influence the effects of cocaine on brain functions involved in cocaine-induced behavior. This may be the basis of gender and estrous cycle differences in response to cocaine.

These results confirm our earlier findings in intact female rats, which suggest an interaction between the endocrine environment and cocaine-induced behaviors. The present study extends our previous findings because estrogen and progesterone were directly manipulated, thus adding to the body of evidence showing an interaction between the endocrine profile of the female at time of drug administration and effects of the drug on the CNS.

CHAPTER 5: ESTROGEN AFFECTS COCAINE-INDUCED BEHAVIORAL SENSITIZATION.

Differences in cocaine-induced alterations have been shown in female during the different stages of their reproductive cycles. In rats, the estrous cycle influences an animal's motivation to self-administer cocaine (Roberts, D. C. S., Dalton, J. C. H., & Vickers, G. J., 1987; Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989) and the intensity of cocaine-induced stereotypic and locomotor activities (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). Thus, it has been hypothesized that gonadal hormones, which fluctuate during the estrous cycle, influence cocaine-induced behavioral alterations. This may be the basis of gender and estrous cycle cocaine-induced behavioral and neurochemical differences.

For example, estrogen enhances behavioral sensitization to cocaine (Peris, J., Decambre, N, Coleman-Hardee, M. L., & Simpkins, J. W., 1991). Progesterone has also been implicated in modulating cocaine-induced behavioral and neuroendocrinological alterations. It has been demonstrated that RU486, a progesterone antagonist, decreases cocaine toxicity in rats (Sharma, A., Plessinger, M. A., Miller, R. K., & Woods, J. R., 1993; Glantz, J. C. & Woods, J. R., 1994). Furthermore, cocaine induces steroid-dependent reproductive behaviors in rats, which are blocked by RU486 (Apostolakis, M. E., Garai, J., Clark, J. H., & O'Malley, B. W., 1996). Cocaine-induced alterations in progesterone plasma levels in intact and pregnant female (Quiñones-Jenab, V, Krey, L. C., Schlussman, S. D., Ho, A., & Kreek, M. J, 2000; Quiñones-Jenab, V et al., 2000) and male rats (Quiñones-Jenab, V, Zhou, Y., Jenab, S., Ho, A., & Kreek, M. J, 2000). Further, progesterone treatment affects cocaine-induced increases in serotonin levels in the medial prefrontal cortex (Perrotti, L. I., Beck, K. D., Luine, V. N., & Quiñones-Jenab, V, 2000). Co-administration of estrogen+progesterone affects cocaine-induced locomotor activity after chronic (Sircar, R. & Kim, D., 1999) or acute "binge" pattern administration

(Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). This evidence suggests that ovarian hormones may be an important component in the cascade of events modulating cocaine-induced behavioral and neurochemical adaptations after cocaine administration.

The present study was designed to further understand how ovarian hormones affect the behavioral and neuroendocrinological response after acute and chronic cocaine administration in ovariectomized female rats. The identification of which cocaine-induced behavioral and hormonal alterations are affected by steroids will enhance the understanding of mechanisms affecting sex differences in response to cocaine. Moreover, chronic steroid replacement in humans can be found in women utilizing estrogen- or progesterone-based anticonceptive or estrogen-replacement treatment after menopause. This study extends our understanding of the effect(s) of cocaine in women utilizing these steroid treatments.

Methods

Animals:

All NIH guidelines for the care and use of laboratory animals were followed. Two separate cohorts of eight-week-old ovariectomized (OVX) female Fischer rats (Charles River, N.C.) were individually housed in standard cages with free access to food and water, and maintained on a 12-hour light:dark cycle (lights on at 9:30 A.M. EST). Two weeks after ovariectomy, rats were randomly assigned to either acute or chronic cocaine or saline administration, and then, further subdivided into one of four hormone pretreatment conditions: cholesterol (vehicle-control), estrogen, progesterone or estrogen+progesterone (n=6 per group). Thirty minutes after the last drug treatment, animals were decapitated following a brief (10-15 seconds) exposure to CO_2.

Drug and hormone treatments:

Estrogen treatment was administered via Silastic capsules. Capsules (0.058 in. i.d. and 0.077 in. o.d., Dow Corning), containing either 0.5cm of packed estradiol (10% estradiol and 90% cholesterol) or cholesterol (100%), were implanted (s.c.) into the interscapular region. Progesterone was administered acutely via s.c. injection (500 μg/rat dissolved in sesame oil) four hours before the last cocaine administration. Cocaine HCl (Sigma) was administered i.p. 15mg/kg dissolved in 0.9% saline. Cocaine was diluted in saline immediately prior to each injection.

For the acute administration: forty-four hours after estradiol implantation, rats received either progesterone or vehicle according to their respective experimental group. Four hours following the administration of progesterone (48 hours post estrogen treatment), rats received a single injection of saline or cocaine. For chronic administration: forty-four hours after estrogen or cholesterol implants, rats received daily injections of saline or cocaine for fourteen consecutive days. On Day 14, rats received s.c. injections of progesterone or vehicle four hours prior to the last drug administration according to their respective experimental group.

Behavioral assays: Behavioral measurements were performed for each rat in its home cage for 30 minutes after saline or cocaine administration. Both stereotypic and locomotive activities were analyzed for each animal.

Locomotor activity: Spontaneous locomotor activity was monitored with a Photobeam Activity System from San Diego Instruments (CA). These monitors consisted of two frames, in which the home cage was placed. The lower frame counted horizontal activity and the upper frame vertical activity. Total locomotor counts were determined by calculating the sum of counts on all four channels detecting horizontal motion.

Ambulatory activity was determined by total counts of two consecutive photobeam interruptions in the lower frame. Rearing activity was represented as total counts of vertical motions.

Stereotypic activity: Rats were videotaped for 25 seconds at 15 and 30 minutes post-injection. The videotapes were later analyzed for behavioral stereotypy by three trained observers blind to each rat's treatment group. The rating for cocaine-induced stereotypic behaviors (summarized in Table 1) was based upon a modification (Daunais, J. B. & McGinty, J. F., 1995) of the Creese and Iversen scale (Creese, I. & Iversen, D., 1974). The scale consists of 10 scores, ranging from a score of 1 (given to an animal that was asleep or inactive) to 10 (given to an animal that exhibited splayed hind limbs). A score of 10 was never observed during the course of this experiment.

Plasma levels of benzoylecgonine and corticosterone: Trunk blood was allowed to clot and then centrifuged at 3,000 RPM for 15 minutes at 4°C. Plasma was collected and stored at -70°C until radioimmunoassays (RIA) were performed. Samples were analyzed for benzoylecgonine and corticosterone with Count-A-Coat RIA kits from Diagnostic Product Corporation (CA).

Data analysis:

Locomotor activity: Total locomotor, ambulatory, and rearing counts were represented as the sum of total photocell counts over 30 minutes. To examine the response to acute and chronic saline and cocaine administration during different hormonal treatments, three-way repeated measures ANOVAs were used (HORMONAL TREATMENT × DRUG TREATMENT × LENGTH OF ADMINISTRATION). In order to detect any effects of drug- and hormone-treatment on the three measures of locomotor activity within acute and chronic treatment conditions, separate two-way ANOVAs (HORMONE × DRUG)

were run. When significant interactions were obtained, Newman-Keuls *post-hoc* tests were used to locate between groups differences.

Stereotypic behaviors: Since the distribution of the cumulative scores of stereotypy does not depart from normality, we examined the effects of drug, hormone, and length of drug treatment on these scores of stereotypic behavior using a three-way ANOVA (HORMONAL TREATMENT × DRUG TREATMENT × LENGTH OF ADMINISTRATION). In order to locate any effects of drug- and hormone-treatment on stereotypy within acute and chronic administration conditions, separate two-way ANOVAs were run. When appropriate, Newman-Keuls *post-hoc* tests were used to locate any differences between groups.

Plasma levels of cocaine metabolite and corticosterone: To examine the effects of acute versus chronic cocaine administration on plasma levels of benzoylecgonine and corticosterone, one and two factor ANOVAs were used, followed by Newman-Keuls *post hoc* tests when significant interactions were obtained.

Results

Cocaine-induced locomotor activity:

Total Locomotor Counts: There was significantly greater total locomotor activity in cocaine-treated rats after both acute and chronic cocaine administration when compared to saline-treated rats ([$F(1,464)=331.405$, $p<0.001$]; Figure 21A and B). Chronic cocaine administration resulted in higher counts of total locomotor activity when compared to acute cocaine administration [$F(1,464)=3.864$, $p<0.05$]. After acute saline or cocaine administration, hormone treatment did not affect total locomotor counts [$F(3,230) = 2.230$, $p > 0.05$]. In contrast, after chronic cocaine administration, hormonal replacement did affect cocaine-induced total locomotor activity [$F(3,232) = 6.153$, $p < 0.001$];

estrogen and estrogen+progesterone replacement increased locomotor activity when compared to progesterone or control treatment groups [$p < 0.005$; $p < 0.001$ estrogen vs. progesterone and cholesterol, respectively; $p < 0.02$, $p < 0.001$; estrogen+progesterone vs. progesterone and cholesterol, respectively].

Ambulatory Counts: Overall, cocaine-treated rats had higher ambulatory activity than saline-treated controls ([F (1,464) = 165.402, $p < 0.002$]; Figure 21C, D). Further, there was an interaction between DRUG and LENGTH OF ADMINISTRATION [F (1,464) = 3.965, $p < 0.05$]; after acute cocaine administration ambulatory counts for cocaine-treated animals were higher than after chronic administration [$p < 0.001$]. Unlike total locomotor behaviors, significant DRUG × HORMONE interactions were found after both acute and chronic administration [F (3,219) = 2.811, $p < 0.05$; F (3,232) = 5.707, $p < 0.001$; acute and chronic, respectively]. After acute co-administration of cocaine and estrogen, increases in ambulatory counts were observed when compared to vehicle-control or progesterone-treated groups [$p < 0.005$; $p < 0.005$, respectively], and after chronic cocaine and estrogen administration ambulatory counts were higher in comparison to all other hormone treatment groups [$p < 0.001$; $p < 0.001$; $p < 0.003$, cholesterol, progesterone, estrogen+progesterone, respectively]. Moreover, after chronic cocaine and saline administration, no differences in ambulatory activity between the saline and cocaine vehicle-control groups were observed [$p > 0.05$].

Rearing Counts: Cocaine-treated rats spent significantly more time rearing than saline controls ([F(1,464)=188.547, p<0.001]; Figure 21E and F). Overall, no differences in hormone or drug-treatment effects on rearing activity after acute vs. chronic administration were observed [F (3,464) = 1.329, $p > 0.2$]. However, after acute cocaine administration, a significant DRUG * HORMONE interaction was observed [F (3,227) = 7.005, $p < 0.001$]; co-administration of estrogen and cocaine induced more rearing

activity when compared to vehicle control or estrogen+progesterone treatments [$p < 0.001$; $p < 0.05$, respectively]. Similarly, after chronic cocaine administration, a significant interaction between drug and hormone treatment was also observed [$F(3,232) = 12.472$, $p < 0.001$], where estrogen treatment induced significantly more rearing activity when compared to all other hormone groups [$p < 0.001$; $p < 0.001$; $p < 0.001$, cholesterol, progesterone, estrogen+progesterone, respectively]. In vehicle-treated rats, rearing activity after chronic cocaine administration was significantly lower than all steroid replacement paradigms [$p < 0.001$; $p < 0.01$; $p < 0.01$, estrogen, progesterone, estrogen+progesterone, respectively] and not statistically different from saline-treated controls [$p > 0.5$].

Stereotypic behaviors:

Overall, cocaine-treated rats had higher scores of stereotypic behaviors than saline-treated groups' ([$F(1,80)=121.851$, $p< 0.001$]; Figure 22). No significant differences in stereotypic behaviors were observed 15 and 30 minutes after cocaine administration for any of the hormone-treatment groups after acute [$F(3,40)=.563$, $p > 0.5$] or chronic [$F(3,40)=1.473$, $p > 0.2$] administration, data not shown. Thus, the average of cumulative scores for both readings (15 and 30 minutes post injection) were used.

As can be seen in Figure 22A, hormone treatment did not affect stereotypic activity in acute cocaine or saline treated rats [$F(3,40)=.937$, $p > 0.3$]. However, after chronic administration, a significant DRUG × HORMONE interaction was found [$F(3,37)=4.133$, $p < 0.02$] as a result of differences between saline-treated groups (Figure 22B). Saline-treated vehicle-control rats had higher scores of stereotypy when compared to progesterone or estrogen+progesterone treated groups' [$p < 0.01$; $p < 0.01$, respectively]. Further, no differences between vehicle-control saline or cocaine rats were

observed [$p > 0.1$]. There were no differences in scores of stereotypic behavior among chronic cocaine-treated animals in the four hormone pretreatment groups [$p > 0.05$].

Interactions between steroids and cocaine-induced alterations in plasma levels of corticosterone and benzoylecgonine:

Overall, levels of benzoylecgonine were higher after chronic cocaine administration when compared to acute cocaine administration ([$F(1,42)=7.842$, $p< 0.01$]; Figure 23). However, plasma levels of benzoylecgonine did not vary across the different hormone treatments [$F(3,42)=2.760$, $p > 0.05$].

Overall, plasma levels of corticosterone were significantly lower for all animals in the chronic treatment condition than those in the acute condition ([$F(1,68) = 31.481$, $p < 0.001$]; Figure 24). After chronic cocaine administration, estrogen-treated rats had higher corticosterone plasma levels than control or progesterone-treatment groups [$p < 0.05$].

Discussion

Similar to previous reports in intact female and male rats (Sircar, R. & Kim, D., 1999) (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) (Kuhn, C. & Francis, M. S., 1997) (Craft, R. M. & Stratmann, J. A., 1996), acute cocaine administration caused increases in both stereotypic and locomotor behaviors in ovariectomized rats. Estrogen caused higher levels of cocaine-induced increases in locomotor activities; while progesterone treatment did not affect these behaviors. Although, Sicar and Kim (1999) previously reported that estrogen+progesterone administration resulted in more locomotor activity in response to acute cocaine, in the present study, co-administration of estrogen+progesterone and acute cocaine did not differentially affect any of the locomotor behaviors measured. It is possible that the

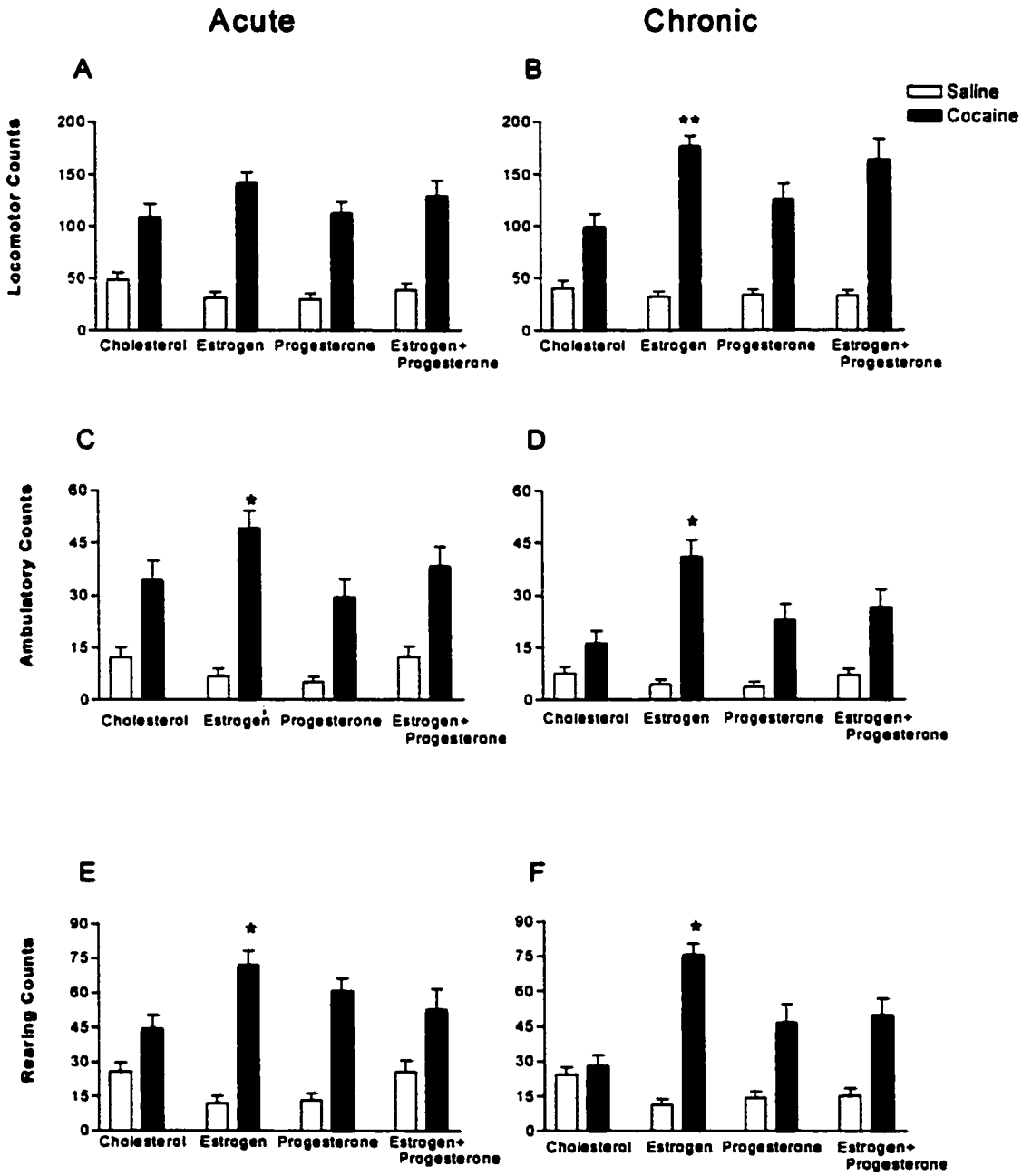


Figure 21: *Locomotor behavior of acute and chronic cocaine- and saline- treated OVX Fischer rats in each of the four hormone pretreatment groups.* Total locomotor (A and B), ambulatory (C) and D) , and rearing (E and F) activities for acute (left column) and chronic (right column) treated animals pretreated with one of the hormone regimens: vehicle-control, estrogen, progesterone, or estrogen+progesterone. Solid black bars represent mean (+ SEM) behavioral activity in cocaine-treated animals and white bars show mean (+ SEM) behavioral activity for saline-treated rats.

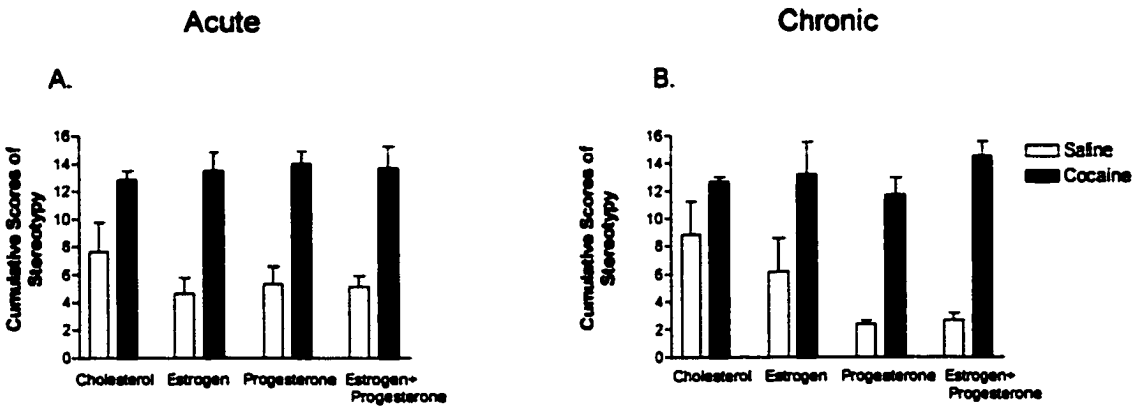


Figure 22: *Behavioral stereotypy of cocaine- and saline-treated OVX female rats in each of the four hormone pretreatment conditions.* Mean (+ SEM) scores of stereotypic behavior after acute (A) or chronic (B) administration of cocaine (black bars) or saline (white bars) in OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen+progesterone.

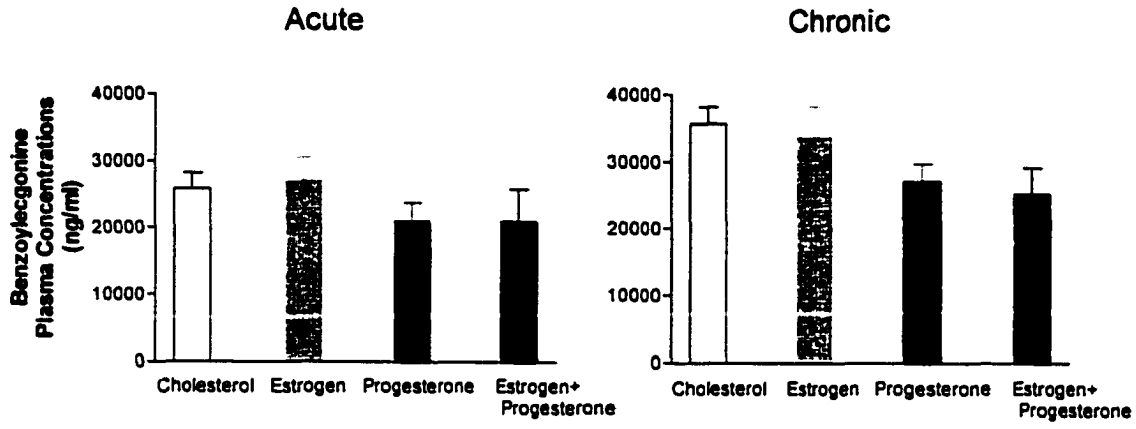


Figure 23: *Plasma levels of benzoylecgonine for hormone pretreated OVX Fischer rats after acute or chronic cocaine administration.* Mean (+ SEM) plasma levels of benzoylecgonine (expressed as μg/ml) for acute (A) and chronic (B) cocaine-treated animals in each of the four hormone-treatment conditions: vehicle-control, estrogen, progesterone or estrogen+progesterone.

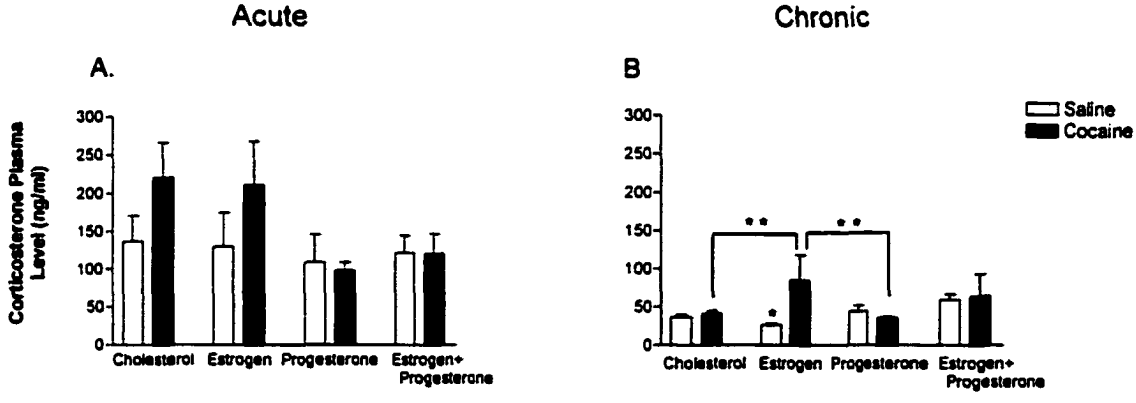


Figure 24: *Plasma corticosterone levels for hormone pretreated OVX Fischer rats after acute or chronic cocaine or saline treatment.* Mean + SEM plasma levels of corticosterone (expressed as ng/ml) after acute (A) and chronic (B) cocaine (black bars) or saline (white bars) treatment for OVX Fischer rats in each of the four hormone-treatment condition; vehicle-control, estrogen, progesterone, and estrogen+progesterone

differences in results between these two studies could be due to inbred differences in Fischer rats as they were purchased from two different commercial sources (Perrotti, L. I., Russo, S., Lagos, F, & Quinones-Jenab, V, 2000). Overall, our study suggests that the endocrinological profile of the female may affect the acute effects of cocaine. Whereas estrogen and progesterone may play a key role in the modulation of behavioral responses to acute cocaine administration.

Repeated cocaine exposure caused ovariectomized rats to develop behavioral tolerance (exhibited by a decrease in activity) in rearing and ambulatory, but not total locomotor, activity. Estrogen treatment not only reversed the development of tolerance in rearing and ambulations, but caused sensitization (defined as a progressive increase in motor stimulation after repeated cocaine administration (Kalivas, P. W. & Stewart, J., 1991)) of cocaine-induced total locomotor activity. However, similar to our findings for acute cocaine administration, progesterone-treatment did not affect any of the locomotor behaviors measured after chronic cocaine administration. While our observations are consistent with those previously reported (Peris, J., Decambre, N, Coleman-Hardee, M. L., & Simpkins, J. W., 1991; Sircar, R. & Kim, D., 1999), which demonstrate that after chronic cocaine administration total locomotor activity is affected by estrogen and not progesterone, our results extend these studies by showing that rearing and ambulatory activities are also affected by estrogen.

After chronic cocaine administration, Sicar and Kim (1999) reported that estrogen+progesterone treatment resulted in sensitization of cocaine-induced locomotor activity. However, similar to the report by Peris et al. (1991), we did not observe sensitization in locomotor activity (total locomotor, ambulatory or rearing) with this hormone replacement regimen. Discrepancies between these studies may reside in the steroid replacement paradigms used. (In both our study and that by Peris et al. (1991),

estradiol was administered via s.c. implanted Silastic capsules, while Sicar and Kim (1999) administered estradiol via s.c. injections 48 and 24 hours prior to each behavioral test session. Route and method of progesterone was different for all three studies). Gender differences in the development of behavioral sensitization in response to cocaine have been reported (Glick, S. D., Hinds, P. A., & Shapiro, R. M, 1983; Post, R. M., Lockfeld, A., Squillace, K. M., & Contel, N. R., 1981; Sircar, R. & Kim, D., 1999; Kuhn, C. & Francis, M. S., 1997). Our study suggests that modulation by ovarian hormones may be a key factor in the development of behavioral sensitization or tolerance to cocaine and a basis for reported gender differences.

Cocaine caused increases in stereotypic activity. However, although hormone treatments did not affect cocaine-induced stereotypy, stereotypic activity was affected by chronic saline and hormone treatments. This is consistent with previous observations after "binge" saline administration, where stereotypic activity was affected by ovarian hormone replacement in saline-treated groups (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). Ovarian hormone modulation on stereotypic activity of saline-groups may be related to hormonal regulation of motor activity associated with the facilitation of reproductive behaviors. The lack of an interaction between cocaine and hormone treatments may be due to a ceiling effect on our scale of stereotypic behavior. Thus, it is possible that hormone modulation of cocaine-induced differences in stereotypic activity may reside in the frequency, rather than the level, of activity. This needs to be elucidated.

Estrous cycle (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) and gender (Bowman, B. et al., 1999) differences in benzoylecgonine plasma levels have been reported. After "binge" cocaine administration, ovarian modulation of benzoylecgonine levels have also been previously reported, where lower

levels of benzoylecgonine were observed after co-administration of estrogen and progesterone (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). Furthermore, gender differences in liver enzymes that metabolize cocaine have also been observed; suggesting that the endocrinological profile of rats and humans may modulate the metabolism of cocaine. In this study, no differences in benzoylecgonine levels were observed after acute or chronic administration. Discrepancies between the steroid effects on benzoylecgonine levels after "binge" (15mg/kg; i.p.; once an hour for three hours) or single dose cocaine administration could be due to differences in the final plasma concentrations of cocaine. Based on the present report, the effects of ovarian hormones on behavioral activity after acute and chronic cocaine administration, as well as previously reported estrous cycle and gender differences, may not completely reside in ovarian modulation of cocaine metabolism.

HPA activation is believed to play a major role in modulating cocaine-induced CNS alterations. Cocaine administration leads to an elevation in plasma corticosterone levels in males and females (Moldow, R. L. & Fischman, A. J., 1987; Zhou, Y. et al., 1998; Koob, G. F. & LeMoal, M., 1997; Kuhn, C. & Francis, M. S., 1997); female rats demonstrate an exaggerated HPA response to cocaine (Kuhn, C. & Francis, M. S., 1997). Moreover, corticosterone levels vary throughout the estrous cycle of the rat (Atkinson, H. C. & Waddell, B. J., 1997). This suggests that reproductive hormones may play an important role in HPA modulation of cocaine-induced alterations. In the present study, corticosterone plasma levels were not significantly affected by acute cocaine-treatment. However, after chronic cocaine administration, estrogen induced increases in corticosterone plasma levels. Interestingly, this is the hormone-treatment group which showed the development of sensitization in cocaine-induced behavior. Thus, suggesting that modulation of ovarian hormones on HPA activated may be a key component in the development of sensitization and tolerance of female rats.

Of key importance is the female addict that may use estrogen- or progesterone-based contraceptives. Based on our observations, interactions between steroids and cocaine may ultimately affect not only the contraceptive treatment but also the behavioral and subjective responses to cocaine. Thus, women using different steroid treatments or at different stages of their menstrual cycles may use higher doses of cocaine to achieve greater subjective effects of the drug. Interactions between cocaine and ovarian hormones may lead to overdoses and other clinical complications. Moreover, the development of sensitization or tolerance to cocaine may be affected according to which steroid-based contraceptive a women is using or where she is in her reproductive cycle. This important clinical issue in females needs further investigation.

CHAPTER 6: TEMPORAL INTERACTIONS BETWEEN ESTROGEN AND PROGESTERONE AFFECT COCAINE-INDUCED BEHAVIORS IN OVARIECTOMIZED FEMALE RATS

In the United State of America 30% of cocaine users are females (Substance Abuse and Mental Health Services Administration , 1998). Evidence is accumulating suggesting that there are sex differences in the behavioral response to cocaine (Kuhn, C. & Francis, M. S., 1997; Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996; Sircar, R. & Kim, D., 1999; Bowman, B. et al., 1999; Caihol, S. & Morméde, P., 1999; Chin, J. et al., 2000a; Van Haaren, F. & Meyer, M. E., 1991; Craft, R. M. & Stratmann, J. A., 1996; Glick, S. D., Hinds, P. A., & Shapiro, R. M, 1983; Lynch, W. J. & Carroll, M. E., 2000), which may reside in endocrinological differences between males and females. Females have a complex endocrinological profile, where estrogen and progesterone concentration ratios vary during each of the stages of the cycle. The interaction between these gonadal hormones is bimodal (Morin, L. P, 1976). For example, in lordosis behavior, progesterone first synergizes with estrogen to potentiate estrogen-induced behaviors, and later it acts to inhibit the behavioral effects of estrogen (Morin, L. P, 1976). This temporal relationship has been postulated to be important to control the different components of reproductive behaviors, such as pacing, lordosis and other motor behaviors (Morin, L. P, 1976). Due to the possible overlap of the motor and reward mechanisms of cocaine-induced and reproductive behaviors, cocaine-induced motor behaviors may be similarly regulated by estrogen and progesterone; where progesterone may exert inhibitory or synergistic modulation on either the motor components of cocaine-induced behaviors or rewarding aspects of the drug according to the length of time it interacts with estrogen.

Although it has been reported that estrogen enhances behavioral sensitization to cocaine (Peris, J., Decambre, N, Coleman-Hardee, M. L., & Simpkins, J. W., 1991), as well as self-administration (Roberts, D. C. S., Dalton, J. C. H., & Vickers, G. J., 1987), and progesterone affects cocaine toxicity (Sharma, A., Plessinger, M. A., Miller, R. K., & Woods, J. R., 1993), little is known about the interactions of both gonadal hormones in the modulation of cocaine-induced behaviors. Using a single cocaine+estrogen+progesterone administration, it has been recently reported that female gonadal hormones modulate cocaine-induced behavioral activity (Sircar, R. & Kim, D., 1999; Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000; Perrotti, L. I., Russo, S., Lagos, F, & Quinones-Jenab, V, 2000). After a "binge" pattern cocaine administration (3 injections, one hour apart), a bimodal interaction between estrogen+progesterone was observed; where after 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, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). This shift from inhibition to enhancement of cocaine-induced behavioral activity may reside in a temporal interaction between estrogen and progesterone. 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.

Methods

Animals:

Three cohorts (each with 48 animals) of ovariectomized (OVX) female Fischer rats purchased from Charles River (N.C.) were individually housed in standard cages, in a stress-minimized facility 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. All NIH Guidelines for the Care and Use of Laboratory Animals were followed.

Drug and hormone treatment:

All chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Two weeks after ovariectomy, rats were randomly assigned to either cocaine or saline treatment. 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 or 1, 4, 20, 24, 30, 43, 44, 45, 46, or 48 hours post estrogen administration (see Figure 25). This administration paradigm has been previously shown to have a bimodal interaction between estrogen and progesterone on reproductive behaviors (Satou, M. & Yamanouchi, K., 1996). The doses of estrogen and progesterone used have also been shown to modulate cocaine-induced neurochemical and behavioral activity (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000).

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). Throughout the study all injections were administered in each rat's home cage. Thirty minutes after

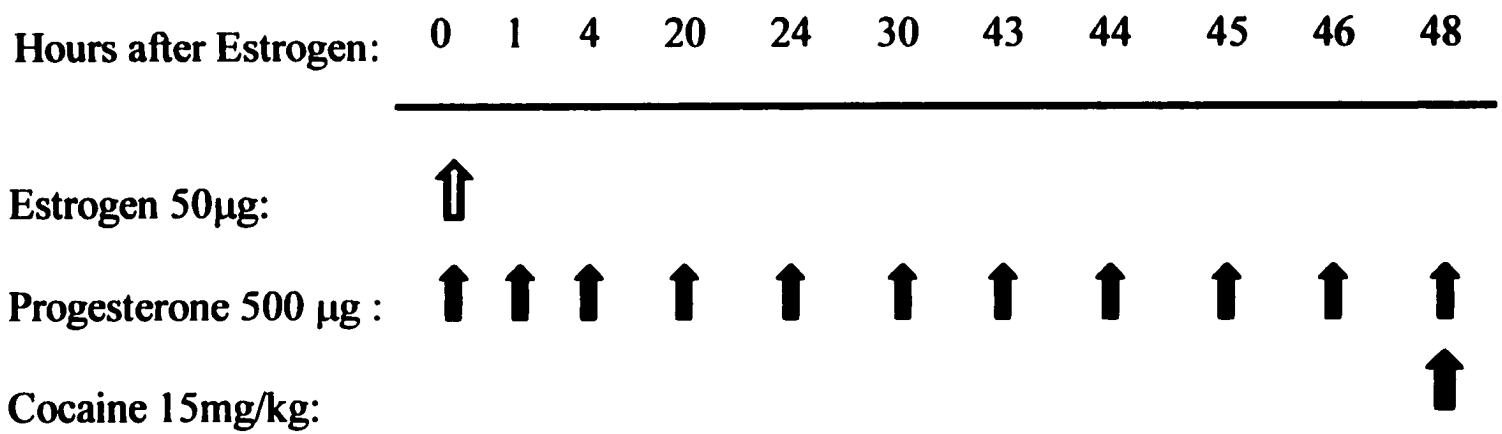


Figure 25: Cocaine and hormone administration paradigm

cocaine/saline administration rats were sacrificed by decapitation, following a brief exposure (30 seconds) to CO_2, and trunk blood was collected.

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.

Locomotor activity:

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, L. I., Russo, S., Lagos, F, & Quinones-Jenab, V, 2000). Total locomotor activity represents all of the behavioral activity of the animal and represents the sum of all counts in the horizontal frame. 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. All three locomotor activities were then subdivided into 5 minutes time intervals.

Stereotypic behaviors:

Each animal was videotaped in its home cage for 30 seconds 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 (Daunais, J. B. & McGinty, J. F., 1995) of the Creese and Iversen scale (Creese, I. & Iversen, D., 1974) (see Table 1). A score of 10 was never observed during the course of this experiment.

Plasma levels of benzoylecgonine and corticosterone:

The trunk blood was allowed to clot and then centrifuged at 3,000 RPM for 15 minutes at 4°C. Plasma was collected and stored at -40°C until radioimmunoassays were run. Samples were analyzed with Count-A-Coat Cocaine Metabolite or Corticosterone radioimmunoassay kits from Diagnostic Products Corporation (CA). Intra-assay coefficients of variance were less than 10%.

Data analysis:

Stereotypic behaviors:

Since the distribution of the scores of stereotypy does not depart from normality, we examined the effects of hormone treatment on scores of stereotypic behavior using parametric analyses of variance (ANOVAs). Scores obtained for 15 and 30 minutes post-injection were examined using a repeated measures ANOVA: DRUG × PROGESTERONE TIME × BEHAVIORAL TEST TIME (15 vs. 30 MINUTES). When a significant effect was obtained for the repeated measures factor, one-way ANOVAs were run to locate significant differences among the treatment groups. Cumulative scores of stereotypy were examined using a two-way ANOVA: DRUG CONDITION × PROGESTERONE TIME.

Locomotor activities:

To examine differences in the drug and hormone treatment groups' in locomotor activities, two-way repeated measures ANOVAs on the sum of total locomotor, rearing or ambulatory counts DRUG CONDITION × PROGESTERONE TIME × TIME AFTER DRUG EXPOSURE. When significant interactions were obtained for the repeated measures factor, one-way ANOVAs were run to locate significant differences among the treatment groups. When appropriate, Newman-Keuls *post hoc* tests were run to locate differences between the groups.

Plasma levels of benzoylecgonine and corticosterone:

To examine the effects of the length of progesterone administration on plasma levels of benzoylecgonine and corticosterone, one and two factor ANOVAs were used, followed by Newman-Keuls *post hoc* tests when significant interactions were obtained.

Results

Total Locomotor Activity: Overall, cocaine significantly increased total locomotor activity ([$F(1,103)=166.658$, $p<0.001$); Figure 26]. When each treatment group was analyzed across the 30 minutes after cocaine administration, a significant difference over time was observed [$F(4,412)=24.708$, $p<0.001$], where total locomotor activity was higher the first six minutes after the injections of cocaine or saline compared to the other four time periods [$p < 0.05$ for all comparisons].

An interaction between the length of progesterone administration and drug treatment was observed [$F(10,103)=3.918$, $p<0.001$], where total locomotor activity after co-administration of 24 hours of progesterone with 48 hours of estrogen administration was significantly higher than all other hormone/drug-induced activity [$p < 0.002$ for all comparisons]. Interestingly, there were no significant differences between saline- and cocaine-treated rats in animals receiving progesterone 1, 44, or 46 hours after estrogen treatment [$p=0.724$; $p=0.084$; and $p=0.50$, respectively]. There were no differences between saline-treated animals receiving progesterone at the different time points [$p>0.5$].

Ambulatory activity: Overall, cocaine significantly increased ambulatory activity when compared to saline-treated controls ([$F(1,103)=153.156$, $p<0.001$], Figure 27). When each treatment group was analyzed across the 30 minutes after drug administration, a significant difference over time was observed [$F(4,412)=38.604$, $p<0.001$]. Similar to locomotor activity, activity during the first six minutes after the injections of cocaine or saline was higher compared to the other four time intervals [$p < 0.05$ for all comparisons].

An interaction between the drug treatment and length of progesterone administration was obtained [$F(10,103)= 3.829$, $p<0.001$). In groups receiving progesterone concurrent with estrogen or 20, 24 or 43 hours after estrogen significant increases in ambulatory activity after cocaine administration were observed. ([$p<0.05$ for all comparisons]; Figure 3). 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 or inhibition of typical cocaine-induced ambulatory activity was observed in rats treated with progesterone 1, 4, 30, 44, 45, 46, 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 [$p>0.5$].

Rearing activity: Overall, cocaine caused increases in rearing activity ([$F(1,103)=153.156$, $p<0.001$], Figure 28). Moreover, across the testing period, a significant change in activity was observed [$F(4,412)=13.909$, $p<0.001$]; rearing activity in saline-treated groups was higher during the first six minutes after injection when compared to the other four time periods [$p < 0.05$ for all comparisons]. No significant differences in rearing activity over time for cocaine- treated rats were observed [$p=0.13$].

A significant Drug x Progesterone interaction was observed [$F(10,103)= 6.005$, $p<0.001$], where cocaine-treated rats given progesterone 4, 20, 24, 30, 44, or 45 hours after estrogen treatment had higher levels of rearing activity when compared to saline treated rats ([$p<0.05$ for all comparisons]; Figure 4). Cocaine-treated rats receiving progesterone concurrent with estrogen (0 hour group) also showed increases in rearing activity when compared to saline treated rats. However, this increase just failed to reach significance [$p=0.051$]. Similar to ambulatory and total 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 receiving progesterone 1, 43, 46 or 48 hours following estrogen [$p>0.5$ for all comparisons]. This bimodal interaction between estrogen and progesterone in rearing activity was not observed in saline-reiterated rats [$p>0.05$].

Stereotypic behaviors: Overall, cocaine induced stereotypic activity ([$F(1,81)=73.763, p<0.01$], Figure 29). No statistically significant differences in stereotypic activity in saline treated rats were observed [$p>0.05$]. A significant interaction between drug and time of stereotypic measurements was observed [$F(1,80)=7.884$, $p<0.001$], where all cocaine-treated groups displayed more stereotypy 15 minutes than 30 minutes after cocaine administration [$p<0.05$]. This temporal relationship in stereotypic activity and drug treatment was not observed in saline treated rats [$p=0.95$]. Steroid replacement administration did not differentially alter stereotypic activity 15 or 30 minutes post cocaine administration [$F(10,80)=1.300$, $p=0.244$].

Benzoylecgonine plasma levels:

A significant interaction between length of progesterone administration and benzoylecgonine (BE) plasma levels was observed ([$F(10,73)$ 5.587, $p < 0.001$]; Figure 30). 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; 43, 44, 46 or 48 hours after estrogen [p <0.05 for all comparisons].

Corticosterone plasma levels:

Plasma levels of corticosterone differed significantly across the different drug and progesterone treatment groups ([F (10,109) 1.929, $p < 0.05$]; Figure 31). 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].

Discussion

Similar to previous reports in intact and ovariectomized female rats, acute cocaine administration affects both stereotypic and locomotor behaviors in estrogen+progesterone treated ovariectomized rats (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000; Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999; Kuhn, C. & Francis, M. S., 1997; Craft, R. M. & Stratmann, J. A., 1996; Sircar, R. & Kim, D., 1999). In reproductive behaviors, a temporal interaction between estrogen and progesterone has been previously reported; estrogen effects on lordosis were either inhibited by progesterone (when administered up to 24 hours after estrogen) or facilitated (when administered 27-48 hours after estrogen) (Satou, M. & Yamanouchi, K., 1996). Similar to lordosis, a bimodal/temporal interaction between estrogen and progesterone in cocaine-induced locomotor behaviors was observed. However, the temporal aspects of this interaction differ with that previously reported for

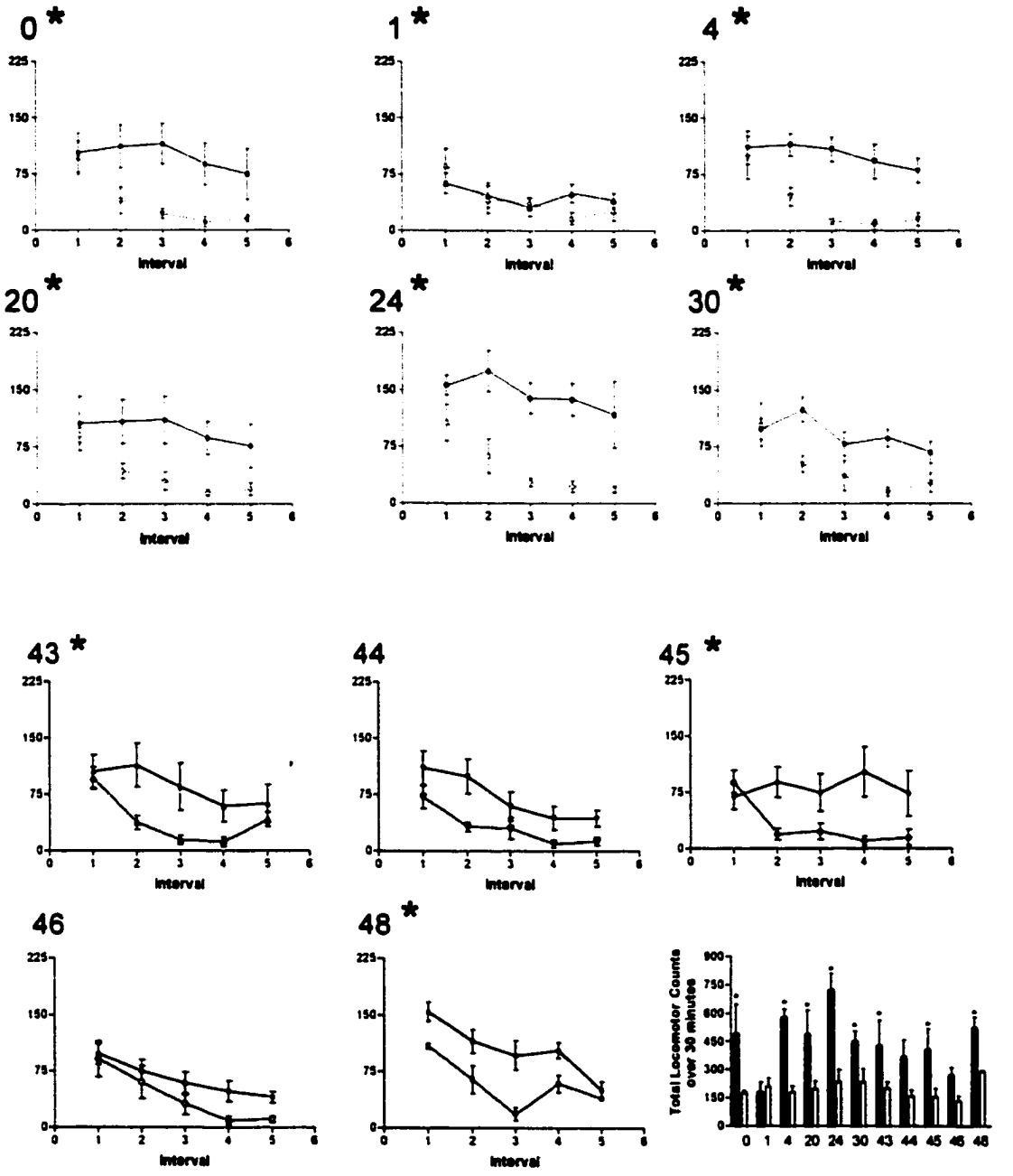


Figure 26: *Total locomotor behavior of ovariectomized cocaine- and saline- treated animals in each of the progesterone time conditions.* Total locomotor activity over 30 minutes following administration of saline (open symbols) or cocaine (closed symbols) for OVX Fischer rats pretreated with estrogen (48 hours before cocaine or saline treatment) and progesterone at 0, 1, 4, 20, 24, 30, 43, 44, 45, 46, or 48 hours after estrogen treatment. Data are represented as a cumulative mean (+ SEM) of five six-minute time bins. The last panel represents cumulative total locomotor activity for saline- (white bars) and cocaine-treated (black bars) animals in each of the progesterone conditions for the 30 minutes of behavioral testing.

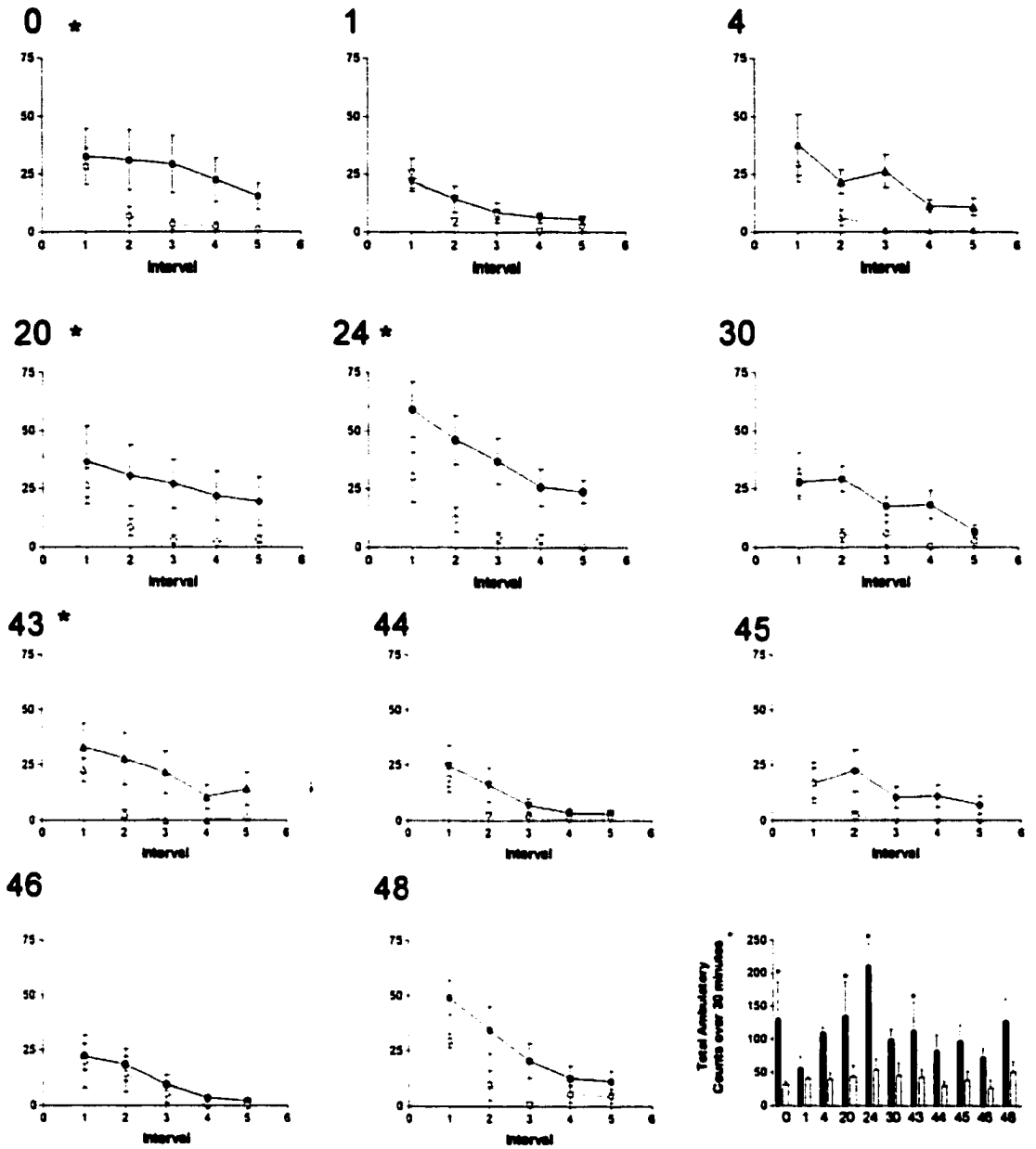


Figure 27: *Ambulatory counts for ovariectomized cocaine- and saline- treated animals in each of the progesterone time conditions.* Ambulatory activity over 30 minutes following administration of saline (open symbols) or cocaine (closed symbols) for OVX Fischer rats pretreated with estrogen (48 hours before cocaine or saline treatment) and progesterone at 0, 1, 4, 20, 24, 30, 43, 44, 45, 46, or 48 hours after estrogen treatment. Data are represented as a cumulative mean (+ SEM) of five six-minute time bins. The last panel represents cumulative ambulatory activity for saline- (white bars) and cocaine-treated animals in each of the progesterone conditions for the 30 minutes of behavioral testing.

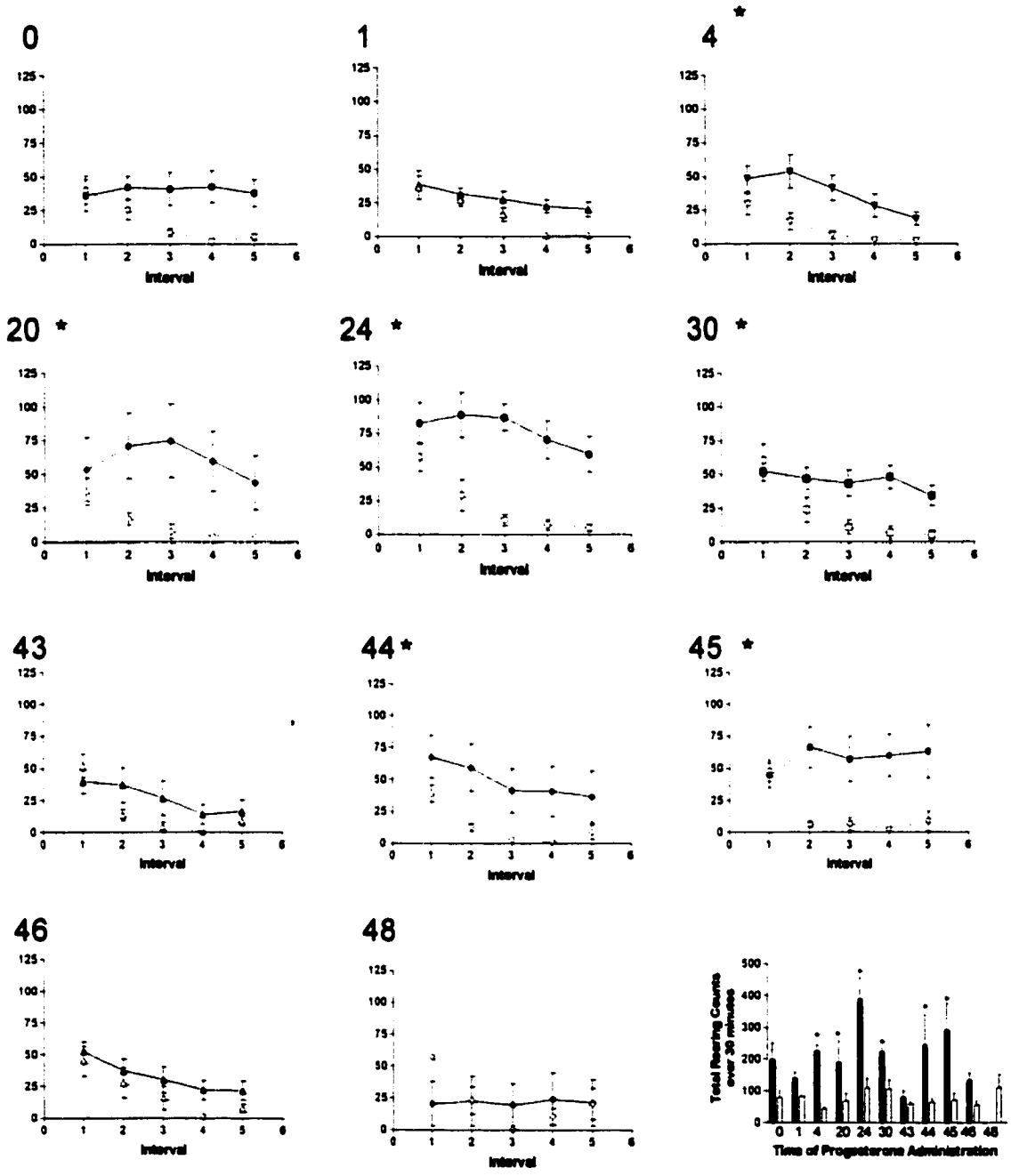


Figure 28: *Rearing activity of ovariectomized cocaine- and saline- treated animals in each of the progesterone time conditions.* Rearing activity over 30 minutes following administration of saline (open symbols) or cocaine (closed symbols) for OVX Fischer rats pretreated with estrogen (48 hours before cocaine or saline treatment) and progesterone at 0, 1, 4, 20, 24, 30, 43, 44, 45, 46, or 48 hours after estrogen treatment. Data are represented as a cumulative mean (+SEM) of five six-minute time bins. The last panel represents cumulative rearing counts for saline- (white bars) and cocaine-treated animals in each of the progesterone conditions for the 30 minutes of behavioral testing.

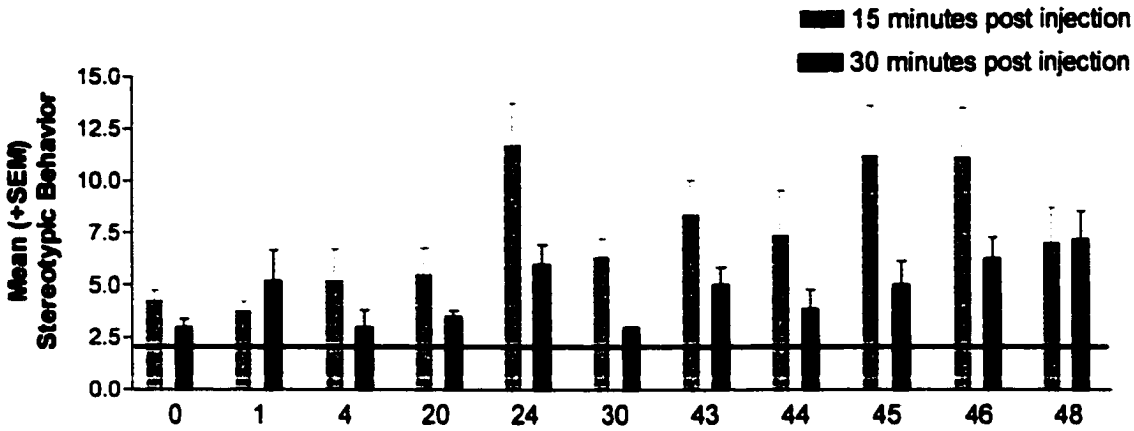


Figure 29: *Behavioral stereotypy of cocaine- and saline-treated female rats in each of the progesterone time conditions.* Mean (+ SEM) scores of stereotypic behavior 15 (gray bar) and 30 (black bar) minutes post cocaine treatment for OVX Fischer rats pretreated with estrogen (48 hours before cocaine treatment) and progesterone at 0, 1, 4, 20, 24, 30, 43, 44, 45, 46, or 48 hours after estrogen treatment. Mean activity of saline animals averaged 2.4 and is represented by a solid line across the graph.

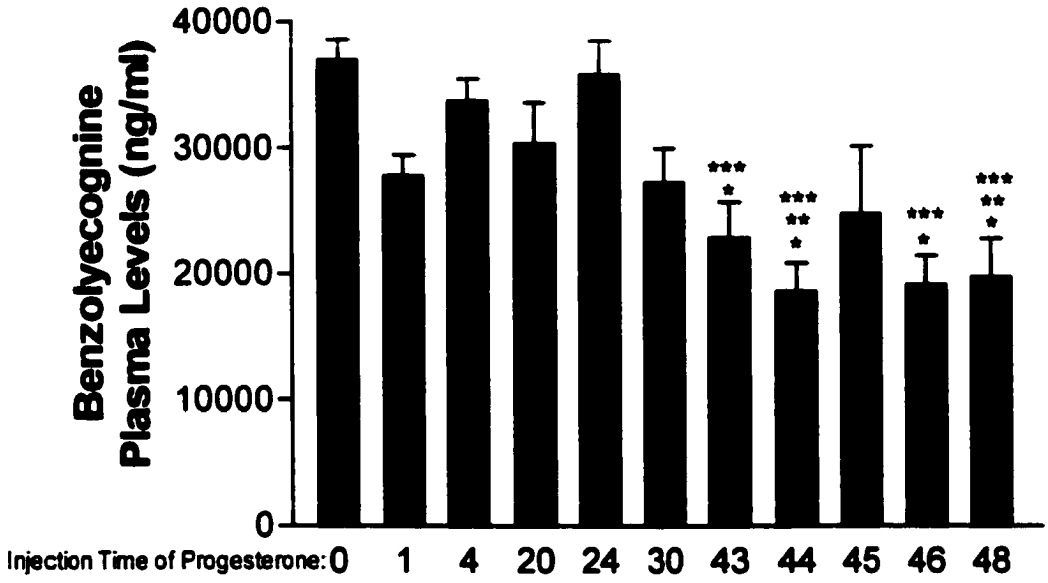


Figure 30. *Plasma levels of benzoylecgonine for hormone pretreated OVX Fischer rats.* Mean (+ SEM) plasma levels of benzoylecgonine for cocaine treated animals in all 11 hormone treatment groups. [* indicates a significant difference vs. 0 hour group, ** indicates a significant difference vs. 4 hour group, *** indicates a significant difference vs. 24 hour group]

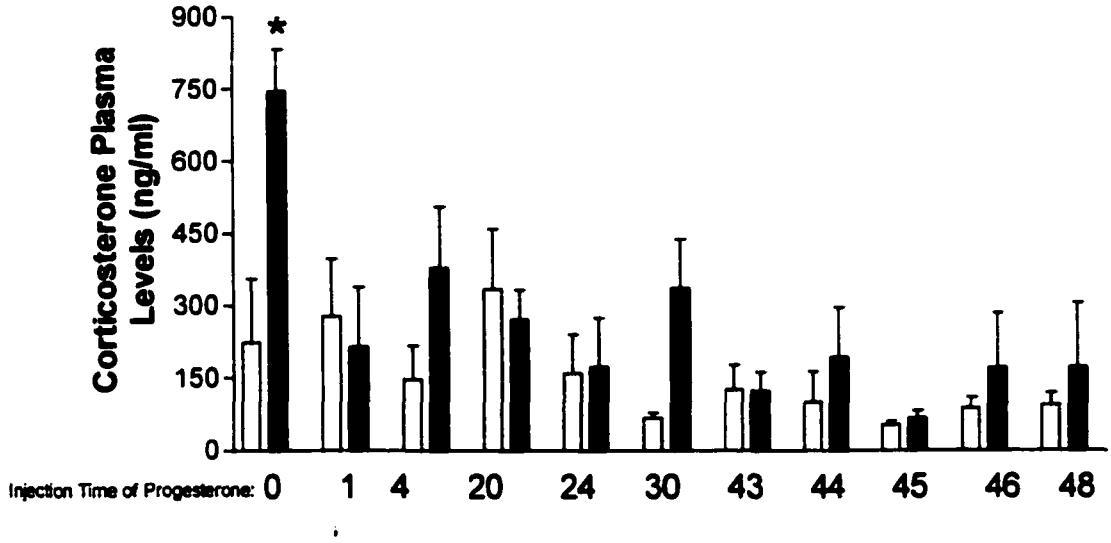


Figure 31. *Plasma corticosterone levels for hormone pretreated OVX Fischer rats after cocaine or saline treatment.* Mean + SEM plasma levels of corticosterone (expressed as ng/ml) for cocaine- (black bars) and saline-treated (white bars) rats in all 11 hormone treatment conditions.

lordosis (Satou, M. & Yamanouchi, K., 1996). First, cocaine-induced increases in locomotor activity (when estrogen and progesterone were co-administered) 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. However, in the case of total locomotor activity a third increase in behavioral activity was observed, when progesterone was administered concurrent with cocaine (48 hours after estrogen), while this same treatment inhibited cocaine-induced increases in rearing and ambulatory activities. The third increase in total locomotor activity may reside in the modulation by estrogen and progesterone of other cocaine-induced behavioral activity, i.e. frequency of stereotypic behaviors. The temporal relationship observed in the present study supports our previous observation during "binge" pattern cocaine administration in terms of a bimodal interaction between estrogen and progesterone in the control of cocaine-induced locomotor activity.

Cocaine-induced behavioral effects are significantly different in the female rat during different stages of the reproductive cycle. For example, the estrous cycle influences a rat's motivation to self-administer cocaine as well as cocaine-induced behavioral activation (Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989; Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). Rats in estrus self-administer more cocaine (Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989) and demonstrate more cocaine-induced locomotor behavior (Quiñones-Jenab,

V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999), while during diestrus rats self-administer cocaine at a lower rate of responding (Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J., 1989) and display less cocaine-induced behavioral activity when compared to rats at other stages of the cycle (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). Based on this study, it is possible that differences in the behavioral response to cocaine during the different stages of the cycle may reside in a temporal interaction of estrogen and progesterone. Thus, the higher cocaine-induced activity during estrus and decreased cocaine-induced activity during diestrus may be based on progesterone's synergistic and inhibitory effects on estrogen-mediated behaviors.

Unlike locomotor activity, no temporal interaction between estrogen and progesterone was observed in the modulation of stereotypic behavior. The lack of gonadal hormone effects on stereotypic behaviors supports our previously published observations after a single administration of estrogen, progesterone, and cocaine (where estrogen and progesterone administration did not modulate stereotypic behavior) (Perrotti, L. I., Russo, S., Lagos, F, & Quinones-Jenab, V, 2000). However, it is also possible that estrogen and progesterone modulate the frequency of components of stereotypic activities rather than the quantity of behaviors. This may account for some of the discrepancies obtained between, ambulatory, and total locomotor activities.

Benzoylecgonine (BE) is an active metabolite of cocaine. Gender and estrous cycle differences in BE plasma levels have been reported (Bowman, B. et al., 1999; Chin,

J. et al., 2000b). Modulation of BE levels by gonadal hormones have also been reported (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000); where 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 also observed where overall, BE plasma levels were lower in animals treated with progesterone for shorter periods of time. Similar to behavioral activity, the liver enzymes that metabolize cocaine may be regulated by estrogen and progesterone in a biphasic manner. The relationship between cocaine metabolism and the interactions between estrogen and progesterone remains to be elucidated. However, this bimodal interaction between estrogen and progesterone in BE levels may account for estrous cycle related differences in cocaine metabolism.

Cocaine administration leads to an elevation in plasma corticosterone levels in males and females (Moldow, R. L. & Fischman, A. J., 1987; Zhou, Y. et al., 1998; Koob, G. F. & LeMoal, M., 1997; Kuhn, C. & Francis, M. S., 1997), where female rats demonstrate an exaggerated HPA response to cocaine (Kuhn, C. & Francis, M. S., 1997). Moreover, corticosterone levels vary throughout the estrous cycle of the rat (Atkinson, H. C. & Waddell, B. J., 1997). Thus, suggesting that reproductive hormones may play an important role in HPA modulation of cocaine-induced alterations. A narrower temporal interaction between estrogen and progesterone in HPA axis activation was observed; where after only concurrent administration of estrogen and progesterone were corticosterone levels in cocaine-treated rats increased. Interestingly, although HPA

activity is believed to play a major role in modulating cocaine-induced behavioral activity (Moldow, R. L. & Fischman, A. J., 1987; Zhou, Y. et al., 1998; Koob, G. F. & LeMoal, M., 1997; Kuhn, C. & Francis, M. S., 1997), this narrow temporal interaction between progesterone and estrogen on corticosterone levels do not correlate with the estrogen and progesterone behavioral alterations. Thus, suggesting that the bimodal interaction of estrogen and progesterone on cocaine-induced behaviors does not reside completely in HPA axis activation or cocaine metabolism.

Gonadal regulation of CNS plasticity has been postulated to occur in stages: an early and rapid modulation via cellular alterations followed by a longer, genomic mediated mechanism. The biphasic regulation by progesterone and estrogen on cocaine-induced behaviors may involve separate mechanisms of neuronal modulation. Thus, the rapid inhibitory effects of progesterone (i.e. observed in animals treated with 48 hours of estrogen and 4-2 hours of progesterone) may be mediated via rapid activation of cellular events, such as DA/5-HT regulation; while the gradual induction of activity observed in animals treated with progesterone (4-40 hours) may activate other mechanisms, such as protein synthesis. This remains to be elucidated.

CHAPTER 7: PROGESTERONE AND COCAINE ADMINISTRATION AFFECT MONOAMINES IN THE MEDIAL PREFRONTAL CORTEX OF OVARIECTOMIZED RATS.

Cocaine has a variety of pharmacological actions; however, its major effect is the inhibition of the reuptake of neuronal monoamines (Heikkila, R. E., Orlansky, H., & Cohen, G., 1975). Cocaine binds with dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporters and prevents the reuptake of these monoamines, thus, increasing their synaptic concentrations (Heikkila, R. E., Orlansky, H., & Cohen, G., 1975). These neurotransmitter systems are believed to be an integral part of the cascade of events that are involved in the psychomotor and subjective effects of cocaine in the CNS.

Cocaine administration has been shown to alter these monoamine levels in the medial prefrontal cortex (mPFC), an area shown to be involved in the initiation or acquisition of cocaine self-administration (Goeders, N. E. & Smith, J. E., 1983). For example, in the mPFC, acute cocaine administration increased extracellular DA levels (Maisonneuve, I. M. & Kreek, M. J., 1994), while repeated administration caused deceases in DA levels (Sorg, B. A., Davidson, D. L., Kalivas, P. W., & Prasad, B. M, 1997). Acute interperitoneal or self-administration of cocaine decreased dopamine and serotonin turnover (Goeders, N. E. & Smith, J. E., 1993; Carey, R. J. & Damianopoulos, E. N., 1994). Moreover, 6-hydroxydopamine lesions of DA neurons in the mPFC decreased self-administration of cocaine into this brain area (Goeders, N. E. & Smith, J. E., 1986), but did not affect intravenous cocaine self-administration (Martin-Iverson, M. T., Szostak, C., & Fibiger, H. C., 1986). However, this effect that was later found to be

dose-dependent (McGregor, A., Baker, G., & Roberts, D. C. S., 1996; Schenk, S., Horger, B. A., Peltier, R., & Shelton, K., 1991).

Evidence suggests that ovarian hormones, estrogen and progesterone, may be an important component in the cascade of events modulating cocaine-induced behavioral and neurochemical adaptations after cocaine administration. For example, estrogen and progesterone modulate cocaine-induced behaviors in ovariectomized rats (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000; Sircar, R. & Kim, D., 1999). Thus, interactions between ovarian hormones, monoamines, and cocaine may be a key mechanism underlying observed gender (Sircar, R. & Kim, D., 1999; Caihol, S. & Morméde, P., 1999) and estrous cycle (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999) differences in response to cocaine and may have important implications in cocaine use and abuse. The purpose of this study was to examine the effect of ovarian hormone pre-treatment in cocaine-induced alterations in monoamine levels of the mPFC.

Two cohorts (each with 24 animals) of ovariectomized (OVX) female Fischer rats purchased from Charles River Laboratories (N.C.) were individually housed in standard cages, in a stress-minimized facility. Rats had free access to food and water, and were maintained on a 12-hour light/dark cycle (lights on at 10:30 AM EST). All NIH Guidelines for the Care and Use of Laboratory Animals were followed.

Two weeks after ovariectomy, rats were randomly assigned to either cocaine- or saline-treatment groups, and then, further divided into one of four hormone pretreatment conditions: vehicle-control (sesame oil), estrogen, progesterone, or estrogen+progesterone (n=6 per group). Forty-eight hours prior to the start of drug treatment, animals (estrogen and estrogen+progesterone groups) received subcutaneous (s.c.) injections of estrogen benzoate (50μg dissolved in sesame oil) or sesame oil (vehicle and progesterone groups). Four hours prior to cocaine treatment animals (progesterone and estrogen+progesterone groups) received s.c. injections of progesterone (500μg dissolved in sesame oil) or sesame oil (vehicle and estrogen groups). This administration paradigm and the doses of steroids used have been shown to induce lordosis behavior in ovariectomized rats (Pfaff, D. W. & Schwartz-Giblin, S., 1995), as well as modulate cocaine-induced locomotor and stereotypic activities (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000).

At 11:00 AM EST (48 hours post estrogen treatment and 4 hours post progesterone treatment) animals received three interperitoneal injections, administered in a "binge" paradigm [one hour apart, of 0.9% saline (1 ml/kg) or cocaine 15 mg/kg dissolved in 0.9% saline at a concentration of 15 mg/ml)]. This "binge" dosing schedule was chosen to mimic the manner in which cocaine is often self-administered by humans, both in terms of temporal pattern and in relation to circadian rhythm (Branch, A. D., Unterwald, E. M., Lee, S. E., & Kreek, M. J., 1996). Throughout the study all injections were administered in each rat's home cage.

Thirty minutes after the last injection of cocaine, brains were removed and frozen at -70°C until used. A sagittal section was obtained between Bregma 4.70 and 3.00 using a razor blade, and under a dissecting microscope, three bilateral punches were made down the median line. As previously described (Beck, K. D. & Luine, V. N., 1999), 60 μl of sodium acetate buffer containing α-methyl-dopamine (internal standard) was added and samples were frozen and thawed to release the transmitters. The samples were centrifuged and pellets were resuspended for protein analysis by the Bradford method (Bradford, M., 1976). Forty μl of supernatant was injected into a high-performance liquid chromatography (HPLC) with electrochemical analysis detection system to quantify levels of monoamines (5-HT, DA, and NE) and metabolites (5HIAA, DOPAC, and HVA). The mobile phase, described elsewhere (Luine, V. N., Bowling, D., & Hearns, M., 1990), was pumped through a Waters Alliance module connected to an ESA Coulochem II detector (+ 0.48-+ 0.50 V potential) via a C-18 reverse phase column (Brownlee Velosep RP-18, 3μm). An additional 100% methanol gradient was introduced into the flow (99.5% mobile phase: 0.5% methanol) to increase peak sharpness. Single sample runs averaged between 12-20 minutes.

Tissue levels for monoamines were calculated using peak areas under the curve by a Waters Millennium computer based program. Levels were expressed as pg/μg protein. Two-way analyses of variance (DRUG * HORMONE) were used to analyze all neurochemical data. Where appropriate, Newman Keuls post hoc tests were used to test for groups differences.

A significant DRUG * HORMONE interaction was obtained for levels of 5-HT in the mPFC [F (3,27) = 3.564, $p < 0.05$]. Co-administration of progesterone and cocaine resulted in higher levels of 5-HT when compared to other cocaine and hormone-treated rats ([Newman Keuls: $p < 0.02$; $p < 0.02$, estrogen and estrogen+progesterone, respectively]; Figure 32A). A significant hormone treatment effect was also obtained for levels of 5-HIAA, a 5-HT metabolite, [F (3,37) = 3.295,$p < 0.05$]. Regardless of drug-treatment condition, progesterone treated rats had overall higher levels of 5-HIAA than rats in the other three groups ([Newman Keuls: $p < 0.05$; $p < 0.05$; $p < 0.02$, vehicle-control, estrogen and estrogen+progesterone, respectively];Figure 32B). When data were analyzed as a turnover ratio, 5-HIAA:5-HT, no significant differences were found [F(3,27)=1.238, p = 0.315].

Although levels of dopamine, DOPAC, and HVA decreased in OVX rats co-administrated estrogen+progesterone and cocaine, these decreases were not statistically significant ([F(3,27)=1.221, p = 0.320;F(3,27)=0.742, p= 0.536; F(3,27)=0.901, p =0.453], Figure 33). When data were analyzed as a turnover ratios, DOPAC and HVA :DA, no significant differences were found [F(3,27)=0.860, p = 0.473;F(3,27)=1.346, p = 0.280]. Furthermore, norepinephrine levels in the medial prefrontal cortex were not affected by drug or hormone treatment [F(3,27)=0.603, p = 0.618], Figure 34).

In male rats, previous studies show that cocaine administration decreases serotonin synthesis (Baumann, M. H., Raley, T. J., Partilla, J. S., & Rothman, R. B., 1993; Galloway, M. P., 1990) and turnover (Carey, R. J. & Damianopoulos, E. N., 1994)

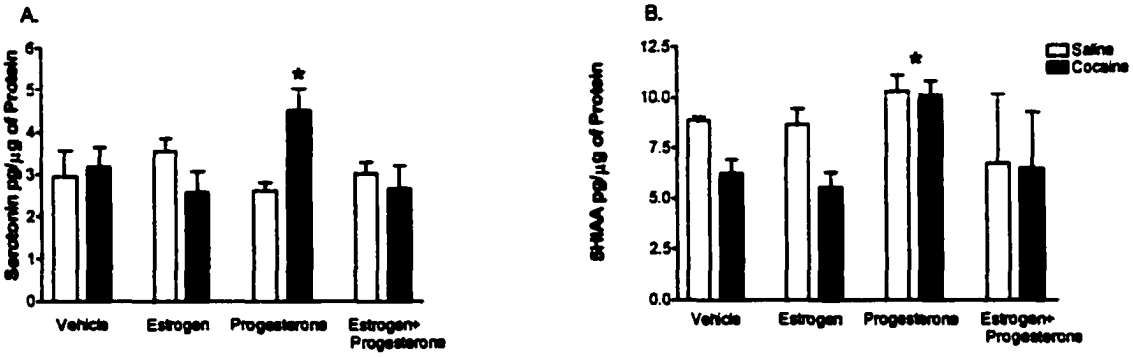


Figure 32: Mean (+ SEM) levels of serotonin (A) and 5-HIAA (B) in the medial prefrontal cortex after administration of cocaine (black bars) or saline (white bars) in OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen+progesterone. * denotes a difference of $p < 0.05$ from estrogen and estrogen+progesterone cocaine groups and progesterone saline control group.

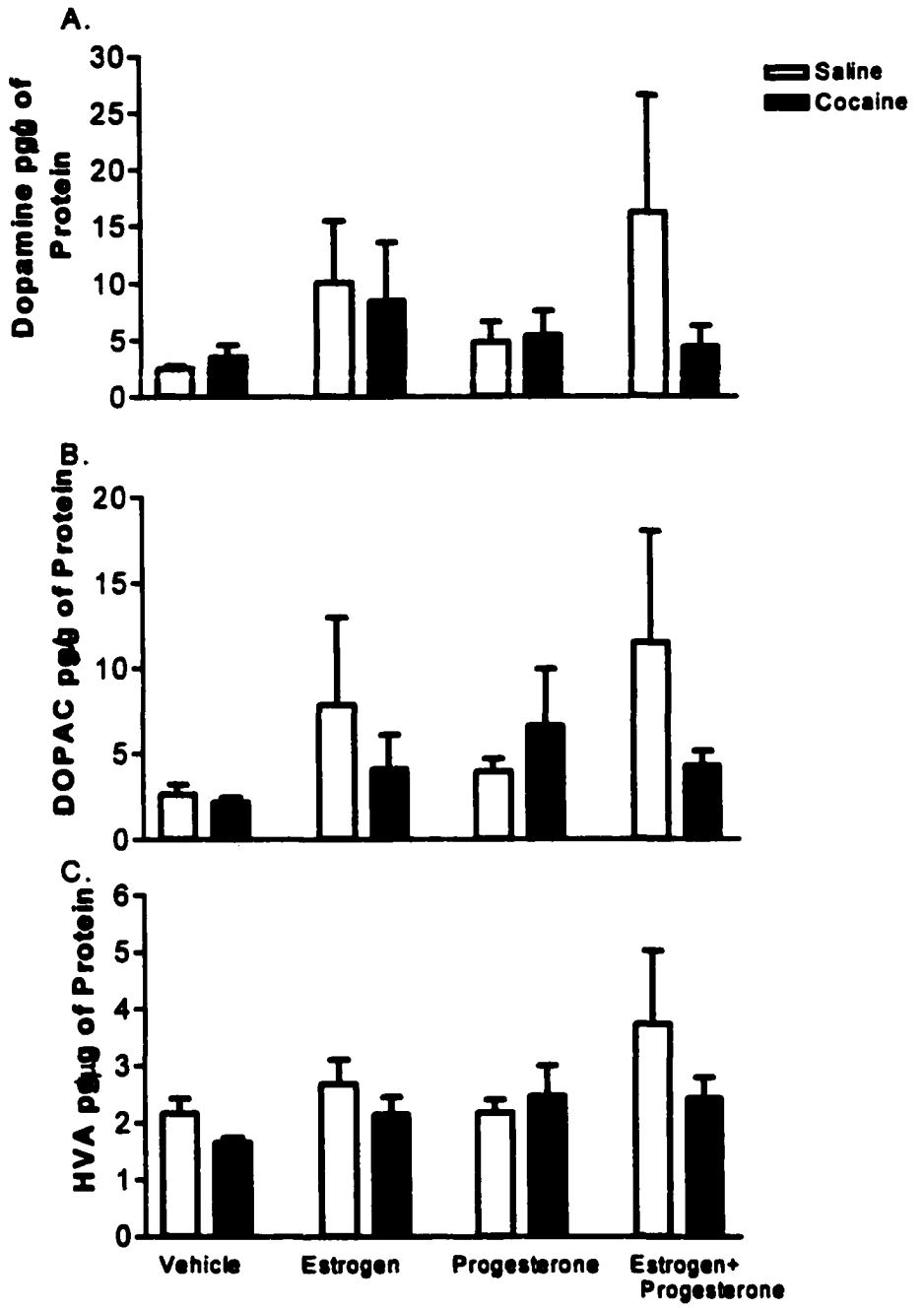


Figure 33: Mean (+ SEM) levels of dopamine (A), DOPAC (B), and HVA (C) in the medial prefrontal cortex after administration of cocaine (black bars) or saline (white bars) in OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen+progesterone.* denotes an overall difference of $p < 0.05$ of progesterone treated animals from all other hormone treated groups.

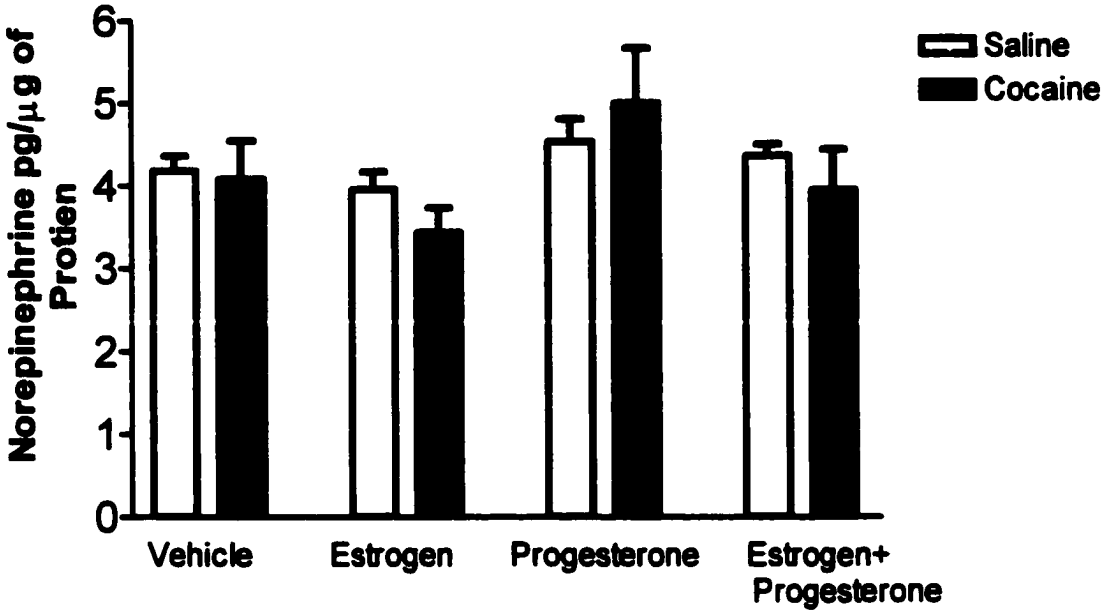


Figure 34: Mean (+; SEM) levels of norepinephrine in the medial prefrontal cortex after administration of cocaine (black bars) or saline (white bars) in OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen+progesterone.

in the mPFC. Thus, consistent with the male data, alterations in the serotonergic system were observed in OVX female rats. However, these alterations were only evident when rats were treated with progesterone. Further, levels of serotonin in the mPFC have been correlated with locomotor activity (Morrow, B. A. & Roth, R. H., 1996; Carey, R. J. & Damianopoulos, E. N., 1994). Previous studies have demonstrated that progesterone or estrogen+progesterone modulate locomotor and stereotypic activity in OVX Fischer rats (Sircar, R. & Kim, D., 1999). Thus, progesterone modulated changes in cocaine-induced alterations of 5-HT levels may be related to previously observed ovarian hormone effects on cocaine-induced locomotor activity in rats (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). Therefore, progesterone modulation of cocaine-induced alterations in the serotonin system may be an important element in the neuronal events underlying the interactions between cocaine and steroid hormones on behavior, and may be a key element in pathways that modulate estrous cycle differences.

The modulation of progesterone on cocaine-induced 5-HT levels without a change in the 5HIAA:5-HT ratio suggests that there is a decrease in turnover rate. This finding is consistent with previous reports in male rats where cocaine decreased 5-HT turnover in the mPFC [5;6]. Carrey et al.(Carey, R. J. & Damianopoulos, E. N., 1994; Goeders, N. E. & Smith, J. E., 1993) suggested that a decrease in 5-HT turnover after acute cocaine administration was probably due to an inhibitory feedback mechanism. Progesterone may potentiate cocaine effects on these inhibitory mechanisms. Future experiments should use in vivo microdialysis and/or pargyline treatment to further determine the effects of progesterone in cocaine-induced 5-HT release or turnover.

Chronic exposure to cocaine causes changes in 5-HT function that mimic depression (Parsons, L. H. & Justice, J. B., 2000; Baumann, M. H. & Rothman, R. B., 1998). Moreover, women who are drug addicts have a high propensity for comorbid depression, and this negatively impacts on treatment success (Boyd, C. J., 1993; Grant, B. F., 1995). Thus, the enhanced effects of cocaine on 5-HT systems in women might explain this observation. Moreover, medications targeting 5-HT neurons could be useful pharmacological adjuncts for treating women drug addicts.

In the mPFC of male rats, dopamine synthesis and turnover are affected by cocaine (Baumann, M. H., Raley, T. J., Partilla, J. S., & Rothman, R. B., 1993; Galloway, M. P., 1990). However, we observed a non-significant decrease in levels of DA, DOPAC, and HVA in OVX rats. Discrepancies between the male and female dopaminergic response to cocaine may be one of the factors that underlies sex differences in the behavioral response to cocaine. A more detailed time course and dose response study may reveal the extent to which the interactions between estrogen, progesterone modulate the DA response to cocaine in the mPFC of females.

Based on these and previous observations, interactions between steroids and cocaine may ultimately affect not only the contraceptive treatment (Lundahl, L. H., Kouri, E. M., & Lukas, S. E., 1999) but also the behavioral (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000; Sircar, R. & Kim, D., 1999) and neurochemical responses to cocaine of female addicts using gonadal-based

anticonceptive methods. Furthermore, results of this study suggest that gonadal hormones affect the ability of cocaine to alter monoamine levels. Thus, the effects of cocaine on neurotransmission may be affected according to which steroid-based contraceptive a women is using or where she is in her reproductive cycle. This important clinical issue in females warrants further investigation.

CHAPTER 8: VENDOR DIFFERENCES IN COCAINE-INDUCED BEHAVIORAL ACTIVITY AND HORMONAL INTERACTIONS IN OVARIECTOMIZED FISCHER RATS

Recently, both Sicar and Kim (1999) and our laboratory, have demonstrated that estrogen and progesterone modulate cocaine-induced behavioral activity in ovariectomized (OVX) Fischer rats (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000; Sircar, R. & Kim, D., 1999). However, we observed an inhibition of cocaine-induced locomotor activity after co-administration of estrogen, progesterone and cocaine (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000), while Sicar and Kim, reported an increase in locomotor activity (Sircar, R. & Kim, D., 1999). Although both groups used OVX Fischer rats, they were obtained from different commercial sources. Vendor differences have been observed in other inbred strains. For example, Wister rats show differences in stress-induced alterations (Pare, W. P. & Kluczynski, J., 1997). Since Fischer rats are an inbred strain, vendor differences may underlie the differences in behavioral observations between both laboratories. This study was designed to test this postulate.

Methods

Animals:

Eight-week-old OVX female Fischer rats were purchased from either Charles River Laboratories (N.C.) or Taconic (N.Y.). Rats from both vendors were group housed prior to ovariectomy; eight-week old rats were packed in crates and shipped to their respective vendor's surgical site where ovariectomy was preformed. After a recovery

period of one-two days animals were packed in wooden crates and shipped to our facilities where they arrived within 24 hours. Once in our facility, rats were individually housed in standard cages with free access to food and water and maintained on a 12-hour light : dark cycle with lights on at 11:00 A.M. EST.

This study was run in two separate cohorts; cohort one consisted of 48 eight-week-old Charles River rats, cohort two consisted of 48 eight-week-old Taconic rats. All animals were housed in the same room in our animal facility under the same conditions and cared for by the same animal technicians at the same time each day. Following a one week acclimatization period in our laboratory, rats were randomly assigned to either cocaine- or saline-treatment groups, and then, further subdivided into one of four hormone pretreatment conditions: vehicle control, estrogen, progesterone or estrogen+progesterone (n=6 per group). Thirty minutes after drug treatment, animals were decapitated after brief exposure to CO_2, and trunk blood was collected. All NIH Guidelines for the Care and Use of Laboratory Animals were followed.

Drug and hormone treatment:

Animals received either subcutaneous (s.c). injections of estrogen benzoate (50 μg/rat; dissolved in sesame oil) or sesame oil, according to their respective experimental groups. Forty-four hours post-estrogen treatment, animals received s.c. injections of either progesterone (500 μg/animal; dissolved in sesame oil) or sesame oil. Forty-eight hours after estrogen treatment (four hours after progesterone injection), animals received one i.p. injection of cocaine (15 mg/kg; dissolved in 0.9% saline) or saline. This

administration paradigm and these doses of steroids have been shown to induce lordosis behavior in ovariectomized rats (Pfaff, D. W. & Schwartz-Giblin, S., 1995) and affect cocaine-induced behavioral alterations (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). All injections were administered in each rat's home cage.

Stereotypic behaviors:

Both stereotypic and locomotor activities were analyzed for each animal. All behavioral assays were performed in each rat's home cage for 30 minutes after cocaine administration. After cocaine or saline administration, each animal's behavior was videotaped for 25 seconds at 15 and 30 minutes post-injection. The videotapes were later analyzed for behavioral stereotypy by three trained observers blind to each animal's treatment group. The rating for cocaine-induced stereotypic behaviors was based upon a modification (Daunais, J. B. & McGinty, J. F., 1995) of the Creese and Iversen scale (Creese, I. & Iversen, D., 1974). This scale consists of 10 scores, ranging from a score of 1 (given to an animal that was asleep or inactive) to 10 (given to an animal that exhibited splayed hind limbs). A score of 10 was never observed during the course of this experiment.

Locomotor activity:

Spontaneous locomotor activity was monitored with a Photobeam Activity System from San Diego Instruments (CA). These monitors consist of two frames, in which the standard cage is placed. One frame counts horizontal activity and the other

vertical activity. With this technique, there was no change in the environment of the animals during measurements, and both stereotypic and locomotor activities were measured simultaneously.

Total locomotor counts were determined by calculating the sum of counts on all four channels detecting horizontal motion. Ambulatory activity was determined by total counts of two consecutive photobeam interruptions in the horizontal frame. Rearing activity was represented as total counts of vertical motions. Cumulative counts over a period of 30 minutes after cocaine or saline administration were used for all three measurements.

Plasma levels of benzoylecgonine and corticosterone:

Trunk blood was allowed to clot and then centrifuged at 3,000 RPM for 15 minutes at 4°C. Plasma was collected and stored at -70°C until radioimmunoassays were preformed. Samples were analyzed with Count-A-Count Cocaine Metabolite or Corticosterone RIA kit from Diagnostic Product Corporation (CA).

Data analysis:

To look for possible interactions of hormone treatment and drug treatment on locomotor and stereotypic behaviors for animals from each vendor separately, two-way ANOVAs were run (Drug × Hormone-treatment group). To examine the effects of vendor (Charles River vs. Taconic) on locomotor and stereotypic activity a three-way

ANOVA was run [Drug × Hormone × Vendor]. When significant, differences between groups were examined using Newman Keuls *post hoc* tests.

To locate differences in body weight of rats from the different vendors and in amount of weight gained over the course of the study a two-way repeated measures ANOVA was used.

To examine the effects of vendor and hormonal treatment on plasma levels of the cocaine metabolite benzoylecgonine and corticosterone two and three factor ANOVAs were used, followed by Newman-Keuls *post hoc* tests.

Results

Charles River Rats' Behavioral Activity: Overall, cocaine-treated rats from Charles River displayed increases in total locomotor [$F(1,40) = 32.076, p < 0.001$], ambulatory [$F(1,40) = 9.079, p < 0.005$], and rearing [$F(1,40) = 16.892, p < 0.001$] activities, when compared to saline-treated animals (Figure 35A, C, E). A significant interaction between drug and hormone treatments on ambulatory activity was observed [$F(2,37) = 3.412, p < 0.03$]. Estrogen+progesterone significantly lowered cocaine-induced ambulatory activity [$p < 0.05$] (Figure 35C), but increased rearing [$p < 0.02$] and total locomotor activity [$p < 0.03$] when compared to corresponding saline/hormone treated rats (Figure 35E and 35A, respectively).

As shown in Figure 36A, cocaine-treated Charles River rats displayed more stereotypic behaviors than saline-treated animals [$F(1, 38) = 81.304, p < 0.001$]. Steroid treatment did not alter stereotypic activity in either saline- or cocaine-treated groups [$F(1,38) = 0.322, p = 0.809$].

Taconic Rats' Behavioral Activity: Similar to Charles River rats, Taconic rats also displayed increases in locomotor and ambulatory activity in response to cocaine ([$F(1,40) = 14.642, p < 0.0005$; $F(1,40) = 8.704, p < 0.01$, respectively]; Figure 35B and D). Interestingly, when compared to saline-treated rats, rearing activity was not altered by cocaine administration ([$F(1,40) = 1.594, p > 0.2$]; Figure 35F). Furthermore, no interaction between drug and hormone treatments on total locomotor, ambulatory, or rearing activity was observed ([$F(3,40) = 0.627, p > 0.5$; $F(3,40) = 1.071, p > 0.2$; $F(3,40) = 0.336, p > 0.5$, respectively]; Figure 35B, D, F).

Cocaine administration caused increases in stereotypic behaviors in Taconic Fischer rats when compared to saline-treated animals ($F(1,N=40) = 38.159, p < 0.001$]; Figure 36B). Similar to Charles River Fischer rats, no differences in stereotypic behaviors were observed in either cocaine- or saline-treated animals after the different steroid replacement paradigms [$F(1,40) = 2.521, p = 0.0714$].

Vendor Differences in Behavioral Activity: Overall, cocaine-treated rats from Charles River had higher total locomotor and rearing activity than Taconic rats ([$F(1,80) = 9.423, p < 0.005$; $F(1,80) = 7.213, p < 0.01$, respectively]; Figure 1A and B, E and F,

respectively). However, no significant differences in cocaine-induced ambulatory activity between vendors were observed ([F (1,80) = 1.626, $p < 0.2$]; Figure 35C and D). No vendor differences were observed in stereotypic behaviors after cocaine or saline administration or hormonal treatment [F (3,78) = 0.495, $p = 0.686$].

Body Weight: As is shown in Figure 37, although rats from both vendors consistently gained weight over time [F (4, 196) = 240.902, $p < 0.001$; F (4, 196) = 27.976, $p < 0.001$, Charles River and Taconic, respectively], Charles River rats weighed significantly more than those obtained from Taconic [F (1,99) = 209.04, $p < 0.001$].

Vendor Differences in Plasma Levels of Benzoylecgonine and Corticosterone: Plasma levels of benzoylecgonine, a cocaine metabolite, were not significantly different between Taconic and Charles River rats ([F (3,26) = 0.371, $p > 0.773$]; Figure 38). Furthermore, the different ovarian hormone replacement paradigms did not alter benzoylecgonine levels in rats from either vendor [F (1,17) = 0.050, $p > 0.5$; F (1,12) = 1.032, $p > 0.2$, Charles River and Taconic, respectively].

Taconic rats had overall higher corticosterone plasma levels than those from Charles River ([F (1,55) = 42.483, $p < 0.01$]; Figure 39). Neither acute cocaine administration nor hormone treatment affected corticosterone levels in Charles River rats [$p > 0.2$]. A significant DRUG * HORMONE treatment interaction in plasma levels of corticosterone was found for Taconic rats [F (3,29) = 3.137, $p < 0.05$]. However, there

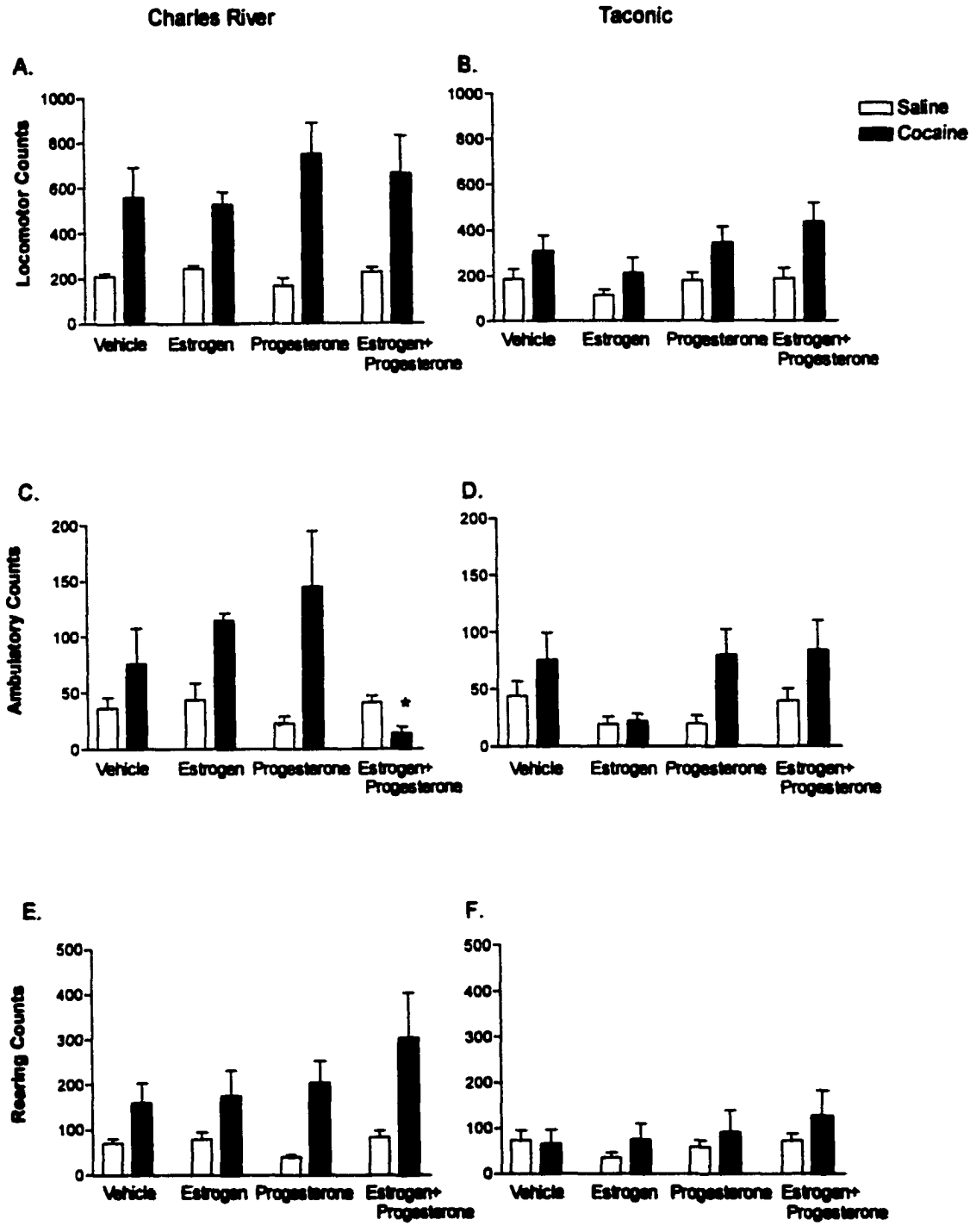


Figure 35. Three measures of locomotor activity (mean + SEM): locomotor counts (A,B), ambulatory activity (C,D) and rearing (E,F) for all cocaine- (black bars) and saline- (white bars) treated animals in all four hormone-treatment groups (n=6 per group) from Charles River (left column A, C, E) and Taconic (right column B, D, F).

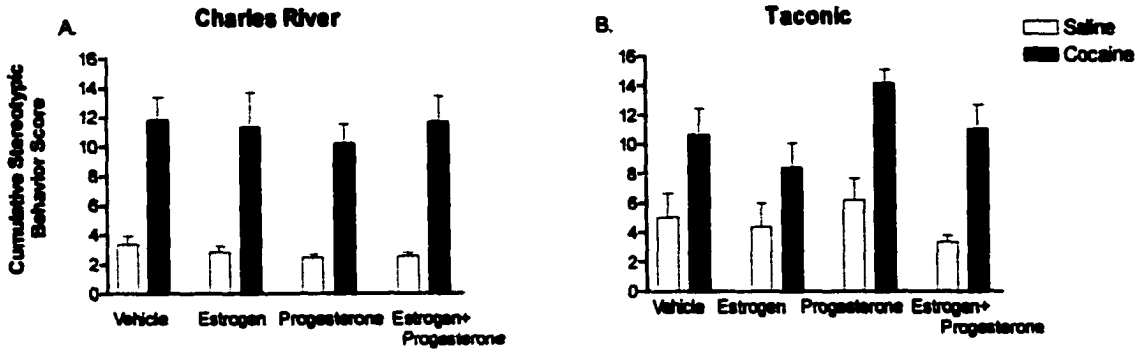


Figure 36: Cumulative scores of stereotypic behavior (mean + SEM) for cocaine- (black bars) and saline- (white bars) treated animals in each of the four hormone-treatment groups (n=6 per group) from both vendors, Charles River (A) and Taconic (B).

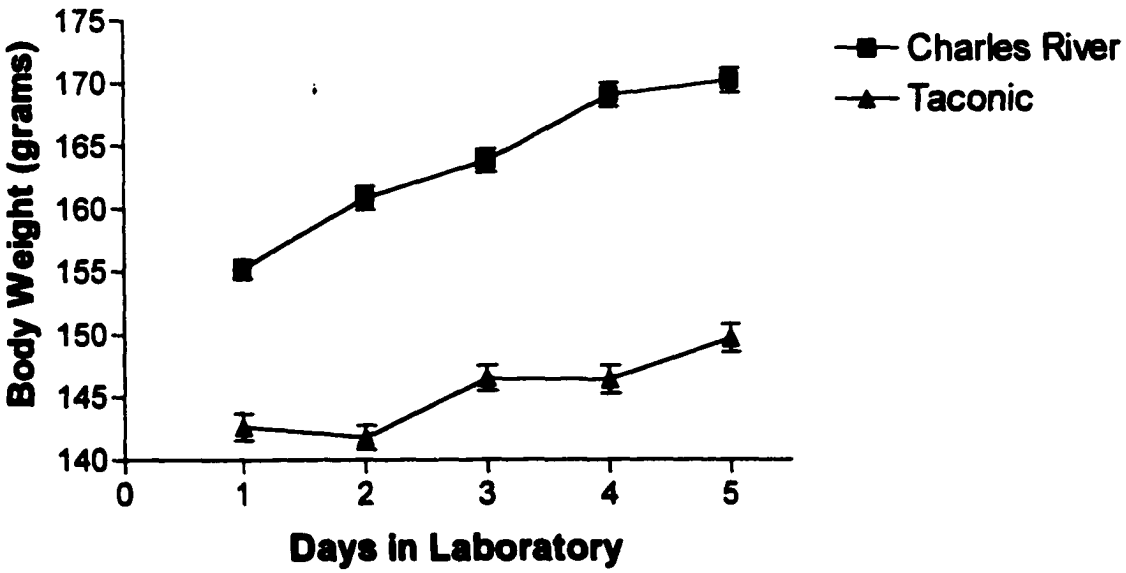


Figure 37: Mean ± SEM body mass in grams of rats from Charles River (squares) and Taconic (triangles) over the five days which they were housed in our laboratory.

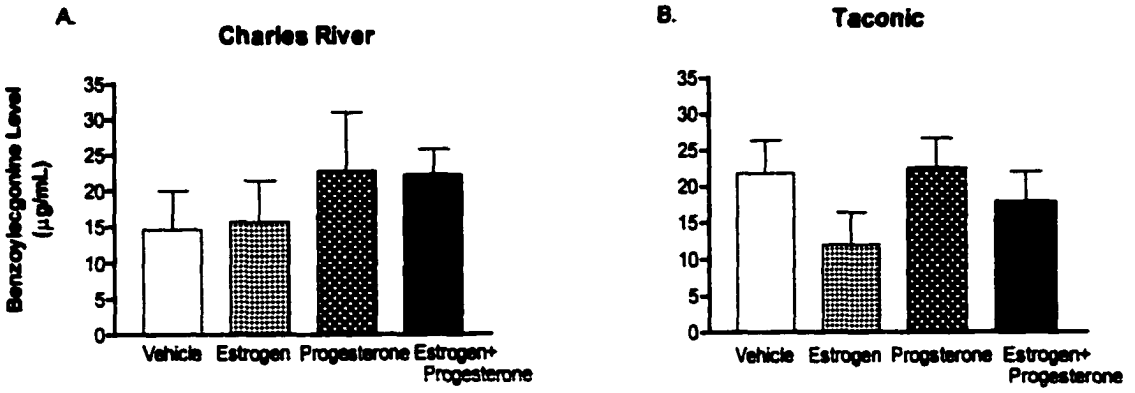


Figure 38: Benzoylecgonine plasma levels (mean + SEM) for all cocaine-treated animals in all four hormone-treatment conditions (n=6 per group) from Charles River (A) and Taconic (B).

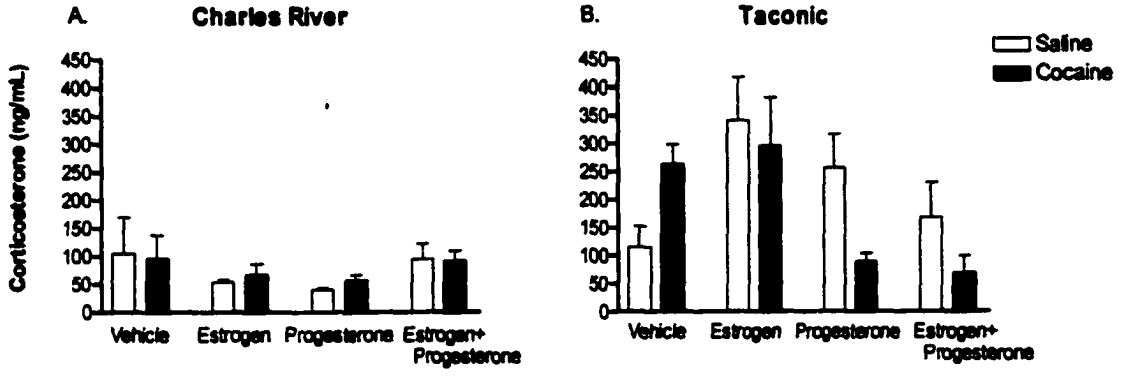


Figure 39: Corticosterone plasma levels (mean + SEM) for all cocaine- (black bars) and saline- (white bars) treated animals given each of the four hormone treatment conditions (n=6 per group). Charles River (A), and Taconic (B).

were no significant differences between steroid replacement or drug treatment groups when examined with Newman Keuls *post hoc* tests.

Discussion

Similar to previous reports (Sircar, R. & Kim, D., 1999; Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000), cocaine administration caused increases in locomotor and stereotypic activities in female Fischer rats. However, differences in cocaine-induced behavioral activation between vendors were observed. Overall, Charles River rats were behaviorally more active in response to cocaine than Taconic rats. Moreover, while Charles River animals demonstrated increases in all three locomotor measurements in response to cocaine, Taconic rats demonstrate only certain aspects of cocaine-induced locomotor activity (ambulatory and total locomotor activity).

In Charles River rats, we have previously observed that estrogen+progesterone pretreatment suppressed cocaine-induced increases in locomotor activity after "binge" (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000) or single dose administration when compared to estrogen- or progesterone-treated animals (Perrotti and Quiñones-Jenab, in preparation). Interestingly, Sicar and Kim (1999) reported that co-administration of estrogen+progesterone enhanced locomotor activities in OVX Fischer rats purchased from Taconic (Sircar, R. & Kim, D., 1999). In this report, we observed that estrogen+progesterone co-administration suppressed cocaine-induced ambulatory activity for Charles River rats, but not Taconic rats. The observed differences in behavioral activities were not due to differences in cocaine metabolism, since no

changes in plasma levels of benzoylecgonine were observed for rats from either vendor or after any of the hormone replacement paradigms.

Taconic saline-treated control rats had higher stereotypic activity than Charles River rats. This may be due to stress-induced stereotypic activity (Spangler, R., Zhou, Y., Schlussman, S. D., Ho, A., & Kreek, M. J., 1997). Overall, we observed that Taconic rats demonstrated more aggressive and stereotypic behaviors. This may account for their higher corticosterone plasma levels. Interestingly after cocaine administration, no differences between vendors were observed in stereotypic behavior. This may be due to a ceiling effect of cocaine-induced stereotypic activity in both lines of rats.

Overall, due to the selective breeding of Fischer rats, Taconic and Charles River rats have different parental lineages. While Fischer rats at both vendors originate from the same source (M.R. Curtis, Columbia University Institute for Cancer Research, 1920), Charles River Laboratories obtained their breeder rats in 1960 at generation F68 directly from Columbia University and Taconic's rats were obtained at generation F143 in 1984 from the NIH Animal Genetic Resource. Thus, differences between the two studies may reside in the inbred differences within these two lines of Fischer rats. It is provocative to postulate that observed differences in corticosterone levels or other inbred differences between these two lines of Fischer rats may account for the differences in cocaine-induced behavioral activation between the rats from different vendors.

CHAPTER 9: PREPRODYNORPHIN mRNA ALTERATIONS IN THE STRIATUM AND HYPOTHALAMUS OF OVARIECTOMIZED FISCHER RATS ARE DIFFERENTIALLY AFFECTED BY ACUTE "BINGE" AND SINGLE-DOSE COCAINE ADMINISTRATION AND GONADAL HORMONE REPLACEMENTS.

Opioid and dopaminergic systems have a collective role in the regulation of motivated and emotional behaviors and the control of locomotor activity. There are interactions between these two systems at the anatomical, behavioral and neurochemical levels. These interactions have been postulated to be important in cocaine-mediated effects in the CNS (for review see (Kreek, M. J, Schluger, J. H., Borg, L., Gunduz, M., & Ho, A., 1999; Naik, R. S., Kelkar, M. R., & Sheth, U. K., 1978)). In male rats, it has been demonstrated that cocaine has significant effects on several components of the endogenous opioid system. Chronic, acute, and self-administration of cocaine regulates levels of preprodynorphin (the precursor to dynorphin) in different areas of the mesocorticolimbic and nigrostriatal pathways (Daunais, J. B. & McGinty, J. F., 1995; Daunais, J. B., Roberts, J. L., & McGinty, J. F., 1999; Hurd, Y. L., Brown, E., Finlay, J. M., Fibiger, H. C., & Gerfen, C. R., 1992; Spangler, R., Unterwald, E. M., & Kreek, M. J., 1993; Spangler, R. et al., 1997b; Steiner, H. & Gergen, C. R., 1993). Cocaine also increases levels of dynorphin immunoreactivity in the striatum, substantia nigra and nucleus accumbens, but not in the hippocampus (Sivam, S. P., 1989; Smiley, P. L., Johnson, M., Bush, L., Gibb, J. W., & Hanson, G. R., 1990). Furthermore, the administration of a dopamine antagonist blocked cocaine-induced increases in levels of dynorphin in the striatum and substantia nigra (Smiley, P. L., Johnson, M., Bush, L.,

Gibb, J. W., & Hanson, G. R., 1990). Cocaine administration also affects the number (Hubner, C. B. & Koob, G. F., 1990; Koob, G. F., Vaccarino, R. J., Amalric, M., & Swerdlow, N. R., 1987; Hammer, R. P., 1989; Unterwald, E. M., Rubenfeld, J. M, & Kreek, M. J., 1994; Unterwald, E. M., 1995; Unterwald, E. M., Horne-king, M. J., & Kreek, M. J., 1992) and mRNA levels (Spangler, R. et al., 1997b; Buzas, R., Rosenberg, J., & Cox, B. M., 1996; Azaryan, A. V., Coughlin, L. J., Buzas, B., Clock, B. J., & Cox, B. M., 1996; Yuferov, V. et al., 1999) of different subtypes of opioid receptors in the mesolimbic and nigrostriatal neurons, including κ- and μ- opioid receptors. It has been postulated that dynorphin/kappa opioid receptor response to cocaine may counter the abrupt elevation in basal dopamine levels initiated by cocaine administration.

Evidence is accumulating suggesting that there are sex differences in the behavioral response to cocaine (Robinson, T. E., Camp, D. M., Jacknow, D. S., & Becker, J. B., 1982; Robinson, T. E., Becker, J. B., & Presty, S. K., 1982; Craft, R. M. & Stratmann, J. A., 1996; Kuhn, C. & Francis, M. S., 1997; Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996; Sofuoglu, M., Duidish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K, 1999). It has been postulated that endocrinological differences between males and females may underlie some of the previously observed differences in cocaine-induced behavioral and neurochemical alterations and be a basis for gender differences in patterns of drug abuse. For example, estrogen and progesterone have been reported to modulate cocaine-induced behavioral activity in the female rat (Quiñones-Jenab, V, Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J., 2000). Previous studies in female mice (Quiñones-Jenab, V, Ogawa, S.,

Jenab, S., & Pfaff, D. W., 1997) and rats (Harlan, R. E., Shivers, B. D., Romano, G. J., Howells, R. D., & Pfaff, D. W., 1987; Romano, G. J., Harlan, R. E., Shivers, B. D., Howells, R. D., & Pfaff, D. W., 1988; Romano, G. J., Mobbs, C. V., & Pfaff, D. W., 1989) have shown that estrogen administration increases preproenkephalin mRNA in the mesolimbic system. In contrast, preprodynorphin levels have been shown to decrease after estrogen replacement in ovariectomized (OVX) rats (Spaminato, S., Canossa, M., Campana, G., Carboni, L., & Bachetti, T., 1995; Wagner, E. J., Manzaneres, J., Moore, K. E., & Lookingland, K. J., 1994).

Thus, it is probable that in the female rat, similar to the male rat, preprodynorphin mRNA levels are modulated by cocaine. However, due to the effects of gonadal hormones on preprodynorphin mRNA levels, cocaine-induced alterations in mRNA levels of preprodynorphin may be altered via interactions with estrogen and progesterone. The possible interactions between progesterone, estrogen, and cocaine in the opioid system remain to be elucidated. The purpose of this study was to determine if, as in male rats, preprodynorphin mRNA levels are increased after acute and "binge" pattern cocaine administration in the striatum and hypothalamus (areas in the CNS known to be involved in the rewarding aspects of cocaine) of OVX female rats. Furthermore, to determine if, as is the case in the behavioral response to cocaine, gonadal hormones modulate cocaine effects on preprodynorphin mRNA levels.

The identification of which of the cocaine-induced CNS alterations at the behavioral and molecular levels are affected by steroids is essential for understanding the

mechanism(s) affecting sex differences in response to cocaine. This knowledge will help to develop effective pharmacological treatments for female addicts. Moreover, the identification of what molecular changes correlate with cocaine-induced behavioral alterations will provide some understanding of the functional significance of any observed molecular alterations. Furthermore, chronic steroid replacement in humans can be found in females utilizing estrogen or progesterone based anti-conceptive or estrogen replacement treatment after menopause. This study will also provide insight into the effects of cocaine in female addicts utilizing these steroid treatments and help to understand some of these postulates.

Methods

Animals: Ovariectomized eight-week-old female Fischer rats purchased from Charles River were individually housed in standard cages in a stress-minimized facility with free access to food and water. Rats were maintained on a 12-hour light/dark cycle. Two weeks after ovariectomy, rats were randomly assigned to either cocaine- or saline- treatment groups, and then further subdivided into one of four hormone pre-treatment conditions: vehicle/control, estrogen, progesterone or estrogen+progesterone (n=6/group). Thirty minutes after their last drug treatment, animals were decapitated, after brief (30 seconds) exposure to CO_2, and the hypothalamus and striatum were dissected. Tissue was homogenized in Trizol reagent (Life Sciences) and RNA was extracted following the manufactures instructions. All NIH Guidelines for the Care of Laboratory Animals were followed.

Drug and hormone treatments: Animals received either subcutaneous (s.c.) injections of estradiol benzoate (50 μg; dissolved in sesame oil) or vehicle (sesame oil), according to their respective experimental groups. Forty-four hours post estrogen treatment animals received s.c. injections of either progesterone (500 μg; dissolved in sesame oil) or vehicle. Forty-eight hours post estrogen/vehicle administration (four hours post progesterone/vehicle), animals received either one (single-dose administration) or three ("binge" pattern administration; one injection per hour for three hours) interperitoneal injections of 0.9% saline (1 ml/kg) or cocaine (15 mg/kg dissolved at a concentration of 15 mg/ml in 0.9% saline). All injections were administered in each rat's home cage.

Solution hybridization and ribonuclease protection assays: Preprodynorphin mRNA levels were measured by solution hybridization following methods previously described by Branch et al (Branch, A. D., Unterwald, E. M., Lee, S. E., & Kreek, M. J., 1996). Briefly, duplicate aliquots of total RNA extracts were dried in a 1.5 ml Eppendorf microcentrifuge tube and resuspended in 30 μl of 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) containing 150,000 dpm of P^{32}-riboprobe.

The hybridization solution was covered with 2 drops of mineral oil and incubated at 75°C overnight. After incubation, the mixture was subjected to 40 μg/ml ribonuclease A and 2 μg/ml ribonuclease T1 for 30 minutes at 30°C in 300 μl of 0.3 M NaCl, 5 mM EDTA and 10 mM Tris-HCl pH 7.4. The ribonuclease digestion was terminated with 1 ml of 5% TCA and 0.75% sodium pyrophosphate and two drops of 0.5% BSA. The

solution was mixed and the TCA precipitable dpms were collected onto glass fiber paper (Brandel, Gaithersburg, MD) using a 24-place cell harvester and counted by liquid scintillation in 4 ml of Hydrofluor (National Diagnostics, Manville, NJ). Comparisons were made with standard calibration curves to quantify the mRNA and total RNA levels. Total RNA concentrations were determined by 260/280 absorption rate.

Data analysis: Planned comparisons, t-tests, were used to examine the effects of cocaine versus saline administration on preprodynorphin levels in animals in each of the hormone conditions.

Results

Single dose cocaine administration:

Preprodynorphin mRNA levels in the striatum were elevated by acute single dose cocaine administration in OVX control rats or after estrogen+progesterone pretreatment when compared to saline-treated groups ($p<0.05$ for both comparisons). No significant differences between saline and cocaine treatment in rats pre-treated with estrogen or progesterone alone were observed ($p>0.05$). Although not statistically significant ($p=0.19$), cocaine caused an elevation of preprodynorphin levels in the hypothalamus of vehicle/OVX rats. However, no significant differences were observed in estrogen, progesterone, or estrogen+progesterone groups when compared to each groups' respective saline treated controls (see Figure 40).

"Binge" pattern cocaine administration:

After "binge" pattern cocaine administration, preprodynorphin mRNA levels appeared to be consistently decreased in both the hypothalamus and striatum in all hormone treatment groups. However, statistically significant changes were only observed in the estrogen and estrogen+progesterone pre-treated rats in the striatum, and in the hypothalamus in estrogen and estrogen+progesterone groups ($p<0.05$ for all comparisons). No statistically significant differences in preprodynorphin mRNA levels after "binge" pattern cocaine administration were observed in either the striatum or the hypothalamus of OVX rats (p=0.4; Figure 41).

Discussion

Our results indicate that acute cocaine administration affects the expression of preprodynorphin mRNA levels in the striatum of female rats. The increase of preprodynorphin mRNA levels after acute single cocaine administration in OVX female rats is consistent with previous reports using male rats, where cocaine induced preprodynorphin levels by 30% (Spangler, R., Unterwald, E. M., & Kreek, M. J., 1993; Spangler, R. et al., 1997b; Daunais, J. B. & McGinty, J. F., 1995). Interestingly, after single cocaine administration we did not observe changes in preprodynorphin mRNA levels after estrogen or progesterone pre-treatments. Thus, suggesting that gonadal hormones may inhibit the cocaine-induced elevation of preprodynorphin mRNA in the striatum. However, when estrogen+progesterone were co-administered increases in preprodynorphin levels were again observed. It has been previously reported that progesterone, when co-administered with estrogen, either potentates estrogenic effects on

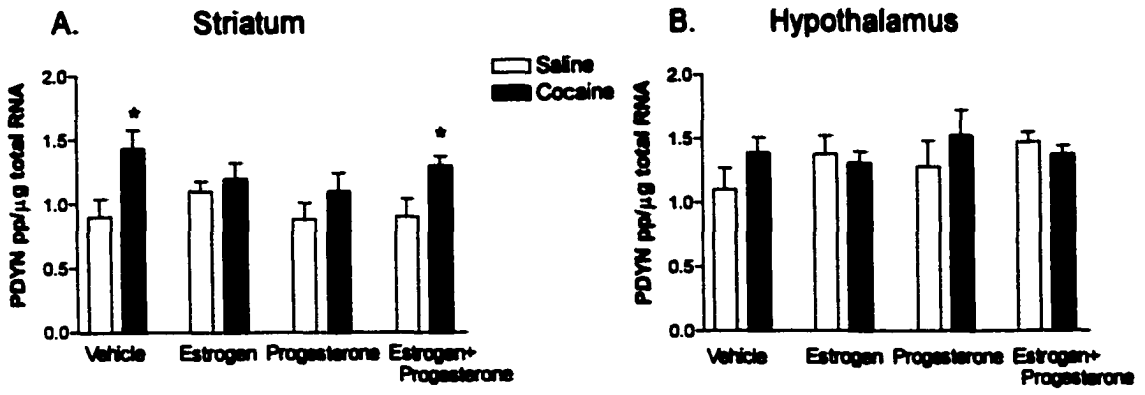


Figure 40: Mean (+) SEM striatal (A) and hypothalamic (B) preprodynorphin RNA levels for single dose cocaine- (black bars) and saline-treated (white bars) animals in each of the four hormone conditions. * $p < 0.05$ vs saline-treated control

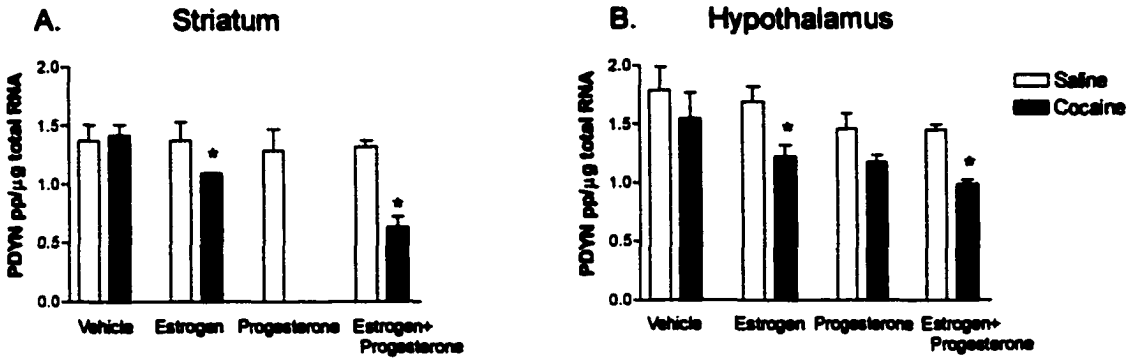


Figure 41: Mean (+) SEM striatal (A) and hypothalamic (B) preprodynorphin RNA levels for "binge" cocaine- (black bars) and saline-treated (white bars) animals in each of the four hormone conditions. * $p < 0.05$ vs saline-treated control

cocaine-induced behaviors or inhibits this behavioral activation (Perrotti, L. I., Russo, S., Lagos, F, Sternin, O., & Quiñones-Jenab, V, 2000). Thus, the observed induction of preprodynorphin mRNA levels after the co-administration of both estrogen and progesterone, but not after estrogen or progesterone alone, may reside in a temporal interaction between the gonadal hormones on cocaine-induced changes in preprodynorphin.

Unlike single dose administration, when cocaine was administered in a "binge" pattern, no alterations in preprodynorphin levels were observed in the striatum of ovariectomized vehicle-treated rats. On the contrary, a significant decrease in preprodynorphin mRNA levels was observed after estrogen+progesterone pre-treatment. Thus, suggesting that the manner in which cocaine was administered influenced the interaction between cocaine and gonadal hormones in the regulation of preprodynorphin levels in the striatum [i.e., either the higher concentration or temporal administration of cocaine or the time when the tissue was collected (3 hours after the first drug administration)]. Estrogen modulation of preprodynorphin mRNA levels has previously been reported in OVX rats; estrogen(Spaminato, S., Canossa, M., Campana, G., Carboni, L., & Bachetti, T., 1995) decreased preprodynorphin mRNA levels in the anterior pituitary(Spaminato, S., Canossa, M., Campana, G., Carboni, L., & Bachetti, T., 1995). Interestingly co-administration of estrogen+progesterone caused a further decrease in the level of striatal preprodynorphin after "binge" pattern cocaine administration. Thus, the down regulation of preprodynorphin by estrogen and progesterone may be consistent with these previous results. Overall, our results suggest an interaction between the

gonadal hormone effects in the modulation of cocaine-induced alterations. Differences between the results presented here and those reported for male rats (Spangler, R., Unterwald, E. M., & Kreek, M. J., 1993; Spangler, R. et al., 1997b), regarding cocaine-induced increases in preprodynorphin levels, may underlie some of the gender differences in behavioral responses to cocaine previously reported (Robinson, T. E., Camp, D. M., Jacknow, D. S., & Becker, J. B., 1982; Robinson, T. E., Becker, J. B., & Presty, S. K., 1982; Craft, R. M. & Stratmann, J. A., 1996; Kuhn, C. & Francis, M. S., 1997; Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996; Sofuoglu, M., Duidish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K, 1999).

It has been demonstrated in male rats that cocaine administration (i.c.v.) causes a 35% decrease in preprodynorphin mRNA levels in the hypothalamus (Romualdi, P., Donatini, A., Izenwasser, S., Cox, B. M., & Ferri, S., 1996). A reduction of preprodynorphin mRNA levels in the hypothalamus has also previously been reported after exposure to opiates (Mochetti, I., Ritter, A., & Costa, E., 1989; Romualdi, P., Lesa, G., & Ferri, S., 1991). Similar to previous reports using male rats (Spangler, R., Unterwald, E. M., & Kreek, M. J., 1993; Spangler, R. et al., 1997b; Daunais, J. B. & McGinty, J. F., 1995), we observed that "binge" pattern cocaine administration and gonadal hormone replacement decreased mRNA levels of preprodynorphin in the hypothalamus by 30% in OVX female rats. However, after single cocaine administration, no differences in the hypothalamic mRNA levels of preprodynorphin were observed in any of the experimental groups. Similar to the effects of cocaine and gonadal hormones in the striatum, differences in cocaine-induced alterations in preprodynorphin mRNA

levels in the hypothalamus were observed between the two administration paradigms. "Binge" pattern cocaine caused a decrease in dynorphin in both the hypothalamus and striatum, while single administration caused dynorphin to increase in the striatum. Therefore, cocaine may modulate dynorphin differently in different brain areas.

It has been previously reported that the estrous cycle influences an animal's behavioral response to cocaine (Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J., 1999). It is provocative to postulate the observed effects of gonadal hormones in cocaine-induced alterations in preprodynorphin levels may underlie some of the estrous cycle related differences. Due to the observed effect of female gonadal hormones in cocaine-induced preprodynorphin regulation, it is provocative to postulate that: 1) the gender differences in cocaine-induced behavioral alterations previously reported may involve differential regulation of molecular adaptations; 2) gonadal hormones play a major role in cocaine-induced alterations in CNS plasticity; 3) female addicts using steroid based contraception may have different regulation of preprodynorphin.

CHAPTER 10: SUMMARY

There are sex differences in behavioral and endocrinological responses to cocaine as well as the development of sensitization; female rats show an exaggerated response to the acute effects of cocaine and a more rapid sensitization than males. This may account differences in the reported subjective effects and pattern of cocaine abuse in humans. If females sensitize to cocaine rapidly, they may use different amounts of cocaine and administer it more or less frequently than males. This suggests that effective treatments for cocaine addiction may need to be different for men versus women.

Additionally, there are sex differences in cocaine metabolism as well as differences in cocaine-induced HPA axis activation. The differences in the endocrinological profile of females versus males could be a mechanism accounting for the observed differences. These differences in cocaine metabolism may be related to differences in the content and activity of the liver enzymes that metabolize cocaine. The esterase mechanisms of degradation may be sensitive to estrogen and progesterone concentrations in the plasma, as is the case of the hepatic P450 related enzymes. This is an important finding, since it may influence differences in the pattern of cocaine abuse between men and women, and may underlie differences in the subjective and behavioral effects of the drug.

Modulation of the endocrine system, HPA axis activation and progesterone plasma levels, were also observed in response to drug and gender or hormone treatment.

This suggests that gonadal hormones and corticosterone may underlie some of the reported sex differences in response to cocaine. Female rats showed exaggerated HPA axis activation in response to cocaine and developed tolerance of this response after chronic cocaine treatment. Following a cocaine challenge, after a period of abstinence, the HPA response of the female was again exaggerated. This observation and the pivotal role of HPA axis activity in cocaine addiction, suggests that stress regulation is a key player in gender differences in response to cocaine. Male and female rats respond differently to stress and this response is reflected by HPA axis activity (Beck, K. D., 1999). This sex difference in the stress response may be a key factor in the observed differences in response to cocaine, an exogenous stressor.

Plasma progesterone levels were increased in female rats. This suggests an interaction between cocaine and activation of the hypothalamic-pituitary-gonadal (HPG) axis. Disregulation of HPG axis endocrine regulation may underlie the known effects of cocaine on the reproductive cycle of humans. Progesterone activation by cocaine may be modulated by two mechanisms; via direct effects of cocaine on the HPG or via effects of the modulation of the HPA axis on HPG axis. Either mechanism of activation may be a key component in gender and estrous cycle related differences in response to cocaine.

Estrogen modulates cocaine-induced alterations at the behavioral and molecular levels. We observed that estrogen potentates the behavioral response to acute and chronic cocaine (and thus, the development of behavioral sensitization). Estrogen has neuroprotective effects in the CNS. It is possible that chronic cocaine use causes

neurodegeneration in the CNS. Co-administration of estrogen and cocaine chronically may protect the brain from the neurodegenerative effects of cocaine; allowing the brain to maintain its capacity to respond to repeated cocaine with the same intensity as the response to an acute exposure. Furthermore, estrogen enhances learning. Alternatively sensitization effects may be due to an enhancement of learning.

Estrogen also modulates HPA and HPG axes activation. The sensitization by estrogen in cocaine-induced activity may underlie the effects of estrogen on the HPA axis. We observed estrogenic effects on HPA axis activation after chronic exposure; suggesting that estrogen effects on the stress response may be a key component involved in sensitization to cocaine. It has been postulated that the dynorphin/kappa opioid receptor response to cocaine may counter the abrupt elevation in basal dopamine levels initiated by cocaine administration. In the prefrontal cortex, estrogen did not modulate monoamine levels. Interestingly, a 30% decrease in mRNA levels of preprodynorphin in the hypothalamus and striatum was observed in female rats after co-administration of "binge" cocaine and gonadal hormones. After single cocaine administration, there was a reduction of preprodynorphin mRNA levels in the striatum, but not the hypothalamus. It is possible that these neuroprotective effects evidence themselves in the behavioral outcome. Perhaps it is the case that this potentiation of cocaine-induced behavioral activity by estrogen may be due to a dysregulation of the dynorphin/dopaminergic regulatory mechanisms.

Progesterone administration did not alter the behavioral response after acute "binge" or single cocaine administration, or after chronic single cocaine administration. Although similar to estrogen, dynorphin levels were reduced by progesterone; unlike estrogen, progesterone affected cocaine-induced changes in monoamine levels in the prefrontal cortex (including serotonin and dopamine levels). The decreased 5-HT rate observed after progesterone and cocaine administration may be due to an inhibitory feedback mechanism potentiated by progesterone's effects on cocaine. Future experiments should use in vivo microdialysis and/or pargyline treatment to further determine the effects of progesterone in cocaine-induced 5-HT release or turnover. It is provocative to postulate that the regulation of both dynorphin and the monoamine systems by progesterone in opposite directions may counter-balance any modulation of behavioral responses. Chronic exposure to cocaine causes changes in 5-HT function that mimic depression (Parsons, L. H. & Justice, J. B., 2000; Baumann, M. H. & Rothman, R. B., 1998). Moreover, women who are drug addicts have a high propensity for comorbid depression, and this negatively impacts treatment success (Boyd, C. J., 1993; Grant, B. F., 1995). Thus, enhanced effects of cocaine on 5-HT systems in women might explain this observation. Moreover, medications targeting 5-HT neurons could be useful pharmacological adjuncts for treating women who are drug addicts.

The manner of estrogen administration affected the modulation of cocaine-induced behavioral activity as well as the interaction between estrogen and progesterone on the activation of cocaine-induced behaviors. The co-administration of estrogen (s.c.), progesterone (s.c.) and cocaine (either acute single or "binge" cocaine administration)

caused a decrease in locomotor behaviors, however, this inhibition of cocaine-induced behavioral activity was not observed after chronic single cocaine administration. Interestingly, when estrogen was administered via silastic capsules, this effect was not observed. This may be due to the effects of a pulse verses chronic steady release of estrogen. Discrepancies between these two manners of estrogen administration may underlie differences in response to cocaine among women using estrogen-based contraceptives and menstrual cycle effects (Lundahl, L. H., Kouri, E. M., & Lukas, S. E., 1999; Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H., 1996).

Moreover, differences in the modulatory interaction between estrogen and progesterone on cocaine-induced behavioral activity were observed in rats from the same strain but different vendors. Due the "genetically pure" nature of Fischer rats, this suggests that there are genetically based differences in the interaction between estrogen and progesterone on cocaine-induced alterations.

Similar to lordosis, there was a bimodal interaction between estrogen and progesterone in the regulation of locomotor activity in Fischer rats, where progesterone either synergizes with or inhibits estrogen modulation of cocaine-induced activity. This temporal interaction was also observed in corticosterone and cocaine metabolite levels; suggesting that estrous cycle effects on cocaine-induced alterations may be influenced by the length of estrogen and progesterone interactions. Thus, there seems to be some overlap in the CNS mechanisms involved in the regulation of cocaine-induced behaviors and female reproductive behaviors.

After progesterone and estrogen administration, preprodynorphin mRNA levels were decreased in the hypothalamus and striatum of rats administered "binge" cocaine but increased in the striatum after a single injection of cocaine. This suggests a delicate interaction between gonadal replacements and the manner, dose and temporal administration of cocaine. Therefore, the observed effects of gonadal hormones in cocaine-induced alterations in preprodynorphin levels may underlie some of the sex and estrous cycle related differences we have previously observed. Due to the observed effects of female gonadal hormones on cocaine-induced preprodynorphin regulation, it is provocative to postulate that: 1) the gender differences in cocaine-induced behavioral alterations previously reported may involve differential regulation of molecular adaptations; 2) gonadal hormones play a major role in cocaine-induced alterations in CNS plasticity; 3) female addicts using steroid-based contraception may have different regulation of preprodynorphin.

Overall, results presented in this thesis support the hypothetical model (Figure 7) discussed in Chapter One. The behavioral responses to cocaine are modulated by gonadal hormones using a rapid/cellular mechanism (i.e., monoamine alterations and endocrine regulations) and a slower/genomic modulation (i.e., preprodynorphin mRNA levels). However, it seems that the interactions between the gonadal hormones and cocaine may be affected by the route and manner of cocaine administration (i.e., observed differences between "binge" vs. single administration), genetic factors (i.e., difference between Fischer rats from two vendors), dose and manner of administration of estrogen (Silastic

vs. s.c. injections) and temporal interactions between estrogen and progesterone. Estrogen modulates acute behavioral response to cocaine as well as the development of sensitization. At the neurochemical endpoint, estrogen does not directly modulate dopamine, serotonin or norepinephrine. Interestingly, although estrogen decreased dynorphin levels, this was only observed after "binge" pattern cocaine administration.

This work completes the model of steroid hormone and cocaine interactions by showing that this interaction does, in fact, affect the behavioral outcome via both rapid cellular alterations where the presence or absence of hormones affects the serotonin system in the medial prefrontal cortex and through a slower genomic mechanism affecting dynorphin expression in the opioid system. Estrogen administration potentates the behavioral response to cocaine, but decreases dynorphin levels, and does not affect neurotransmitter levels in the medial prefrontal cortex. Perhaps it is the case these estrogenic effects on behavior are due to increases in activity of the dopaminergic system, which are caused by or cause decreases in levels of dynorphin.

Due to the effects of female gonadal hormones in cocaine-induced behavioral, neurochemical and molecular alterations, it is provocative to postulate that: 1) the gender differences in cocaine-induced behavioral alterations previously reported may involve differential regulation by gonadal hormones; 2) the interactions between estrogen and progesterone determine the behavioral and neurochemical response to cocaine during each stage of the menstrual/estrous cycle; 3) stage of the estrous/menstrual cycle at the

time of cocaine administration may influence the effects of cocaine on brain functions involved in cocaine-induced behavior.

Of key importance is the female addict who may use estrogen- or progesterone-based contraceptives. Based on these observations, interactions between steroids and cocaine may ultimately affect not only the contraceptive treatment but also the behavioral and subjective responses to cocaine. Thus, women using different steroid treatments or at different stages of their menstrual cycles may use higher doses of cocaine to achieve greater subjective effects of the drug. Interactions between cocaine and ovarian hormones may lead to overdoses and other clinical complications. Moreover, the development of sensitization or tolerance to cocaine may be affected according to which steroid-based contraceptive a women is using or where she is in her reproductive cycle. This important clinical issue in females needs further investigation.

Reference List

Alburges, M. E., Narang, N., & Wamsley, J. K. (1993). Alterations in the dopaminergic receptor system after chronic administration of cocaine. Synapse, 14, 314-323.

Apostolakis, M. E., Garai, J., Clark, J. H., & O'Malley, B. W. (1996). *In vivo* regulation of central nervous system progesterone receptors: cocaine induces steroid-dependent behavior through dopamine transporter modulation of D5 receptors in rats. Molecular Endocrinology, 10, 1595-1604.

Arnold, A. P. & Breedlove, S. M. (1985). Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. Hormones and Behavior, 19, 469-498.

Atkinson, H. C. & Waddell, B. J. (1997). Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: Sexual dimorphism and changes across the estrous cycle. Endocrinology, 138, 3842-3848.

Azaryan, A. V., Coughlin, L. J., Buzas, B., Clock, B. J., & Cox, B. M. (1996). Effect of chronic cocaine treatment on mu and delta opioid receptor mRNA levels in dopaminergically innervated brain regions. Journal of Neurochemistry, 66, 443-448.

Bain, G. T. & Kornetsky, C. (1977). Naloxone attenuation of the effect of cocaine on rewarding brain stimulation. Life Sciences, 40, 1119-1126.

Baumann, M. H., Raley, T. J., Partilla, J. S., & Rothman, R. B. (1993). Biosynthesis of dopamine and serotonin in the rat brain after repeated cocaine injections: A microdissection mapping study. Synapse, 14, 40-50.

Baumann, M. H. & Rothman, R. B. (1998). Alterations in serotonergic responsiveness during cocaine withdrawal in rats: similarities to major depression in humans. Biological Psychiatry, 44, 578-591.

Beck, K. D. (1999). Housing environment influences on chronic stress-induced changes in behavior and neurochemistry: Sex differences in Sprague-Dawley rats. The Graduate School and University Center of the City University of New York.

Beck, K. D. & Luine, V. N. (1999). Food deprivation modulates chronic stress effects on object recognition in male rats: role of monoamines and amino acids. Brain Research, 830, 56-71.

Becker, J. B. (1990b). Direct effect of 17β-estradiol on striatum: sex differences in dopamine release. Synapse, 5, 157-164.

Becker, J. B. (1990a). Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. Neuroscience Letters, 118, 169-171.

Becker, J. B. & Beer, M. E. (1986). The influence of estrogen on nigrostriatal dopamine activity: behavioral and neurochemical evidence for both pre- and postsynaptic components. Behavioural Brain Research, 19, 27-33.

Becker, J. B. & Cha, J. (1989). Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. Behavioural Brain Research, 35, 117-125.

Becker, J. B. & Ramirez, V. D. (1981). Sex differences in the amphetamine stimulated release of catecholamines from rat striatal tissue in vitro. Brain Res, 204, 361-372.

Beigon, A., Fischette, C. T., Rainbow, T. C., & McEwen, B. S. (1983). Serotonin receptor modulation by estrogen in discrete brain nuclei. Neuroendocrinol., 80, 5134-5137.

Benmansour, S., Tajani-Butt, S. M., Hauptman, M., & Brunswick, D. J. (1992). Lack of effect of high-dose cocaine on monoamine uptake sites in rat brain measured by quantitative autoradiography. Psychopharmacology, 106, 459-462.

Bilsky, E. J., Montegut, M. J., Delon, C. L., & Reid, L. D. (1992). Opioidergic modulation of cocaine conditioned place preference. Life Sciences, 50, PL85-PL92.

Booze, R. M., Wood, M. L., Welch, M. A., Berry, S., & Mactutus, C. F. (1999). Estrous cyclicity and behavioral sensitization in female rats following repeated intravenous cocaine administration. Pharmacology Biochemistry and Behavior, 64, 605-610.

Bowman, B., Vaughan, S. R., Walker, D. Q., Davis, S. L., Little, P. J., Scheffler, N. M., Thomas, B. F., & Kuhn, C. M. (1999). Effects of sex and and gonadectomy on cocaine metabolism in the rat. The Journal of Pharmacology and Experimental Theraputics, 290, 1316-1323.

Boyd, C. J. (1993). The antecedents of women's crack cocaine abuse: family substance abuse, sexual abuse, depression and illicit drug use. J.Subst.Abuse Treat., 10, 433-438.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. Annal.Biochem, 72, 248-254.

Branch, A. D., Unterwald, E. M., Lee, S. E., & Kreek, M. J. (1996). Quantitation of preproenkephalin mRNA levels in brain regions from male Fischer rats following chronic cocaine treatment using a recently developed solution hybridization procedure. Molecular Brain Research, 14, 231-238.

Brown, R. E. (1994). An Introduction to Neuroendocrinology. Cambridge: Cambridge University Press.

Budziszewska, B., Jaworska-Feil, L., & Lason, W. (1996). The effect of repeated amphetamine and cocaine administration on adrenal, gonadal and thyroid hormone levels in the rat plasma. Exp.Clin.Endocrinl.Diabetes, 104, 334-338.

Buzas, R., Rosenberg, J., & Cox, B. M. (1996). Mu and delta opioid receptor gene expression after chronic treatment with opioid agonist. Neuro.Report, 7, 1505-1508.

Cabrera, R., Diaz, A., Pinter, A., & Belmar, J. (1993). In vitro progesterone effects on 3H-dopamine release from rat corpus striatum slices obtained under different endocrine conditions. Life Sciences, 53, 1767-1777.

Caihol, S. & Morméde, P. (1999). Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. Brain Research, 842, 200-205.

Camp, D. M. & Robinson, T. E. (1988). Susceptibility to sensitization. I. Sex differences in the enduring effects of chronic D-amphetamine treatment on locomotion, stereotyped behavior and brain monoamines. Behavioural Brain Research, 30, 55-68.

Carey, R. J. & Damianopoulos, E. N. (1994). Conditioned cocaine induced hyperactivity: an association with increased medial prefrontal cortex serotonin. Behavioural Brain Research, 62, 177-185.

Carr, L. A. & Voogt, J. L. (1980). Catecholamine synthesizing enzymes in the hypothalamus during the estrous cycle. Brain Res, 196, 437-445.

Carroll, M. E., Lac, S. T., Asencio, M., & Kragh, R. (1990). Fluoxetine reduces intravenous cocaine self-administration in rats. Pharmacology Biochemistry and Behavior, 35, 237-244.

Carter, C. S. (1993). Neuroendocrinology of sexual behavior in the female. In J.B.Becker, S. M. Breedlove, & D. Crews (Eds.), Behavioral Endocrinology (3 ed., pp. 71-95). Cambridge: MIT Press.

Castner, S. A., Xiao, L., & Becker, J. B. (1993). Sex differences in striatal dopamine: *in vivo* microdialysis and behavioral studies. Brain Research, 610, 127-134.

Chen, C. & Vandenbergh, J. G. (1994). Effect of chronic cocaine on reproduction in female house mice. Pharmacology Biochemistry and Behavior, 48, 909-913.

Chin, J., Sternin, O., Fletcher, H., Jenab, S., Perrotti, L. I., & Quinones-Jenab, V. (2000b). Effects of sex and gonadectomy on cocaine-induced locomotor and stereotypic behaviors. Society for Neuroscience Abstracts, 26.

Chin, J., Sternin, O., Fletcher, H., Jenab, S., Perrotti, L. I., & Quiñones-Jenab, V. (2000a). Sex differences in cocaine-induced locomotor and stereotypic behaviors in rats: An acute, chronic, and challenge study. Submitted.

Claye, L. H., Akanne, H. C., Davis, M. D., DeMattos, S., & Soliman, K. F. A. (1995). Behavioral and neurochemical changes in the dopaminergic system after repeated cocaine administration. Molecular Neurobiology, 11, 55-66.

Craft, R. M. & Stratmann, J. A. (1996). Discriminative stimulus effects of cocaine in female versus male rats. Drug and Alcohol Dependence, 42, 27-37.

Creese, I. & Iversen, D. (1974). The role of forebrain dopamine system in amphetamine induced stereotypic behaviors in the rat. Psychopharmacology, 39, 345-357.

Crowley, W. R. (1982). Effects of ovarian hormones on norepinephrine and dopamine turnover in individual hypothalamic and extrahypothalamic nuclei. Neuroendocrinol., 34, 381-386.

Crowley, W. R., O'Donohue, T. L., & Jacobowith, D. M. (1978). Changes in catecholamine content in discrete brain nuclei during the estrous cycle of the rat. Brain Res, 147, 315-326.

Cunningham, K. A. (1995). Modulation of serotonin function by acute and chronic cocaine: neurophysiological analyses. In R.P.Hammer (Ed.), The neurobiology of cocaine. (pp. 121-144). Boca Raton: CRC Press.

Cunningham, K. A., Paris, J. M., & Goeders, N. E. (1992). Chronic cocaine enhances serotonin autoregulation and serotonin uptake binding. Synapse, 11, 112-123.

Daunais, J. B. & McGinty, J. F. (1995). Cocaine binges differentially alter striatal preprodynorphin and zif/268 mRNAs. Molecular Brain Research, 29, 201-210.

Daunais, J. B., Roberts, J. L., & McGinty, J. F. (1999). Cocaine self-administration increases preprodynorphin but not c-fos in the striatum. Neuro.Report, 4, 543-546.

Davis, C. F., Davis, B. F., & Halaris, A. E. (1977). Variations in the uptake of [3H]dopamine during the estrous cycle. Life Sciences, 20, 1319-1332.

deWit, H. & Wise, R. A. (1977). Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide but not with the noradrenergic blockers phentolamine or phenoxybnezaime. Can.J.Psychol., 31, 195-201.

Di Paolo, T., Carmichael, R., Labrie, F., & Raymond, J. P. (1979). Effects of estrogen on the characteristics of {3-H}spiroperidol and [3-H]RU24213 binding in rat anterior pituitary gland and brain. Mol Cell Endo, 16, 99-112.

Di Paolo, T., Poyet, P., & Labrie, F. (1981). Effects of chronic estradiol and haloperidol treatment on striatal dopamine receptors. Euro.J.Pharm., 73, 105-106.

Di Paolo, T., Poyet, P., & Labrie, F. (1982). Effects of prolactin and estradiol on rat striatal dopamine receptors. Life Sciences, 31, 2921-2929.

Di Paolo, T., Rouillard, G., & Bedard, P. (1985). 17β-estradiol at a physiological dose acutely increases dopamine turnover in rat brain. Euro.J.Pharm., 117, 197-203.

Diaz-Veliz, G., Baeza, R., Benavente, F., Dussaubat, N., & Mora, S. (1994). Influence of the estrous cycle and estradiol on the behavioral effects of amphetamine and apomorphine in rats. Pharmacology Biochemistry and Behavior, 49, 819-825.

Falk, J. L., Fang, M. A., & Lau, C. E. (1991). Chronic oral cocaine self-administration: pharmacokinetics and effects on spontaneous and discriminative motor functions. J.Pharmacol.Exp.Ther., 257, 457-465.

Fludder, J. M. & Tonge, S. R. (1975). Variations in the concentrations of monoamines and their metabolites in eight regions of rat brain during the estrous cycle: a basis for interactions between hormones and psychotropic drugs. J.Pharm.Pharmacol., 27, 39.

Funabashi, T., Brooks, P. J., & Pfaff, D. W. (1996). Preproenkephalin regulation during the estrous cycle of the female rat. Molecular Brain Research, 33.

Galloway, M. P. (1990). Regulation of dopamine and serotonin synthesis by acute administration of cocaine. Synapse, 6, 63-72.

Glantz, J. C. & Woods, J. R. (1994). Cocaine LD50 in Long-Evans rats is not altered by pregnancy or progesterone. Neurotoxicol.Teratol., 16, 297-301.

Glick, S. D., Hinds, P. A., & Shapiro, R. M. (1983). Cocaine-induced rotation: Sex-dependent differences between left- and right-sided rats. Science, 221, 775-777.

Goeders, N. E. & Kuhar, M. J. (1987). Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. Alcohol Drug Res., 7, 207-216.

Goeders, N. E. & Smith, J. E. (1983). Cortical dopaminergic involvement in cocaine reinforcement. Science, 221, 773-775.

Goeders, N. E. & Smith, J. E. (1986). Reinforcing properties of cocaine in the medial prefrontal cortex: Primary action on dopaminergic terminals. Pharmacology Biochemistry and Behavior, 25, 191-199.

Goeders, N. E. & Smith, J. E. (1993). Intracranial cocaine self-administration into the medial prefrontal cortex increases dopamine turnover in the nucleus accumbens. The Journal of Pharmacology and Experimental Theraputics, 265, 592-600.

Goetz, C., Bourgoin, F., Cesselin, A., Brandi, A., Bression, D., Marinet, M., Peillo, F., & Hamon, M. (1983). Alterations in central neurotransmitter receptor binding sites following estradiol implantation in female rats. Neurochem.Int., 5, 375-383.

Grant, B. F. (1995). Comorbidity between DSM-IV drug use disorders and major depression: results of a national survey of adults. J.Subst.Abuse, 7, 481-497.

Grimm, J. W. & See, R. E. (1997). Cocaine self-administration in ovariectomized rats is predicted by response to novelty, attenuated by 17-β estradiol, and associated with abnormal vaginal cytology. Physiology and Behavior, 61, 755-761.

Hammer, R. P. (1989). Cocaine alters opiate receptor binding in critical brain reward regions. Synapse, 3, 55-60.

Hammer, R. P. (1990). μ-opioid receptor binding in the medial preoptic area is cyclical and sexually dimorphic. Brain Research, 515, 187-192.

Hammer, R. P., Zhou, L., & Cheung, S. (1994). Gonadal steroid hormones and hypothalamic opioid circuitry. Hormones and Behavior, 28, 431-437.

Harlan, R. E., Shivers, B. D., Romano, G. J., Howells, R. D., & Pfaff, D. W. (1987). Localization of preproenkephalin mRNA in rat brain and spinal cord by in situ hybridization. J.Comp.Neurol., 258, 159-184.

Heikkila, R. E., Orlansky, H., & Cohen, G. (1975). Studies on the distinction between uptake inhibition and release of [3H]dopamine in rat brain tissue slices. Biochem.Pharmacol., 24, 847-852.

Houdi, A. A., Bardo, M. T., & Van Loon, G. R. (1989). Opioid mediation of cocaine-induced hyperactivity and reinforcement. Brain Research, 497, 195-206.

Hruska, R. E. (1986). Elevation of striatal dopamine receptors by estrogen: Dose and time studies. Journal of Neurochemistry, 47, 1908-1915.

Hruska, R. E. & Pitman, K. T. (1982). Distribution and localization of estrogen-sensitive dopamine receptors in the rat brain. J.Neurochem., 39, 1418-1423.

Hruska, R. E. & Silbergeld, E. K. (1980). Increased dopamine receptor sensitivity after estrogen treatment using the rat rotation model. Science, 208, 1466-1468.

Hubner, C. B. & Koob, G. F. (1990). The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. Brain Research, 508, 20-29.

Hurd, Y. L., Brown, E., Finlay, J. M., Fibiger, H. C., & Gerfen, C. R. (1992). Cocaine self-administration differentially alters mRNA expression of striatal peptides. Molecular Brain Research, 13, 165-170.

Isenschmid, D. S., Levine, B. S., & Caplan, Y. H. (1989). A comprehensive study of the stability of cocaine and its metabolites. Journal of Analytical Toxicology, 13, 250-256.

Izenwasser, S. & Cox, B. M. (1990). Daily cocaine treatment produces a persistent reduction of [3H]dopamine uptake in vitro in rat nucleus accumbens but not in the striatum. Brain Research, 531, 338-341.

Javaid, J. I., Sahni, S. K., Pandey, S. C., & Davis, J. M. (1993). Repeated cocaine administration does not affect 5-HT receptor subtypes (5-HT1A, 5HT 2) in several brain regions. European Journal of Pharmacology, 238, 425-429.

Johanson, C. E. & Fischman, M. W. (1989). The pharmacology of cocaine related to its abuse. Pharmacological Reviews, 41, 3-52.

Johnson, R. G., Fiorella, D., & Rabin, R. A. (1993). Effects of chronic cocaine administration on the serotonergic system in the rat brain. Pharmacology Biochemistry and Behavior, 46 , 289-293.

Jori, A. & Cecchetti, G. (1973). Homovanillic acid levels in rat striatum during the oestrous cycle. J.Endo., 58, 341-342.

Kalivas, P. W. & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization to motor activity. Brain Research Reviews, 16, 223-244.

Kazandjian, A., Spyraki, C., Sfikakis, A., & Varonos, D. D. (1987). Apomorphine-induced behavior during the oestrous cycle of the rat. Neuropharmacology, 26, 1037-1045.

King, T. S., Schenken, R. S., Kang, I. S., Javors, M. A., & Riehl, R. M. (1990). Cocaine disrupts estrous cyclicity and alters the reproductive neuroendocrine axis in the rat. Neuroendocrinology, 51, 15-22.

Kleven, M. S., Perry, B. D., & Woolverton, W. L. (1999). Effects of repeated injection of cocaine on D1 and D2 dopamine receptors in rat brain. Brain Research, 532, 265-270.

Koob, G. F. (1992). Drugs of abuse: anatomy, pharmacology, and function of reward pathways. Trends Pharmacol.Sci., 13, 177-184.

Koob, G. F. & LeMoal, M. (1997). Drug abuse hedonic homeostatic dysregulation. Science, 278, 52-58.

Koob, G. F., Vaccarino, R. J., Amalric, M., & Swerdlow, N. R. (1987). Neural substrates for cocaine and opiate reinforcement. In A.R.U.S.Fisher (Ed.), Cocaine: Clinical and behavioral aspects (pp. 80-100). New York: Oxford Press.

Kornetsky, C., Huston-Lyons, D., & Porrino, L. J. (1991). The role of the olfactory tubercle in the effects of cocaine, morphine and brain-stimulation reward. Brain Research, 541, 75-81.

Kreek, M. J., Schluger, J. H., Borg, L., Gunduz, M., & Ho, A. (1999). Dynorphin A1-3 causes elevation of serum levels of prolactin through an opioid receptor mechanism in humans: Gender differences and implications for modulation of dopaminergic tone in the treatment of addictions. The Journal of Pharmacology and Experimental Theraputics, 288, 260-269.

Kuhar, M. J., Boja, J. W., Patel, A., Pilotte, N. S., Cerruti, C., & Lever, J. (1995). Cocaine and dopamine transporters. In R.P.Hammer (Ed.), Neurobiology of cocaine: Cellular and Molecular Mechanisms (pp. 201-213). Boca Raton: CRC Press.

Kuhn, C. & Francis, M. S. (1997). Gender differences in cocaine-induced HPA axis activation. Neuropsychopharmacology, 16, 399-407.

Kuhn, C. M., Francis, R. S., & Walker, Q. D. (1999). Cocaine stimulates progesterone but not estradiol secretion in female and male rats. Society for Neuroscience Abstracts, 25, 304.

Kula, N. S. & Baldessarini, R. J. (1991). Lack of increase in dopamine transporter binding or function in rat brain tissue after treatment with blockers of neuronal uptake of dopamine. Neuropharmacology, 30, 89-92.

Lauber, A. H., Romano, G. J., Mobbs, C. V., Howells, R. D., & Pfaff, D. W. (1990). Estradiol induction of proenkephalin messenger RNA in hypothalamus: dose-response and relation to reproductive behavior in the female rat. Molecular Brain Research, 8, 47-54.

Laurier, L. G., Corrigall, W. A., & George, S. R. (1994). Dopamine receptor density and mRNA levels are altered following self-administration of cocaine in the rat. Brain Research, 634, 31-40.

Luine, V. N. (1993). Serotonin, catecholamines and metabolites in discrete brain areas in relation to lordotic responding on proestrus. Neuroendocrinol., 57, 946-954.

Luine, V. N., Bowling, D., & Hearns, M. (1990). Spatial memory deficits in aged rats: contributions of monoaminergic systems. Brain Research, 537, 271-278.

Lukas, S. E., Sholar, M. B., Fortin, M., Wines, J., & Mendelson, J. H. (1996). Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers. Psychopharmacology, 125, 346-356.

Lundahl, L. H., Kouri, E. M., & Lukas, S. E. (1999). Oral Contraceptives alter cocaine plasma levels and subjective responses in females: A preliminary report. NIDA Res.Monograph, 180.

Lynch, W. J. & Carroll, M. E. (2000). Reinstatement of cocaine self-administration in rats: Sex differences. Psychopharmacology, 148, 196-200.

Maggos, C., Spangler, R., Zhou, L., & Kreek, M. J. (1995). Dopamine transporter mRNA levels in the rat substantia nigra and ventral tegmental area immediately following and at two days and ten days after binge cocaine administration. NIDA Res.Monograph, 153, 508.

Maisonneuve, I. M. & Kreek, M. J. (1994). Acute tolerance to the dopamine response induced by a binge pattern of cocaine administration in male rats: an *in vivo* microdialysis study. The Journal of Pharmacology and Experimental Theraputics, 268, 916-921.

Marinelli, M., Piazza, P., Derouche, V., Maccari, S., Le Moal, M., & Simon, H. (1994). Corticosterone circadian secretion differentially facilitates dopamine-mediated psychomotor effects of cocaine and morphine. Journal of Neuroscience, 14, 2731.

Marinelli, M., Piazza, P., Derouche, V., Maccari, S., LeMoal, M., & Simon, H. (2000). Cocaine sensitivity in roman high and low avoidance rats is modulated by sex and gonadal hormone status. Journal of Neuroscience, 14, 2731.

Martin-Iverson, M. T., Szostak, C., & Fibiger, H. C. (1986). 6-Hydroxydopamine lesions of the medial prefrontal cortex fail to influence intravenous self-administration of cocaine. Psychopharmacology, 88, 310-314.

Mateo, A. R., Hijazi, M., & Hammer, R. P. (1992). Dynamic patterns of medial preoptic μ-opiate receptor regulation by gonadal steroid hormones. Neuroendocrinology, 55, 51-58.

Mayfiled, R. D., Larson, G., & Zahniser, N. R. (1992). Cocaine-induced behavioral sensitization and D1 dopamine receptor function in rat nucleus accumbens and striatum. Brain Research, 572, 331-335.

McCabe, J. T. & Pfaff, D. W. (1989). In situ hybridization: a methodological guide. Methods in Neurosci, 1, 98-126.

McGregor, A., Baker, G., & Roberts, D. C. S. (1996). Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on intravenous cocaine self-administration under a progressive ratio schedule of reinforcement. Pharmacology Biochemistry and Behavior, 53, 5-9.

McKim, W. A. (1996). Psychomotor Stimulants. In W.A.McKim (Ed.), Drugs and Behavior: An Introduction to Behavioral Pharmacology (Third ed., pp. 212-234). Upper Saddle River, NJ: Prentice Hall.

Mello, N. K., Mendelson, J. H., Drieze, J. M., & Kelly, M. (1990). Acute effects of cocaine on prolactin and gonadotropins in female Rhesus monkey during the follicular phase of the menstrual cycle. The Journal of Pharmacology and Experimental Theraputics, 254, 815-823.

Mello, N. K., Mendelson, J. H., Kelly, M., & Bowen, C. A. (2000). The effects of cocaine on basal ovarian steroid hormones in female rhesus monkeys. CPDD Abstracts, 62, 108.

Mello, N. K., Mendelson, J. H., Kelly, M., Diaz-Migoyo, N., & Sholar, J. W. (1997). The effects of chronic cocaine self-administration on the menstrual cycle in rhesus monkeys. The Journal of Pharmacology and Experimental Theraputics, 281, 70-83.

Mello, N. K., Sarnyai, Z., Mendelson, J. H., Drieze, J. M., & Kelly, M. (1993). Acute effects of cocaine on anterior pituitary hormones in male and female rhesus monkeys. The Journal of Pharmacology and Experimental Theraputics, 266, 804-811.

Mendelson, J. H., Mello, N. K., & Negus, S. S. (1999). Effects of luteinizing hormone-releasing hormone on plasma cocaine levels in Rhesus monkeys. The Journal of Pharmacology and Experimental Theraputics, 289, 791-799.

Mendelson, J. H., Mello, N. K., Sholar, J. W., Seigel, A. J., Kaufman, M. J., Levin, J. M., Renshaw, P. F., & Cohen, B. M. (1999). Cocaine pharmacokinetics in men and in women during the follicular and luteal phases of the menstrual cycle. Neuropsychopharmacology, 21, 294-303.

Mobbs, C. V., Harlan, R. E., Burrous, M. R., & Pfaff, D. W. (1988). An estradiol-induced protein synthesized in the ventral medial hypothalamus and transported to the midbrain central gray. J.Neurosci., 8, 113-118.

Mochetti, I., Ritter, A., & Costa, E. (1989). Down-regulation of proopiomelanocortin synthesis and b-endorphin utilization in hypothalamus of morphine-tolerant rats. Journal of Molecular Neuroscience, 1, 33-38.

Moldow, R. L. & Fischman, A. J. (1987). Cocaine induced secretion of ACTH, beta-endorphin , and corticosterone. Peptides, 8, 819-822.

Morin, L. P. (1976). Progesterone: Inhibition of Rodent Sexual Behavior. Physiology and Behavior, 18, 701-715.

Morishima, H. O., Abe, Y., Matsuo, M., Akiba, K., Masaoka, T., & Cooper, T. B. (1993). Gender-related differences in cocaine toxicity in the rat. Journal of Labortory and Clinical Medicine, 122, 157-163.

Morissette, M. & Di Paolo, T. (1993). Sex and estrous cycle variations of rat striatal dopamine uptake sites. Neuroendocrinology, 58, 16-22.

Morrow, B. A. & Roth, R. H. (1996). Serotonergic lesions alter cocaine-induced locomotor behavior and stress-activation of the mesocorticolimbic dopamine system. Synapse, 23, 174-181.

Naik, R. S., Kelkar, M. R., & Sheth, U. K. (1978). Attenuation of stereotyped behavior by sex steroids. Psychopharmacology, 57, 211-214.

Pare, W. P. & Kluczynski, J. (1997). Differences in the stress response of Wistar-Kyoto (WKY) rats from different vendors. Physiology and Behavior, 62, 643-648.

Parsons, L. H. & Justice, J. B. (2000). Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area, and dorsal raphe nucleus following repeated cocaine administration. J.Neurochem., 61, 1611-1619.

Peris, J., Decambre, N., Coleman-Hardee, M. L., & Simpkins, J. W. (1991). Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [^{3}H]dopamine release. Brain Research, 566, 255-264.

Perret, G., Schluger, J. H., Unterwald, E. M., Kreuter, J., Ho, A., & Kreek, M. J. (1998). Downregulation of 5-HT1A receptors in rat hypothalamus and dentate gyrus after "binge" pattern cocaine administration. Synapse, 30, 166-171.

Perrotti, L. I., Beck, K. D., Luine, V. N., & Quiñones-Jenab, V. (2000). Effects of ovarian hormones on catecholamine levels in the medial prefrontal cortex of ovariectomized cocaine-treated rats. Neuroscience Letters, 291, 1-4.

Perrotti, L. I., Russo, S., Lagos, F., & Quinones-Jenab, V. (2000). Vendor differences in cocaine-induced behavioral activity and hormonal interactions in ovariectomized Fischer rats. Brain Research Bulletin, 53.

Perrotti, L. I., Russo, S., Lagos, F., Sternin, O., & Quiñones-Jenab, V. (2000). Temporal interactions between estrogen and progesterone affect cocaine-induced locomotor behaviors in ovariectomized Fischer rats. Society for Neuroscience Abstracts, 26.

Perrotti, L. I., Webb, T., Russo, S., Fletcher, H., Chin, J., Jenab, S., & Quinones-Jenab, V. (2000). Estrogen affects cocaine-induced behavioral sensitization. Submitted, The Journal of Pharmacological and Experimental Therapeutics.

Pfaff, D. W. & Schwartz-Giblin, S. (1995). Cellular mechanism of female reproductive behavior. In E.Knobil & J.Neill (Eds.), The physiology of reproduction (pp. 1487-1568). New York: Raven.

Pfaus, J. & Pfaff, D. W. (1992). μ–, δ-, and κ-opioid receptor agonists selectively modulate sexual behaviors in the female rat: differential dependence on progesterone. Hormones and Behavior, 26, 457-473.

Pilotte, N. S. (1994). Dopamine transporter. Molecular Brain Research, 22, 132-138.

Platt, J. J. (1997). The Problem of Cocaine Abuse and Addiction. In J.J.Platt (Ed.), Cocaine Addiction: Theory, Research, and Treatement. (pp. 3-21). Cambridge, MA: Harvard University Press.

Post, R. M., Lockfeld, A., Squillace, K. M., & Contel, N. R. (1981). Drug-environment interaction: context dependency of cocaine-induced behavioral sensitization. Life Sciences, 28, 755-760.

Priest, C. A. & Pfaff, D. W. (1995). Actions of sex steroids on behaviours beyond reproductive reflexes. In Non-reproductive Actions of Sex Steroids (pp. 74-89). Chichester: Wiley.

Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J. (2000). Cocaine alters progesterone but not prolactin serum levels in pregnant rats; relationship to maternal behaviors. In press, Neuroscience Letters.

Quiñones-Jenab, V., Jenab, S., Ogawa, S., Inturrisi, C., & Pfaff, D. W. (1996). Estrogen regulation of mu- and delta- but not kappa-opioid receptor messenger RNA in the forebrain of female rodents. Society for Neuroscience Abstracts, 22.

Quiñones-Jenab, V., Jenab, S., Ogawa, S., Inturrisi, C., & Pfaff, D. W. (1997). Estrogen regulation of μ-opioid receptor mRNA in the forebrain of female rats. Molecular Brain Research, 47, 134-138.

Quiñones-Jenab, V., Krey, L. C., Schlussman, S. D., Ho, A., & Kreek, M. J. (2000). Chronic "binge" pattern cocaine alters the neuroendocrine profile of pregnant rats. Neuroscience Letters, 17, 120-122.

Quiñones-Jenab, V., Ogawa, S., Jenab, S., & Pfaff, D. W. (1997). Estrogen regulation of preproenkephalin messenger RNA in the forebrain of female mice. J.Chem.Neuroanatomy, 12, 29-36.

Quiñones-Jenab, V., Perrotti, L. I., Ho, A., Jenab, S., Schlussman, S. D., Franck, J., & Kreek, M. J. (2000). Cocaine affects progesterone plasma levels in female rats. Pharmacology Biochemistry and Behavior, 66, 449-453.

Quiñones-Jenab, V., Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J. (2000). Steroid replacement affects cocaine-induced behaviors in ovariectomized Fischer rats. Pharmacology, Biochemistry and Behavior, In Press.

Quiñones-Jenab, V., Zhou, Y., Jenab, S., Ho, A., & Kreek, M. J. (2000). Cocaine affects testosterone and progesterone plasma levels in male rats. CPDD Abstracts, 62, 128.

Quiñones-Jenab, V., Ho, A., Schlussman, S. D., Franck, J., & Kreek, M. J. (1999). Estrous cycle differences in cocaine-induced stereotypic and locomotor behaviors in Fischer rats. Behavioural Brain Research, 101, 15-20.

Richardson, N. R. & Roberts, D. C. S. (1991). Fluoxetine pretreatment reduces breaking points on a progressive ratio schedule reinforced by intervenous cocaine self-administration in the rat. Life Sciences, 49, 833-840.

Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J. (1989). The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. Psychopharmacology, 98, 408-411.

Roberts, D. C. S., Dalton, J. C. H., & Vickers, G. J. (1987). Increased self-administration of cocaine following haloperidol: effect of ovariectomy, estrogen replacement, and estrous cycle. Pharmacology Biochemistry and Behavior, 26, 37-43.

Roberts, D. C. S., Koob, G. F., Klonoff, P., & Fibiger, H. C. (1980). Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacology Biochemistry and Behavior, 12, 1387-1395.

Robinson, T. E., Becker, J. B., & Presty, S. K. (1982). Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. Brain Research, 253, 231-241.

Robinson, T. E., Camp, D. M., Jacknow, D. S., & Becker, J. B. (1982). Sex differences and estrous cycle dependent variation in rotational behavior elicited by electrical stimulation of the mesostriatal dopamine system. Behavioural Brain Research, 6, 273-287.

Rocha, B. A., Fumagalli, F., Gainetdinov, R. R., Jones, S. R., Ator, R., Giros, B., Miller, G. W., & Caron, M. G. (1998). Cocaine self-administration in dopamine-transporter knockout mice. Nat.Neurosci., 1, 132-137.

Romano, G. J., Harlan, R. E., Shivers, B. D., Howells, R. D., & Pfaff, D. W. (1988). Estrogen increases proenkephalin messenger ribonucleic acid levels in the ventromedial hypothalamus of the rat. Mol.Endo., 2, 1320-1328.

Romano, G. J., Mobbs, C. V., Lauber, A. H., Howells, R. D., & Pfaff, D. W. (1990). Differential regulation of proenkephalin gene expression by estrogen in the ventromedial hypothalamus of male and female rats: implications for the molecular basis of a sexually differentiated behavior. Brain Research, 536, 63-68.

Romano, G. J., Mobbs, C. V., & Pfaff, D. W. (1989). Estrogen regulation of proenkephalin gene expression in the ventromedial hypothalamus of the rat: temporal qualities and synergism with progesterone. Molecular Brain Research, 5, 57-58.

Romualdi, P., Donatini, A., Izenwasser, S., Cox, B. M., & Ferri, S. (1996). Chronic intracerebroventricular cocaine differentially affects prodynorphin gene expression in rat hypothalamaus and caudate-putamen. Molecular Brain Research, 40, 153-156.

Romualdi, P., Lesa, G., & Ferri, S. (1991). Chronic opiate agonists down-regulate prodynorphin gene expression in rat brain. Brain Res, 63, 132-136.

Rough-Pont, F., Marinelli, M., Le Moal, M., Simon, H., & Piazza, P. V. (1995). Stress-induced sensitization and glucocorticoids II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. Journal of Neuroscience, 15, 7195.

Sarnyai, Z., Dhabhar, F. S., McEwen, B. S., & Kreek, M. J. (1998). Neuroendocrine-related effects of long-term "binge" cocaine administration: Diminished individual differences in stress-induced corticosterone response. Neuroendocrinology, 68, 334-344.

Satou, M. & Yamanouchi, K. (1996). Inhibitory effect of progesterone on sexual receptivity in female rats: A temporal relationship to estrogen administration. Zoological Science, 13, 609-613.

Schenk, S., Horger, B. A., Peltier, R., & Shelton, K. (1991). Supersensitivity to the reinforcing effects of cocaine following 6-hydroxydopamine lesions to the medial prefrontal cortex in rats. Brain Research, 543, 227-235.

Sell, S. L., Scalzitti, J. M., Thomas, M. L., & Cunningham, K. A. (2000). Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J.Pharmacol.Exp.Ther., 293, 879-886.

Sell, S. L., Thomas, M. L., Clarke, C. H., & Cunningham, K. A. (1998). Antisense to estrogen receptor ERα reduces locomotor hyperactivity in response to cocaine in female rats. Society for Neuroscience Abstracts, 24, 495.

Sharma, A., Plessinger, M. A., Miller, R. K., & Woods, J. R. (1993). Progesterone antagonist mifepristone (RU 486) decreases cardiotoxicity of cocaine. P.S.E.B.M., 202, 279-287.

Sidell, F. R. & Kaminskis, A. (1975). Influence of age, sex, and oral contraceptives on human blood cholinesterase activity. Clinical Chemistry, 21, 1393-1395.

Sircar, R. & Kim, D. (1999). Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in Fischer, Lewis, and Sprague-Dawley rats. The Journal of Pharmacology and Experimental Theraputics, 289, 54-65.

Sivam, S. P. (1989). Cocaine selectively increases striatonigral dynorphin levels by a dopaminergic mechanism. J.Pharmacol.Exp.Ther., 250, 818-823.

Smiley, P. L., Johnson, M., Bush, L., Gibb, J. W., & Hanson, G. R. (1990). Effect of cocaine on extrapyramidal and limbic dynorphin system. The Journal of Pharmacology and Experimental Therapeutics, 253, 938-943.

Sofuoglu, M., Duidish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K. (1999). Sex and menstrual cycle differences in the subjective effects from smoked cociane in humans. Experimental and Clinical Psychopharmacology, 7, 274-283.

Sorg, B. A., Davidson, D. L., Kalivas, P. W., & Prasad, B. M. (1997). Repeated daily cocaine alters subsequent cocaine-induced increase of extracellular dopamine the the medial prefrontal cortex. The Journal of Pharmacology and Experimental Theraputics, 281, 54-61.

Spaminato, S., Canossa, M., Campana, G., Carboni, L., & Bachetti, T. (1995). Estrogen regulation of prodynorphin gene expression in the rat adenohypohysis: effects of the antiestrogen tamoxifen. Endocrinology, 136, 1589-1594.

Spangler, R., Ho, A., Zhou, Y., Maggos, C., Yuferov, V., & Kreek, M. J. (1996). Regulation of kappa opioid receptor mRNA in the rat brain by "binge" pattern cocaine administration and correlation with preprodynorphin mRNA. Molecular Brain Research.

Spangler, R., Unterwald, E. M., & Kreek, M. J. (1993). 'Binge' cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen. Molecular Brain Research, 19, 323-327.

Spangler, R., Zhou, L., Maggos, C., Schlussman, S. D., Ho, A., & Kreek, M. J. (1997b). Prodynorphin, proenkephalin, and kappa opioid receptor mRNA responses to acute "binge" cocaine. Molecular Brain Research, 44, 139-142.

Spangler, R., Zhou, L., Maggos, C., Zlobin, A., Ho, A., & Kreek, M. J. (1997a). Dopamine antagonist and "binge" cocaine effects on rat opioid and dopaminergic transporter mRNA. Neuro.Report, 7, 2196-2200.

Spangler, R., Zhou, Y., Schlussman, S. D., Ho, A., & Kreek, M. J. (1997). Behavioral stereotypies induced by 'binge' cocaine administration are independent of

drug-induced increased in corticosterone levels. Behavioural Brain Research, 86, 201-204.

Steiner, H. & Gergen, C. R. (1993). Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. Journal of Neuroscience, 13, 5066-5081.

Substance Abuse and Mental Health Services Administration . National household survey on drug abuse. 1998. Rockville, M.D., U.S. Department of Health and Human Services.

Unterwald, E. M. (1995). Cocaine interactions with the endogenous opioid system. In R.P.Hammer (Ed.), The neurobiology of cocaine (pp. 145). New York: Hammer.

Unterwald, E. M., Ho, A., Rubenfeld, J. M., & Kreek, M. J. (1994). Time course of the development of behavioral sensitization and dopamine receptor up-regulation during binge cocaine administration. The Journal of Pharmacology and Experimental Theraputics, 270, 1387-1396.

Unterwald, E. M., Horne-king, M. J., & Kreek, M. J. (1992). Chronic cocaine alters brain mu opioid receptors. Brain Research, 584, 314-318.

Unterwald, E. M., Rubenfeld, J. M., & Kreek, M. J. (1994). Repeated cocaine administration up regulates kappa and mu, but not delta opioid receptors. Neuroreport, 5, 1613-1616.

Unterwald, E. M., Rubenfeld, J. M., & Kreek, M. J. (1995). Time course of cocaine induced alterations in opioid and dopamine receptors and transporter sites. NIDA Res.Monograph, 153, 507.

Van Haaren, F. & Meyer, M. E. (1991). Sex differences in locomotor activity after acute and chronic cocaine administration. Pharmacology Biochemistry and Behavior, 39, 923-927.

Wagner, E. J., Manzaneres, J., Moore, K. E., & Lookingland, K. J. (1994). Neurochemical evidence that estrogen induced suppression of kappa-opioid receptor mediated regulation of tuberoinfundibular dopaminergic neurons is prolactin independent. Neuroendocrinol., 59, 197-201.

Walker, Q. D., Li, S., & Kuhn, C. M. (1997). Gender differences in cocaine responsivity in rats. CPDD, 59, 160.

Warner, A. & Norman, A. B. (2000). Mechanisms of cocaine hydrolysis and metabolism in vitro and in vivo: A clarification. Ther Drug Monit, 22, 266-270.

Weiland, N. G. & Wise, P. M. (1990). Estrogen and progesterone regulate opiate receptor densities in multiple brain regions. Endocrinology, 126, 804-808.

Weiss, F., Parsons, L. H., & Markou, A. (1995). Neurochemistry of cocaine withdrawal. In R.P.Hammer (Ed.), The neurobiology of cocaine (pp. 163-180). Boca Raton: CRC Press.

White, F. J., Henry, D. J., Hu, X. T., Jeziorski, M., & Ackerman, J. M. (1991). Electrophysiological effects of cocaine in the mesoaccumbens dopamine system. In Cocaine: Pharmacology, physiology and clinical strategies. (pp. 221). Boca Raton: CRC Press.

Wilkinson, M., Bhanot, R., Wilkinson, D. A., & Brawer, J. R. (1983). Prolonged estrogen treatment induces changes in opiate, benzodiazepine, and beta-adrenergic binding sites in female rat hypothalamus. Brain Res Bull, 11, 279-287.

Wilkinson, M., Brawer, J. R., & Wilkinson, D. A. (1985). Gonadal steroid-induced modification of opiate binding sites in anterior hypothalamus of female rats. Biol Reprod, 32, 501-506.

Yuferov, V., Zhou, Y., Spangler, R., Maggos, C., Ho, A., & Kreek, M. J. (1999). Acute "binge" cocaine increases mu-opioid receptor mRNA levels in areas of the rat mesolimbic mesocortical dopamine system. Brain Research Bulletin, 48, 109-112.

Zeigler, S. J., Lipton, J., Toga, A., & Ellison, G. (1991). Continuous cocaine administration produces persisting changes in brain neurochemistry and behavior. Brain Research, 552, 35.

Zhang, J., Dean, R. A., Brzezinski, M. R., & Bosron, W. F. (1996). Gender-specific differences in activity and protein levels of cocaine carboxylesterase in rat tissues. Life Sciences, 59, 1175-1184.

Zhou, Y., Yuferov, V., Spangler, R., Maggos, C., Ho, A., & Kreek, M. J. (1998). Effects of memantine alone and with acute "binge" cocaine on hypothalamic pituitary adrenal activity in the rat. Euro.J.Pharm., 352, 65-71.