Sex differences in progestational effects on cocaine-induced behaviors and neural plasticity

by

Samantha E. Diaz

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Vanya Quinones-Jenab, Ph.D.

Date_____ _____
Chair of Examining Committee

Maureen O'Connor, Ph.D.

Date _____

_____
Executive Officer

Shirzad Jenab, Ph.D
Victoria Luine, Ph.D
Rachel Bowman, Ph.D
Jim Gordon, Ph.D
Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK

Abstract

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Samantha E. Diaz

Advisor: Dr. Vanya Quinones-Jenab

Both clinical and rodent models have shown sexually dimorphic patterns in all phases of drug use and addiction (acquisition, maintenance and relapse). These sexually dimorphic responses to psychostimulants are hypothesized to be due to ovarian hormones. Progesterone has been reported to attenuate many of the behaviors associated with cocaine, in females. Progesterone inhibited cocaine-induced locomotor responses in intact male and female rats. Although progesterone attenuated cocaine-induced behavioral responses, it failed to alter cocaine-induced neural plasticity. Progesterone increased dendritic spine densities in the shell and core of the Nucleus Accumbens (NAcS, NAcC) of male rats. Chronic cocaine increased dendritic spines in NAcC, NacS, CA1 region of the hippocampus. In our third experiment, administration of progesterone and finastesteride, an Allopregnalone antagonist, inhibited the expression of cocaine-induced CPP in female but not male rats. In conclusion, progesterone reduces cocaine-induced locomotor activity and learned associations in rats, without reducing neural plasticity.

Acknowledgements

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Chapter 1: Background
A. Cocaine, Monoamines, and Mesolimbic system:

Cocaine is an active alkaloid found in the leaves of the native South American, Erythroxylon coca tree, which has been used as a psychostimulant among the indigenous people of South America for at least 2,000 years (Karch, 1999). Cocaine mediates its addictive properties through the mesocorticolimbic dopamine system, which is responsible for reward, motivation, and incentive based behaviors. This reward circuit includes dopamine projections from the ventral tegmental area and substantia nigra to the nucleus accumbens (NAc) and striatum, as well as glutamate inputs from the prefrontal cortex, amygdala and hippocampus (see Figure 1).

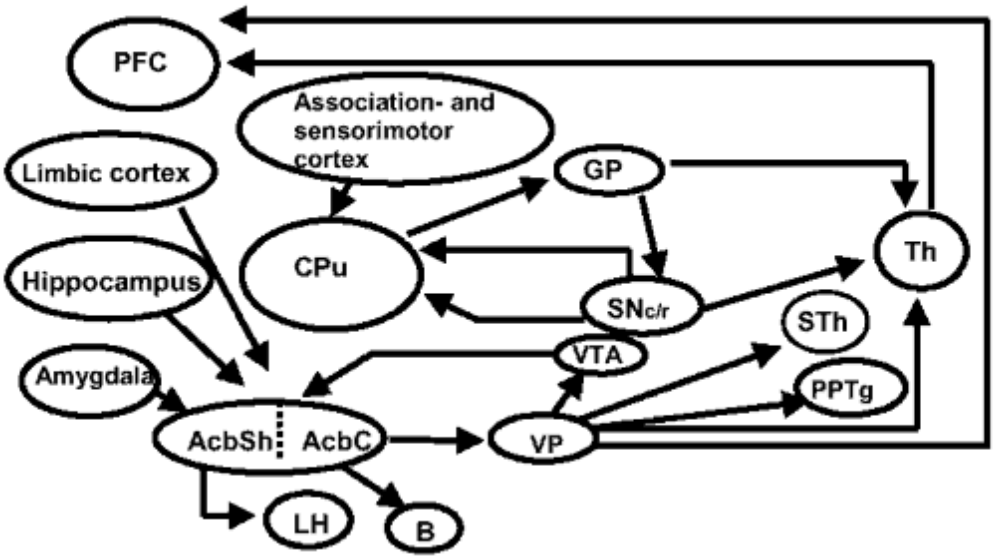

Figure 1: Schematic presentation of the brain structures and their connections between them involved in the regulation of psychomotor and reinforcement processes. The nucleus accumbens receives glutamatergic afferents from some cortical areas and also from the amygdala and hippocampus (Heimer et al.. 1995). CPu = caudate-putamen, GP = globus pallidus, Th = thalamus, STh = subthalamic nucleus, PPTg = Pedunculopontine Tegmental nucleus, SNc = substantia nigra pars compacta, VTA = ventral tegmental area, PCF = prefrontal cortex, VP = ventral pallidum, B = nucleus basalis of Meynert, LH = lateral hypothalamus, NAcSh = nucleus accumbens, shell; NAcC = nucleus accumbens core (modified from Gerfen 1992; Pulvirenti et al. 1991; Robbins and Everitt 1996)).

Internal stimuli such as hunger, thirst, and reproductive impulses activate the mesolimbic dopamine system by stimulating the ventral tegmental area (VTA), which in turn activates the Nucleus Accumbens (NAc) via dopamine releasing axonal projections (Berendse et al., 1992; Brog et al., 2007; Heimer et al., 1991; Klitenick et al., 1992; Schmidt et al., 2005). Cocaine alters this system by inhibiting the reuptake of neural monoamines such as dopamine and thereby increasing their synaptic concentrations (Robbins and Everitt, 1996; Wise and Rompre, 1989) (See Figure 2). The increase in dopamine and other monoamines, in the striatum and NAc, leads to psychostimulant related behaviors, such as an increase in locomotion and various neural adaptations (Becker, 1999). The extent and magnitude of cocaine-induced changes is highly dependent on both behavioral and biological factors, such as the schedule of drug exposure and interactions with other reagents such as hormones.

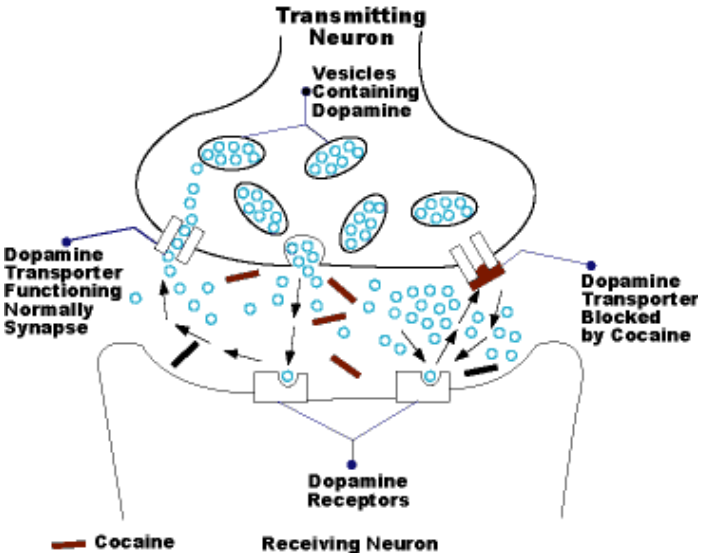

Figure 2: Cocaine's effects on intracellular dopamine: Cocaine increases intracellular dopamine levels by binding to the DAT to inhibit dopamine reuptake. Illustration adapted from the Nat Institute on Drug Abuse Research Advances 13(2), 1998.

II. Responses to psychostimulant administration

A. Behavioral responses to acute and repeated administration of cocaine

Schedules, of cocaine administration, play important roles as mediators of behavioral responses. Acute cocaine administration leads to an increase in locomotion due to the initial synaptic increase of monoamines (Sarti et al., 2007). Chronic or repeated exposure to cocaine results in sensitization or tolerance. Sensitization is an increase in response to a repeated dose of a psychostimulant. Sensitized responses to chronically administered drugs can occur with doses that are equal to or less than what was originally administered. In clinical studies, sensitized participants show longer regular cocaine use and also less dose escalation over prolonged amounts of time (Bartlett et al., 1997).

Contrastingly, tolerance occurs when an organism's response, to an established or previously administered drug dosage, decreases. An organism which has developed a tolerance for a particular stimulus or drug requires an escalation in dosage or stimuli intensity to achieve the initial level of response.

Sensitization and tolerance rest on the idea that both humans and animals learn from environmental cues and make pavlovian associations based on these cues (Bartlett et al., 1997; Carey and Damianopoulos, 2006; Haracz et al., 1995; Post et al., 1981). Sensitization is expressed in locations where animals establish a connection between the place of administration and the drug effects. Priming exposure may be minimal, making the learning of environmental cues an important mediator of the behavior (Carey and Damianopoulos, 2006; Todtenkopf and Carlezon, 2006). Several types of context dependant cues, other than just location, may facilitate sensitization. These cues include the presence of the experimenter, the sensations caused by the injections, rate of cocaine administration, and any promptings associated with handling and drug

administration (Samaha et al., 2002; Todtenkopf and Carlezon, 2006). Without the presence of these cues, sensitization may be experienced neurally but not be behaviorally (Todtenkopf and Carlezon, 2006). A change in temperature, location, olfactory input, or environmental visual cues may lead to a cessation of increased behavioral response without a diminishment in neural adaptations. Context or environment specific behavioral sensitization is preceded by neural sensitization (Robinson and Berridge, 2000; Robinson et al., 2001).

B. Neural Sensitization

Neural adaptations in the mesolimbic system have been reported in response to both acute and chronic administration of psychostimulants. Acute cocaine administration leads to increases in dendritic spine densities in the VTA whereas repeated administration of cocaine and amphetamine results in increases in dendritic spine densities and increased dendritic arborization in the NAc (Sarti, 2007; Robinson & Kolb,1999). The neurochemical and morphological adaptations to the exposure of cocaine and subsequent increase in monoamines, such as dopamine and glutamate, has been termed neural sensitization (Robinson et al., 2001). It includes changes such as increases in dendritic length, arborization, and spine densities. Dopamine and glutamate levels are affected by cocaine exposure and are involved in the physiological adaptations to chronic cocaine, leading to behavioral sensitization. Most spines on NAc medium spiny neurons receive asymmetric glutamate synapses onto the head of the spine with a dopaminergic symmetric input nearby forming a “triad” arrangement (Robinson et al., 2001; Smith et al., 1998). This triad arrangement is thought to represent the structural means by which dopamine inputs modulate the excitatory drive on these neurons while glutamate receptors and transmission have been reported to be modified during drug-associated, Pavlovian, and

instrumental associative learning (Jones and Bonci, 2005; Robbins and Everitt, 1996; Robinson et al., 2001).

Evidence for neural sensitization includes first, animals receiving drug treatments in an environment other than the one in which they ordinarily receive their doses will display behaviors associated with sensitization once placed back in their home or drug associated environment suggesting that the mechanisms which allow for the hypersensitivity were already in place. Secondly, neural sensitization has been reported in both in vitro tissue samples and anesthetized animals (Robinson et al., 2002; Robinson and Kolb, 2004).

Changes in neuronal structure, such as spine density fluctuations, are important because they are hypothesized to be involved in changes in synaptic connectivity and ultimately behavioral responses (Robinson & Kolb, 1999, 2004). For instance, repeated administrations of both amphetamine and cocaine have been shown to lead to sensitized locomotor behavior in both male and female rats (Chin et al., 2002; Zhao & Becker, 2009; Chin et al., 2001; Robinson & Kolb, 1999). This behavioral sensitization has been associated with increases in dendritic spine densities in the NAc core, hippocampus, and prefrontal cortex (Robinson & Kolb, 1999; Robinson & Kolb, 2004; Ferrario et al., 2005; Martin et al., 2004; Li et. al., 2004). See Fig 3. Increases in dendritic spine densities in the Nac have been shown to be in direct response to drug exposure rather than unbiased reward related occurrences. For example, rats which were allowed to self-administer cocaine (0.33 mg/infusion) for 1 h a day, for 1 month, had significantly greater dendritic spine densities in the Nac than did animals which were allowed to bar press for food (Robinson et al., 2001). Similarly, changes in neural structure have been argued to be a result of drug-induced increases in locomotor activity. Rats receiving repeated administration of cocaine

or amphetamine displayed significant increases in dendritic spine densities, in the Nac, compared to animals given free access to running wheels for four weeks (Robinson & Kolb, 1999).

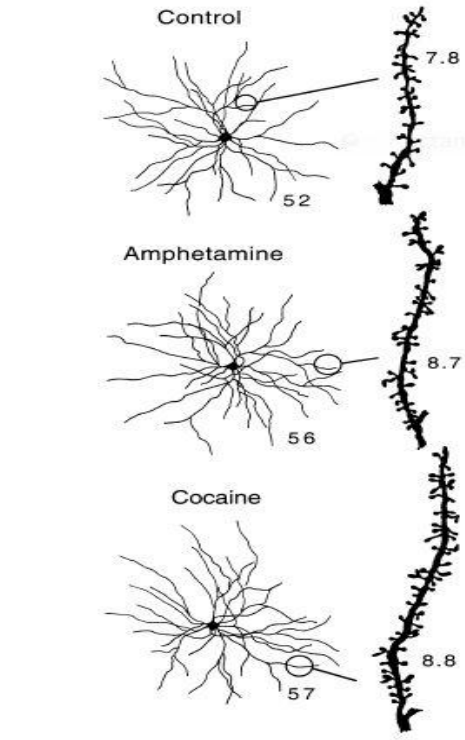

Figure 3: Camera Lucida drawings of medium spiny neurons from the NAc from rats treated with chronic amphetamine, chronic cocaine, and controls. Modified from Robinson et. al (1999).

Dendritic spine increases reflect the amount of drug an animal has been exposed to and corresponding behavioral output. Chronic administration in male rats resulted in an increase of dendritic spine densities in both the core and shell of the NAc, only if animals showed behavioral sensitization (Li, Y., Acerbo, MJ, and Robinson, TE , 2004).

Similarly, Ferrario et al. (2005) has reported that animals given extended access to cocaine have almost double the dendritic spine densities in the NAc core than animals which received limited access to cocaine. Those with increased densities in the Core were also more likely to show sensitized locomotor behavior when given a challenge dose of cocaine a month later.

Changes in dendritic spine density and morphology may be the most direct reflection of drug induced synaptic plasticity because their reorganization fundamentally alters the interaction between dopamine and glutamate neurotransmission, potentially resulting in behaviors that are typical of sensitized animals. For example, the NAc becomes more responsive to stimuli due to the neuroadaptive changes such as the increase of glutamate receptors on NAc neurons (neural sensitization) (Prasad et al., 1999). The increase of receptors, suggests that the redistribution of AMPARS in the NAc provides a mechanism to account for augmentation of the locomotor response and for sensitivity to reinstatement of drug seeking behavior so that when a trigger such as stress; sucrose; a priming injection of cocaine; or cocaine-conditioned cues appear, the limbic system is automatically activated becoming more sensitized over time in a Hebbian manner (Boudreau and Wolf, 2005; Gosnell, 2004). Changes in dendritic length and arborization have also been reported in medium spiny neurons in the NAc after cocaine exposure (Adinoff, 2004; Hyman, 2005; Jones and Bonci, 2005; Kalivas and Volkow, 2005; Li et al., 2004; Nestler and Aghajanian, 1997; Robinson et al., 2001; Robinson et al., 2002; Robinson and Kolb, 1997). Theoretically, increases in length and arborization allow for more connections in the neural circuitry to form. Cocaine administration increases dendritic branching and spine density regardless of whether it is self-administered or administered by an experimenter (Robinson et al., 2001; Robinson et al., 2002; Robinson and Kolb, 1997; Robinson and Kolb, 2004).

The reported increases in branching and spine densities, in the limbic system after chronic drug exposure, are specific changes related to psychostimulants and not with other reward system related stimuli. Robinson (2001) tested the effects of self-administering food and cocaine on spine densities in the NAc. In this experiment, one group of rats bar pressed for food while the other group self-administered cocaine via bar pressing. After acquisition, animals given

access to food, bar pressed more than those given access to cocaine. Although food was more rewarding, it did not increase dendritic spine density or dendritic branching in the NAc. The neural morphology of animals in the food group did not differ from that of controls. Contrastingly, animals which self-administered cocaine showed increases in density and branching (Robinson et al., 2001). The specificity of stimuli, which correlates with the changes in morphology, indicates that drugs override innate natural reward systems and alters them in ways that naturally rewarding stimuli does not. Amphetamine and cocaine exposure have also been shown to override and limit other forms of structural plasticity such as increased branching and spine density that is usually accrued from exposure to living in complex environments (Comery et al., 1996; Kolb et al., 2003; Xiao and Becker, 1997). The psychostimulant induced changes are long lasting and sometimes permanent. After 21 days of withdrawal, cocaine sensitized rats show higher ratios of intercellular and surface glutamate receptors with a corresponding increase in behavior (Boudreau and Wolf, 2005; Robinson et al., 2001). Rats have been shown to maintain cocaine induced behavioral and morphological changes for up to a month and a half after drug exposure (Robinson and Kolb, 2004).

C. Sex Differences in Clinical Models

In clinical studies, similar sexual dimorphisms, to drugs of abuse, have been found. Sex differences are reported in occurrence, addiction, and relapse to cocaine addiction. Although men have more opportunities for cocaine exposure and therefore use, men and women progress to equal use once exposure has occurred (Van Etten and Anthony, 1999; Van Etten and Anthony, 2001; Van Etten et al., 1999) but women consume the drug by more addictive routes and experience more rapid progression of drug dependence (McCance-Katz et al., 1999).Women

report more nervousness after intranasal administration, take longer to feel the subjective effects, report less euphoria and have more severe drug use at greater amounts (Griffin et al., 1989; Kosten et al., 1996; Singha et al., 2000). Women also have more intense cravings for cocaine in the presence of drug associated cues suggesting that feelings of "wanting" associated with sensitization are greater (Kosten et al., 1996). Gender differences are also seen in treatment outcome and relapse rates (Gallop et al., 2007; McCance-Katz et al., 1999; Wong et al., 2002). Women report shorter abstinence periods between cocaine uses than men (Roth et al., 2004).

D. Sex Differences in behavioral responses to acute and repeated administration of cocaine

Sexually dimorphic behaviors have been reported in both acute and chronic schedules of psychostimulant administration. Under acute schedules of psychostimulant administration, greater behavioral effects have consistently been observed and reported in female rodents. Early studies with amphetamine reported that female rats show more intense and prolonged stereotypy and greater locomotor activity than do male rats (Schneider and Norton, 1979; Savageau and Beatty, 1981). Females elicit greater rotational behavior than do males after acute d-amphetamine injections (Becker et al., 1982; Robinson et al., 1980). Studies involving acute cocaine illustrate that female rats and mice demonstrate more robust behavioral responses to exposure than do males (Chin et al., 2002). Following acute cocaine administration, female rats show significantly more horizontal activity, rearing, and stereotypic behaviors than males (Walker et al., 2001). Female rats require lower doses of cocaine to achieve male-like behavioral responses, which persist for a longer duration of time (Festa et al., 2003; Festa et al., 2004).

Models utilizing chronic administration schedules also report dimorphic responses to psychostimulants. Females demonstrate higher levels of behavioral sensitization to cocaine

(Sharma et al., 1993; Festa et al., 2003; Festa et al., 2004) at faster rates, to lower doses of drug, and for longer periods of time (Festa et al., 2003). After 13 days of chronic cocaine administration, female rats exhibit more rearing and total activity than males (Harrod et. al, 2005). Sex differences in the sensitization of orafacial behaviors have also been reported. Female rats exhibit more orofacial incidence by day 13 than do males (Harrod et. al, 2005). Sex differences have been seen after intermittent injections of psychostimulants, which have produced more robust behavioral sensitization in female rats than in male rats (Robinson et al., 1982a; Robinson et al., 1982b; Robinson et al., 1982c)

E. Sex Differences in Neural Sensitization

The role of ovarian hormones in the process of cocaine-induced neuroplasticity is still unclear but we hypothesize that they may have a role in mediating synaptic plasticity for two reasons. The first is that sex steroids have been implicated to be at least partially responsible for drug-induced behavioral sex differences (Festa et al., 2003; Festa et al., 2004; Walker et al., 2001). Secondly, ovarian hormones have been shown to mediate neural plasticity in portions of the mesolimbic dopamine reward system (Gould et al., 1990). Specifically, spine densities have been shown to fluctuate in the Hippocampus during the estrous cycle in female rats, with higher levels of estradiol correlating with higher synaptic densities (Woolley & McEwen, 1992; Woolley et al., 1990). This hormone induced plasticity occurs over a 5 day period. McEwen and Woolley (1994) have shown that there is a decline of spine densities between proestrus and estrus, which is due to a drop in estradiol and rise in progesterone levels. Administration of progesterone quickens the decrease in dendritic spine densities where administration of the progesterone

antagonist, Ru486, blocks the natural decline of synaptic densities (Woolley and McEwen, 1994).

Sex differences in neural sensitization, to cocaine or amphetamine, have yet to be reported in adult rats. Prenatal cocaine exposure has been reported to increase dendritic spine densities in the NAc, CA1, striatum, and prefrontal cortex without producing sex differences in any region in rat pups (Frankfurt, et al., 2009). However, sex hormones, in mature rats, have been reported to affect dendritic spine densities in regions such as the hippocampus (Woolley et al., 1990; Gould et al., 1990; Dalla et al., 2009). Gould et al. (1990) demonstrated that OVX animals had a significant reduction in spine densities in the CA1 region of the hippocampus. Within 5 hours of progesterone and 3 days of estradiol- benzoate administration, spine densities were increased in OVX animals (Gould et al., 1990). In another study, rats administered two injections, every 24 hours, of 10ug of estradiol benzoate, 48 hours post-OVX showed a 32% increase in apical dendritic spine density on the CA1 pyramidal cells (Wolley & McEwen, 1992).

Changes in spine densities are correlated with neural sensitization. Neural sensitization is postulated to be responsible for the psychological process of compulsive drug seeking and drug taking, labeled as "wanting", which is separate from a hedonistic approach to addiction in which addicts intensely like the drug and drug induced feelings (Koob GF et al., 1997; Robinson and Berridge, 2000). This subjective "wanting" is linked to the changes that occur in the mesocorticolimbic dopamine system responsible for addiction and relapse.

III. Mechanisms involved in sexually differentiated responses to cocaine

A. Sex Differences and Hormones

The observed sexually dimorphic responses to psychostimulants are rooted in a biological basis. Circulating hormones are postulated to be the main mediators of sexually dimorphic responses through activational mechanisms. Both Bowman et al, (1999) and Festa et al., (2003) have similarly reported that brain concentrations of cocaine, after systematic administration, are comparable for male and female rats, ruling out sex differences in cocaine metabolism as the leading cause for differentiation in response. Although, organizational effects also play a role, the evidence for activational effects is much stronger. First, the removal of gonadal hormones in female rodents, leads to a decrease in psychostimulant-induced behaviors typically seen in females. For example, ovariectomized rats display less rearing, total activity, and locomotor incidence relative to intact females following acute cocaine injection (Harrod et al., 2005). Ovariectomy also alters locomotor response in chronic models. OVX has been reported to negate any significant difference in locomotor responses between cocaine and saline treated groups after 7 and 14 days (Chin et al, 2002). Surgical or chemical estrogen blockage significantly decreases responding for cocaine selfadministration (Lynch et. Al., 2001). Animals which were OVX or received tamoxifen self-administered significantly less cocaine than did those which were OVX and received estrogen or where sham operated (Lynch et al, 2001).

Secondly, the administration of exogenous female hormones reinstates or increases behavioral responses. Sell et al (2000) reported that the administration of estradiol or estradiol and Progesterone (E+P) to OVX animals increased horizontal locomotor activity in response to cocaine compared to OVX animals or those which were OVX and received Progesterone (OVX+ P). Administration of estradiol to OVX rats has also been reported to augment acute cocaine-

induced rearing and ambulatory behaviors (Perroti et al., 2001). In chronic administration models, estrogen replacement enhances behavioral sensitization and locomotor responses in OVX rats to levels which are statistically similar to intact rats (Peris et. Al, 1991; Sell et. Al., 2000). Perroti et al. (2001) found that OVX rats built tolerance to chronic cocaine exposure but those which were administered estradiol and progesterone (E+P) displayed sensitized total locomotor responses. Estrogen replacement (OVX + EB) also increases the number of infusions and percentage acquisition for cocaine self-administration, compared to animals which were OVX and treated with vehicle (OVX +VEH) (Lynch et al, 2001).

The third piece of evidence, that activational effects of hormones is a strong mediator of sexually dimorphic responses to psychostimulant, is seen in developmental studies. Sexually dimorphic responses to cocaine are not observed in early stages of development in rodents. Seven day old male and female pups, treated both chronically and acutely with cocaine, failed to show any sex differences in locomotor behavior or cocaine brain levels (Bowman et al, 1996). Pups showed very similar locomotor response to adult female rats, after acute cocaine administration. After chronic administration, adult females demonstrated sensitized behavioral responses but rat pups did not (Bowman et al, 1996). Adolescent female rats show the same behavioral responses as due adult females (Lynch, 2007). Adolescent females acquired cocaine self-administration more readily than did males and responded at higher levels under progressive ratio testing (Lynch, 2007). The lack of sex differences reported in infants, suggests that hormonal changes at puberty may account for the differences seen in adolescents and adults.

There are also sex differences seen in HPA responses to cocaine. Intact female rats have higher levels of corticosterone release after cocaine administration (Walker et al, 2001; Chin et al, 2001; Kuhn and Francis, 1997). Females also show enhanced HPA response to monoamine uptake

inhibitors (Kuhn and Francis, 1997). The reported sex differences in HPA activity are postulated to be hormone based. Corticosterone levels fluctuate throughout the estrous cycle, peaking in the proestrous phase (Walker et al, 2001). Ovariectomy and hormone replacement alter baseline and cocaine induced levels of corticosterone. OVX rats show higher corticosterone levels than controls (Chin et al, 2002). Estrogen replacement increases baseline serum levels of corticosterone in saline treated controls (Niyomchai et al, 2005). Estrogen also increases CORT levels in animals receiving cocaine administration (Niyomchai et al, 2005). In contrast, administration of progesterone does not alter corticosterone levels in animals receiving saline or cocaine (Niyomchai et al, 2005).

In humans, the onset of menstruation defines the beginning of a cycle, the follicular phase, in which the development of the ovarian follicle begins and culminates in ovulation. The site of the ruptured ovarian follicle becomes the corpus luteum, and there is an increase in progesterone during this luteal phase. In the next step, the corpus luteum will produce progesterone in addition to estrogens for approximately the next two weeks. It is during this time frame, the luteal phase, where women have reported less subjective effects of cocaine and less desire to smoke cocaine (Evans et al, 2002; Sofuoglu et al, 1999).

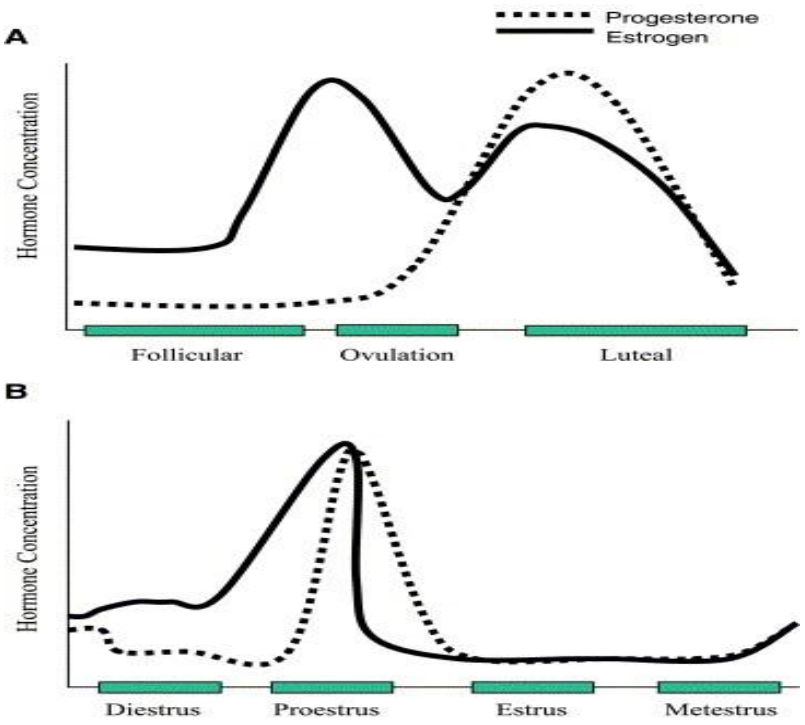

Figure 4: Phases of the menstrual and estrous cycles. During the human menstrual **(A)** and rat estrous **(B)** cycle, levels of the ovarian hormones estrogen and progesterone fluctuate across three phases (follicular, ovulation, and luteal) for the menstrual cycle and four phases (metestrus, diestrus, proestrus, and estrus) for the female rat estrous cycle. Data were redrawn from those presented by Carter (1993). (Festa & Quinones-Jenab, 2003).

B. Progesterone

Progesterone is a steroid hormone involved in the female reproductive cycle, which supports pregnancy. It contains 4 cyclic hydrocarbons and contains ketone and 2 methyl branches (see Figure 4). See Figure 4.

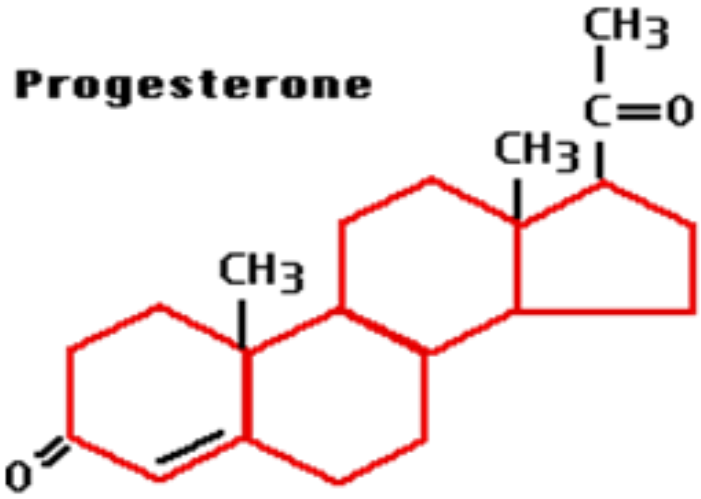

Figure 5: The progesterone molecule: progesterone consists of four interconnected cyclic hydrocarbons. Progesterone contains ketone and oxygenated functional groups, as well as two methyl branches.

Progesterone is synthesized from pregnenolone, a derivative of cholesterol. This conversion takes place in two steps. The 3-hydroxyl group is converted to a keto group and the double bond is moved to C-4, from C-5. Progesterone is the precursor of the mineralocorticoid aldosterone; and after conversion to 17-hydroxyprogesterone (another natural progestogen) becomes a precursor of cortisol and androstenedione. Cortisol levels rise in response to cocaine and stress inducing stimuli. Androstenedione can be converted to testosterone, estrone and estradiol (Falkenstein et al, 2000; McEwen, 2001). As shown in Figure 5, Progesterone is also converted to dihydroprogesterone which binds with high affinity to intracellular progestin receptors in the hypothalamus and other areas (Iswari et al, 1986).Progesterone is converted to 5alpha-pregnan-3alpha-ol-20-one (3alpha, 5 alpha-THP) which is also referred to as allopregnalone (Gago, et al, 2004). 3alpha, 5alpha, THP has actions via GABAa, NMDA, and dopamine receptors which play a role in cocaine mediated reward responses (Frye et al, 2004).

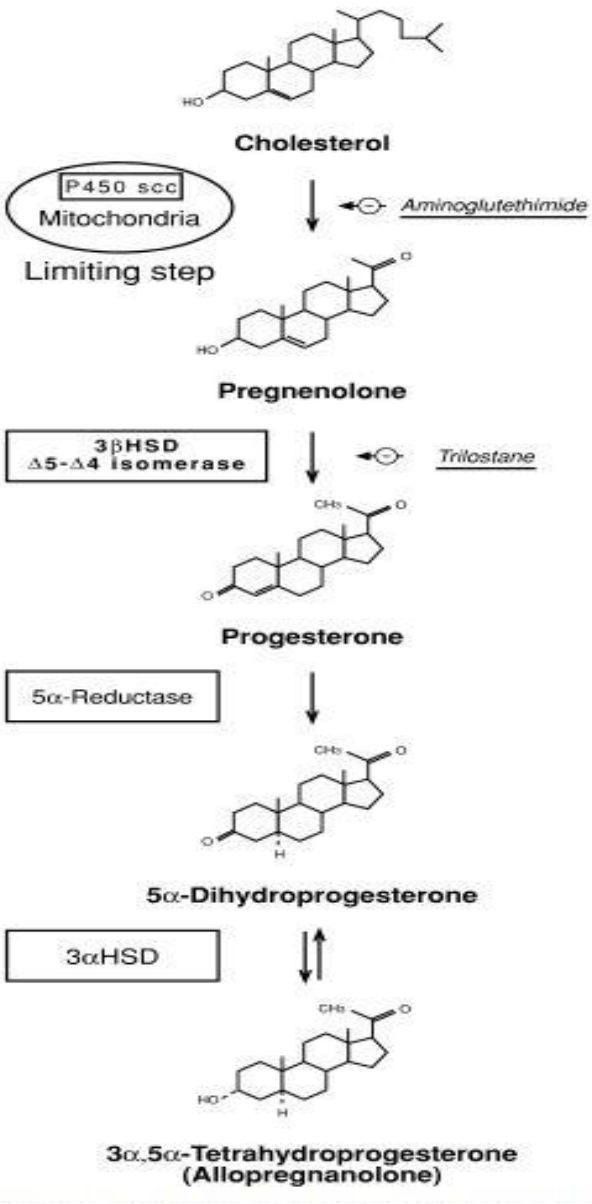

Figure 6: The biosynthesis and metabolism of progesterone. P450scc converts cholesterol to pregnalone, which is then converted to 3BHSD to progesterone. The 5a-reductase converts progesterone to 5a-dihydroprogesterone and the 3a-hydroxysteroid oxidoreductase (3aHSD) catalyzes the conversion of 5a-dihydroprogesterone to 3a,5atetrahydroprogesterone (ALLOP). (Schumacher et al, 2001).

C. Progesterone's effects on cocaine-induced locomotor and reward responses

Progestins influence the expression of naturally motivated behaviors such as feeding, fighting, fleeing, and mating as well as those pertaining to psychostimulants (Frye, 2006). In both clinical

and animal studies, elevated progesterone levels alter cocaine-mediated responses. For example, women cocaine users have attenuated subjective responses and less desire to smoke cocaine during the progesterone dominant luteal phase than during the follicular phase of the menstrual cycle (Evans S.M., 2007; Evans S.M. and Foltin, 2006; Evans et al., 2002; Sofuoglu et al., 1999). Oral administration of progesterone has been shown to attenuate some of the subjective and cardiovascular effects of cocaine self-administration in both men and women (Evans et al., 2002; Sofuoglu et al., 2002; Sofuoglu et al., 2003).

As stated previously, many of the sex differences reported in behavioral responses to cocaine are mediated by gonadal hormones, particularly estrogen. Administration of estradiol to OVX animals has repeatedly increased locomotor activity, cocaine seeking, and administration (Niyomchai et al., 2005; Hu et al., 2004; Jackson et al., 2006; Lynch et al., 2001). Progesterone antagonizes estradiol's increase of behavioral effects. Feltenstein and See (2007) found an inverse relationship between cocaine seeking and progesterone plasma levels. Naturally elevated progesterone levels, in intact female rats, attenuated cocaine-seeking, during self-administration and reinstatement trials following a 10 mg/kg priming dose of cocaine (Feltenstein and See, 2007). Progesterone pretreatment also significantly reduced cocaine-primed reinstatement behavior in intact females in estrus (Feltenstein and See, 2008).

In OVX animals, acute progesterone administration reverses estradiol's effects on acquisition of cocaine self-administration and cocaine primed reinstatement (Anker J.J. et al., 2006; Anker J.J. et al., 2007; Feltenstein and See, 2007; Jackson et al., 2006). Jackson et al (2006) reported that co-administration of P and E, prevented the estradiol-induced acquisition enhancement of cocaine self-administration. OVX rodents receiving P + E self- administered similar amounts of cocaine as OVX females, intact males, castrated and sham males. P + E

administration also reduced reinstatement of cocaine self-administration, in intact females (Anker et al., 2007). Quinones-Jenab et al. (2000) found, in OVX rats, that estrogen and progesterone administered 4 hours before 15 mg/kg of cocaine, significantly decreased locomotor responses compared to animals which were pretreated with estradiol.

Progesterone alone may have attenuating affects on cocaine-induced behaviors. Niyomchai et al. (2005) found that progesterone administered 24 hours before cocaine administration decreased locomotor activity, in OVX animals. Female rats receiving 50 or 500 ug of progesterone displayed a significant reduction in rearing compared to those receiving sesame oil. In intact female rats, administration of P after behavioral extinction, reduced reinstatement of cocaine self-administration compared to animals treated with hormone vehicle and cocaine (Anker et al, 2007).

Progesterone has also been reported to affect the associative memory process linked with psychostimulants and reward. Allopregnanolone, a progesterone metabolite, has been shown to inhibit learning and memory in rodents (reviewed in Maurice et al., 2006). Russo et al (2008) demonstrated that intact female rats, subcutaneously treated with 500 ug of progesterone 4 hours before cocaine administration, failed to acquire and express cocaine-induced conditioned place preference (Russo et al., 2008; Russo et al., 2003). Progesterone, via Silastic capsules, also inhibited CPP expression in intact female rat (Russo et al., 2003) Most studies involving progesterone's effects on reward and associative learning have been performed on female animal models but progesterone has been reported to inhibit CPP in male mice (Romieu et al , 2003). Administration of P, 10 minutes prior to cocaine, inhibited the acquisition of CPP dose dependently. Sircar and Kim (1998) reported that rodents which were OVX and administered progesterone failed to show any behavioral sensitization (Sircar and Kim, 1999). Sircar and Kim

(1999) controlled for individual variability among strains by testing and comparing Sprague Dawley, Fischer 344, and Lewis rats. Sensitization was inhibited in all strains, with the administration of P. The attenuation of sensitization is important because factors that render organisms, both human and non-human, susceptible to sensitization will also contribute to susceptibility and reinstatement of addiction (Robinson and Berridge, 2000; Robinson et al., 2001).

Progesterone's effect on cocaine-induced neural plasticity is still unclear. Hormone studies without the administration of psychostimulants have shown that administration of progesterone affects dendritic spine densities in the CA1 region of the hippocampus. Woolley & McEwen (1993, 1994) have reported that progesterone's effects on dendritic spine densities may be biphasic. In OVX rats, administering estradiol + progesterone (E + P), results in a greater increase in dendritic spine density than that which is caused by estradiol alone. However, 18 hours after the administration of estradiol + progesterone (E + P), dendritic spine densities dropped to that of control OVX animals. It is hypothesized that the sharp decline in dendritic spine densities seen in intact female rats, between proestrus and estrus is due to changing levels of progesterone. Intact rats in proestrus, treated with RU486, a progesterone receptor antagonist, do not display decreases in dendritic spine densities during the proestrus to estrus shift (Woolley & McEwen, 1993, 1994). Progesterone's effects on neural plasticity in the NAc are still unclear.

Allopregnalone and Cocaine

Allopregnalone (5 alpha-pregnan-3alpha-ol-20-one**,** ALLOP) is a progesterone metabolite. It is hypothesized that progesterone is converted, in the brain and peripheral tissues, into ALLOP by enzymatic metabolism (Armstrong et al., 1975; Bixo et al., 1997; Morrow et al.,

1998). Progesterone administration increases ALLOP levels in men and women (Soderpalm et al., 2004). Cocaine administration also increases ALLOP and progesterone levels in serum, hippocampus formation, and striatum in male and female rats, indicating a relationship between the two (Quinines-Jenab et al., 2008).

In cocaine studies, ALLOP administration has been reported to attenuate drug-induced behaviors. For example, chronic administration of ALLOP (30 mg/kg) decreased the percentage of mice exhibiting clonic seizures after successive cocaine injections (Kaminski et al., 2003). Pretreatment with lower doses of ALLOP (17 mg/kg) attenuated sensitization to the convulsive effects of cocaine in cocaine-kindled mice (Kaminski et al., 2003). Pretreatment with ALLOP (15 mg/kg) significantly reduces cocaine-primed reinstatement in females, when compared to treatment groups receiving progesterone + finesteride or vehicle (Anker et al., 2009). In male mice, 2-8 ug of ALLOP pretreatment, inhibited spontaneous locomotor activity and attenuated amphetamine-induced motor hyperactivity (Khisti et al., 2002)

Both progesterone and ALLOP have the potential to mediate behaviors in humans and rodents. Soderpalm et al. (2004) report that increases, in ALLOP levels, produce mild and sedative-like effects in both men and women. In the elevated plus maze, allopregnanolone (8 μg/i.c.v.) induced a significant increase in the time spent and the number of entries in open arms compared with the vehicle-infused controls (Akwa, 1999**).** Beauchamp et al. (2000) reported that 5 ug of ALLOP, administered via i.c.p., caused behavioral aversion in intact male rats, during conditioned place preference procedures (Beauchamp et al., 2000). Interestingly, the effects of ALLOP were dose dependent. Smaller doses caused greater reductions in preference expression. For example, 25 ug of ALLOP caused near aversion whereas 30 ug caused neither preference nor aversion (Beauchamp et al., 2000). I.V. administration of ALLOP, has also been shown to cause

cognitive or memory impairments. For example, I.V. administration of ALLOP was reported to inhibit water maze learning in intact male rats (Johansson et al., 2002). Many of progesterone's attenuating effects may be due to ALLOP since it has been shown to actively mediate behaviors related to addiction such as learning, memory, and locomotion.

IV. Neuroanatomy of reward and addiction

A. The Role of the Nucleus accumbens in cocaine-induced sensitization

Although several areas are important in the process of sensitization and reward learning, presently the focus of this paper and project is on the NAc and Hf because of their large role in reward and learning, as well as extensive influence by hormones, in both function and morphology (Wise, 1998). The NAc is divided into two functionally distinct areas, termed the shell (NAcS) and core (NAcC). The shell is strongly interconnected with the hypothalamus and VTA and is often described as part of the extended amygdala (Kalivas and Volkow, 2005; Schmidt et al., 2005). Reciprocal dopamine innervations from the VTA to the NAc shell are important in controlling motivational salience and contribute to establishing learned associations between motivational events and important coexisting environmental cues (Kalivas and Volkow, 2005). It is also highly tied to the basolateral amygdala (BLA) through dopamine afferents. Modulation of memory consolidation is effected through simultaneous activation of DA receptors within the NAcS and BLA (LaLumiere et al., 2005) but not the NAcC.

In contrast, the core compartment is anatomically associated with the dorsal striatum/basal ganglia and orbitofrontal cortex (Alheid and Heimer, 1988; Kalivas and Volkow, 2005; Schmidt et al., 2005). It is a site mediating the expression of learned behaviors in response to stimuli predicting motivationally relevant events (Kalivas and Volkow, 2005). It mediates the

incentive value of reward-conditioned stimuli and contributes to drug associated cue-induced cocaine seeking (Di Ciano and Everitt, 2004; Fuchs and See, 2002; Schmidt et al., 2005). Another main distinction between the NAcS and NAcC is in negotiation of neurotransmitters. The NAcS is highly dopamine dependant. The NAcC in contrast depends on glutamatergic afferents from the prefrontal cortex to express adaptive behavior (Kalivas and Volkow, 2005). Dopamine is also released into the NAcC but the monumental distinction lies in that it is not necessary in order for the NAcC to mediate behaviors (Kalivas and Volkow, 2005).

Another key distinction between the NAcC and NAcS is that increased spine densities have been reported in both areas after chronic exposure to cocaine, but only after behavioral sensitization had been induced, did the density increase in the core, reinstating its importance in maintaining conditioned types of learning, both behaviorally and morphologically (Li et al., 2004).

Alteration of dendritic morphology is reported in both the NAc and prefrontal cortex, following repeated treatment with psychostimulants such as amphetamine and cocaine (Robinson and Kolb, 2004). Repeated exposure to either psycho stimulant results in an increase of distal dendritic branches and dendritic spine densities on medium spiny neurons, in the nucleus accumbens and in apical dendrites of the prefrontal cortex (Robinson et al., 2001; Robinson et al., 2002; Robinson and Kolb, 1997). Robinson and Kolb (1999) reported that 15 mg/kg of cocaine, administered by I.P. injection, for four consecutive weeks, increased dendritic branches in the NAcS by 6.9%, in intact female rats. The same experiment also found that cocaine significantly increased the density of spines on medium spiny neurons by 13.5%; increased the total number of spines; and the number of multiple-headed spines.

Most studies involving the morphological effects of chronic drug administration, on the NAc, use intense schedules of administration. These treatment regimens include 5 weeks of amphetamine administration or 20 injections of 15 mg/kg of cocaine (Robinson and Kolb, 1997; Robbinson and Kolb, 1999). Kolb et al. (2003) and Li et al. (2004) have reported increases in spine densities with les drug. A single injection of 2 mg/kg of amphetamine and 8 daily injections of 15 mg/kg of cocaine have been shown to produce changes in neuronal morphology. Animals were studied 2 weeks after the last treatment (Kolb et al., 2003; Li et al., 2004).

B. Sex Differences in NAc

Sex difference in neurochemical responses to psychostimulants exist in the nucleus accumbens. For example, previous research in this lab has found that male and female rats showed different monoamine levels in the NAc, after acute cocaine administration (Festa et al, 2004). 20 mg/kg of cocaine increased DA levels in the NAc of male rats, while decreasing DA, DOPAC, 5-HT and HVA levels in females. DOPAC/DA turnover was decreased in both sexes. Sex differences in synaptic plasticity are still to be determined.

C. The Hippocampal Formation (HF) and cocaine-induced environmental learning

A third region, the HF is a brain region that has been strongly connected with spatial learning and memory, more so than addiction or reward, and therefore the literature on its biological adaptations in response to drugs is scarce. Certain drugs have been shown to be readily self-administered by rats into the hippocampus (Vetulani, 2001) suggesting that it may play a larger role in addiction than previously thought. It is also key in learning environmental cues, which is essential for the expression of sensitization. Environmental information is

processed by the thalamus and cortex, relayed to the amygdala and hippocampus, and then to the NAc. The hippocampus and amygdala integrate memories of previously experienced stimuli and past actions, which contain incentive and biological salience. The behavioral output is in response to stimuli such as drugs, drug paraphernalia, or environment. The outcome of this experience is stored as a memory by the hippocampus and other learning systems. This model rests heavily on the importance of both environmental cues and the ability to learn about those cues through proper function of the hippocampus; proper balance of its neuropetides and endocrines; as well as neural morphology to relate and transmit necessary signals.

The ventral portion of the hippocampus is innervated by dopaminergic projections from the VTA and contributes to goal oriented behavior (Di Ciano and Everitt, 2004; Fuchs and See, 2002; Schmidt et al., 2005). Chemical stimulation of the ventral hippocampus activates dopaminergic cell bodies in the VTA, leading to increased dopamine transmission in NAc (Legault et al., 2000; Schmidt et al., 2005).

D. Sex Differences in HF

Alterations and sex differences, based on endocrine levels, have been observed in the hippocampus. Specifically, gonadectomized rodents displayed decreases in CA1 dendritic field responses (Smith et al., 2002) showing that hormones play a crucial role in proper hippocampal function. These observed fluctuations in ovarian hormones alter behavioral processes in rodents and humans, including cognitive performance and responses to drugs (Frye et al., 2000). Elevations in E2 and progestins are associated with enhanced cognitive performance on tasks that are highly hippocampus dependant. Data from tasks that involve hippocampal function

suggests that it is a target site for E2 and its effects on cognitive enhancement (Rapp et al., 2003; Shughrue et al., 2002; Fader et al., 1998).

Sex differences are also seen in the dendritic spines of neurons in the hippocampus. Dendritic spines are the site of termination for most excitatory synaptic inputs in the brain (Gazzaley et al., 2002). The morphology serves to compartmentalize calcium and signaling molecules (Gazzaley et al., 2002). Normal development and morphology is linked to cognition. Changes in morphology, density, and enervation, of spines, has also been linked to changes in long term potentiation, learning, and hormone levels, making them a target site to investigate when looking at neuronal changes associated with sensitization (Gazzaley et al., 2002).

Dendritic spines from hippocampal CA1 pyramidal neurons of proestrus rats have been reported to be more numerous than in estrus (Gonzalez-Burgos et al., 2005; Woolley et al., 1990). Between proestrus and estrous, spine density was shown to drop by 30%. This elevation in dendritic spines is thought to favor better synaptic communication and integration although proestrus rats have been reported to perform less efficiently than estrus animals in the Morris water maze, a highly hippocampal dependant task (Harris and Kater, 1994; Warren and Juraska, 1997). This conflict may be due in part to the difference in shape of the spines across the cyclic phases. Gonzalez-Burgos et al (2005) suggest that not only the amount but the shape of the spines in the hippocampal CA1 pyramidal neurons could contribute to differences in hippocampal-dependant cognitive performance, at proestrus and estrus stages. Mushroom-shaped spines predominated in proestrus rats, while thin spines dominated during estrous (Gonzalez-Burgos et al., 2005). Rats in proestrus have been shown to learn slower than during estrus in the Morris Maze (Kasai et al, 2003). The changes in learning abilities as a function of exposure to gonadal hormones may set a precedence for progesterone's ability to attenuate the

long term associative memory processes associated with context specific behavioral sensitization and the accompanying neural sensitization.

V. Significance of work:

Sex differences have been reported in behavioral responses to both acute and chronic cocaine exposure. These sexually dimorphic behavioral responses are hypothesized to be mediated by fluctuating sex steroids, particularly estrogen and progesterone. Sex hormones may also play a role in mediating neural plasticity which has been associated with cocaine sensitization. However, few studies have addressed the effects of endogenous and exogenous sex steroids on drug-induced neural plasticity in the mesocorticolimbic system. **We hypothesize that after acute and chronic cocaine administration, progesterone will attenuate psychomotor responses, reward-learned associations and cocaine-induced neuronal plasticity in both male and female rats.** Specifically**, we hypothesize that progesterone's overall effects in attenuating psychomotor responses, reward-learned associations and cocaine-induced neuroplasticity will have a bigger magnitude in females rather than males.** To this end, the following aims are proposed.

Specific Aim One: To test the postulate that regardless of the length of cocaine administration, progesterone attenuates cocaine-induced psychomotor responses in both males and females. To this end, psychomotor responses (ambulations, total locomotor activities, rearing and stereotypic behaviors) will be measured after acute and chronic cocaine administration plus progesterone in male and female rats.

Specific Aim Two: *To test the postulate that in males and females, progesterone attenuates cocaine-induced neuronal plasticity in reward- and memory-associated areas.* To this end, progesterone effects in dendritic spine densities in the NAcC, NAcS, and CA1 regions will be measured after acute and chronic cocaine administration in both sexes.

Specific Aim Three: *To test the postulate that progesterone attenuation of psychomotor and reward associated behaviors occur via its metabolite allopregnalone.* To this end, the effects of progesterone and finasteride (an Allo antagonist), and progesterone + finasteride in cocaine-induced conditioned place preferences and psychomotor responses will be determinate in both sexes.

Chapter 2: Progesterone's effects on acute and chronic cocaine-induced behavioral responses in intact male and female rats

Clincal and animal studies have reported sex differences in all phases of cocaine addiction including inception, maintenance, and reinstatement (McCance-Katz et al., 1999;

Singha et al., 2000; Walker et al., 2001). Women report greater dependence and more adverse subjective effects in response to cocaine (Grifin et al., 1989; Kosten et al., 1996). In rodent studies, females show more robust behavioral responses and motivation to seek and self-administer cocaine after both acute and chronic cocaine administration (Walker et al., 2001; Chin et al., 2002; Sharma et al., 1999; Festa et al., 2003; 2004; Anker et al., 2009). Estradiol augments these responses in females (Sell et al., 2000; Perroti et al., 2001). However, the administration of progesterone has been shown to have attenuating effects on cocaine-induced responses. In clinical studies, progesterone weakens cocaine-induced subjective and physiological responses in women (Evans and Foltin, 2006; Sofuoglu et al., 2004). Experiments utilizing CPP and reinstatement paradigms have demonstrated that progesterone administration also attenuates cocaine's rewarding and reinforcing effects in both intact and GDX females (Russo et al., 2003; Anker et al., 2009; Feltenstein et al., 2007).Progesterone's effects on cocaine mediated locomotor activity remains unclear. Although silastic administration of progesterone has been reported to have no or little effect on cocaine-induced responses in OVX females, Niyomchai et al.(2005) showed that i.p. administration of progesterone significantly decreased locomotor responses locomotor responses induced by acute administration of cocaine (Niyomchai et al., 2005; Perroti et al., 2001).Our study aims to clarify and extend these findings by examining progesterone's effects on the increases in psychomotor activity caused by cocaine in intact females.

A second issue which remains poorly understood is whether progesterone affects cocaine-induced responses in males. In male rats, some cocaine-induced physiological responses, such as genital reflexes associated with sleep deprivation, are reduced by progesterone administration (Anderson et al., 2004; 2005). The reports on progesterone's effects on cocaine

induced reward and motivation are confounding. Romieu et al. (2003) demonstrated that progesterone blocked cocaine-induced CPP. However, Anker et al. (2009) has reported that progesterone's metabolite ALLOP has no effect on curbing the reinstatement of cocaine self-administration in males. The discrepancies in results may be due to either species differences or perhaps differences in the effects of progesterone and its metabolite. Since more is known and understood about progesterone's role in mediating cocaine-induced responses in females, our study intends to elucidate progesterone's effect on cocaine-induced psychomotor responses in males.

Knowing whether progesterone's attenuating effects on cocaine induced reward and motivation processes extend to drug-induced increases in psychomotor behaviors, will allow us to better understand progesterone's potential as a therapeutic aid in cocaine-addiction. It is the aim of this study is to investigate the effects of exogenous progesterone on acute and chronic cocaine induced behaviors in male rats. **We hypothesize that after acute and chronic cocaine administration, progesterone will attenuate psychomotor responses and reward-learned associations in both male and female rats.** We further **hypothesize that progesterone's overall effects in attenuating psychomotor responses and reward-learned associations will have a bigger magnitude in females than males.** To this end, psychomotor responses (ambulations, total locomotor activities, rearing and stereotypic behaviors) will be measured after acute and chronic cocaine administration plus progesterone in male and female rats.

Methods:

Subjects:

Eight week old male and female Fischer rats (Charles River, Raleigh, NC, USA) were individually housed in standard cages with free access to food and water. Rats were maintained on a 12-hour light/dark cycle (lights on 8:00 am). All rats were weighed and handled for 5 days prior to

experimental manipulations. After testing, animals were decapitated following a 20 second exposure to CO2. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD, USA) and approved by the Institutional Animal Care and Use Committee at Hunter College.

Drug and hormone administration:
Cocaine solutions were prepared daily. Cocaine hydrochloride was obtained from Sigma Chemical (St. Louis, MO). For experiments 1-3, animals received a daily i.p. injection of cocaine (15 mg/kg of body weight dissolved in 0.9% saline). Animals also received a daily subcutaneous dose of progesterone (500ug/1 cc). For the CPP experiment, female rats received 5 mg/kg of cocaine; whereas males received 15 mg/kg. Previous findings from our lab show that 5mg/kg will induce cocaine-induced CPP in females, whereas 15 mg/kg is needed in males (Russo et al., 2008).

Drug administration and experimental groups for acute cocaine administration:

Eight week old intact male and female Fischer rats (Charles River) were individually housed in standard cages, with free access to food and water. Animals were handled for one week prior to all experiments and maintained on a 12 hour light/dark cycle with lights on at 7:30 a.m. Progesterone or vehicle was administered subcutaneously four hours prior to administration of cocaine or saline. Animals received only one day of injections. As shown in Table 1, the experimental design resulted in four treatment groups: Acute Progesterone (Progesterone + Saline); Acute Progesterone + Cocaine (Progesterone + Cocaine); Acute Cocaine (Vehicle + cocaine); and Control (Vehicle + Saline).

Table 1: Treatment groups in for males and females receiving a single administration of progesterone and/or vehicle four hours prior to i.p. administration of cocaine and/or saline.

Group name	**Days of Hormone Administration**	**Days of Drug Administration**
Progesterone	1 Day of Progesterone	1 Day of Saline
Progesterone + Cocaine	1 Day of Progesterone	1 Day of Cocaine
Acute Cocaine	Vehicle	1 Day of Cocaine
Control	Vehicle	Saline

Drug administration paradigm and experimental groups for chronic cocaine administration:

In order to investigate and compare the effects of acute and chronic administration of Progesterone on cocaine-induced effects, eight week old intact male and female Fischer rats (Charles River) were individually housed in standard cages, with free access to food and water. Animals were handled for one week prior to all experiments and maintained on a 12 hour light/dark cycle with lights on at 7:30 a.m. At the onset of the second week, animals were injected twice daily for 14 consecutive days. The first injection was a subcutaneous administration of either progesterone or its vehicle (sesame oil). Four hours later, cocaine or saline was administered via i.p. injection. On test day, Day 14, animals were placed into locomotor photobeam cages for one hour after the cocaine/saline injection. This experimental design resulted in the following seven groups: Chronic Progesterone (14 days of progesterone + 14 days of saline); Acute Progesterone (13 days of vehicle/1 day of progesterone + 14 days of saline); Chronic Progesterone and Cocaine (14 days of progesterone + 14 days of cocaine);

Acute Progesterone and Cocaine (13 days of vehicle, 1 day of Progesterone + 13 days of saline, 1 day of cocaine); Acute Cocaine (14 days of vehicle + 13 days of saline, 1 day of Cocaine); and Control (14 days of vehicle + 14 days of saline). See Table 2.

Table 2: Treatment groups for males and females receiving acute and/or chronic progesterone and/or cocaine administration for 14 days.

Group name	**Days of hormone administration**	**Days of drug administration**
Chronic progesterone	14 days of progesterone	14 days of saline
Acute progesterone	13 days of vehicle + 1 day of progesterone	14 day of saline
Chronic progesterone + Chronic cocaine	14 days of progesterone	14 days of cocaine
Acute progesterone + acute cocaine	13 days of vehicle + 1 day of progesterone	13 days of saline + 1 day of cocaine
Acute cocaine	14 days of vehicle	13 days of saline + 1 day of cocaine
Chronic cocaine	14 days of vehicle	14 days of cocaine
Control	14 days of vehicle	14 days of saline

Behavioral assays:

Total locomotor, ambulatory, and rearing activities were monitored in the home cages with a Photobeam Activity System from San Diego Instruments (San Diego, CA). There were a total of 16 frames which recorded locomotor activity in 6-min bins. For each cohort of animals, behavior was recorded across 2 days (25 animals per day). Animal groups were balanced for sex

and hormone/drug treatment across all cohorts (n = 6-8). Total locomotor activity represents the sum of counts in the horizontal frame. Ambulatory activity represents the number of counts produced by two consecutive photobeam interruptions in the horizontal frame. Rearing activity represents total counts of vertical motion (Festa, et al, 2004).

Stereotypic behaviors:
For stereotypic behaviors animals were videotaped in their cages approximately 30 minutes after testing began to allow for adaptation to their new environment. This time point was chosen based on prior results from the lab (Quinones-Jenab et al., 1999; Perrotti et al., 2000). The videotapes were analyzed for behavioral stereotypy by three trained observers blind to each animal's treatment condition. The rating for cocaine-induced stereotypic behaviors is based on a modified version of the behavioral scale proposed by Creese and Iversen (1974), which has been previously used in the lab (see Table 3).

Table 3: Modified rating scale from Creese and Iverson (1974) describes the numerical values observers assigned to behaviors during 15 second video clips of animas, 30 minutes post treatment administration.

Score	Behavior
1	Asleep, inactive
2	Alert, actively grooming
3	Increased sniffing 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 s
10	Splayed hind limbs

Statistical analysis Total locomotor, ambulatory, and rearing data are collapsed across five six minute time bins. Statistical analyses to determine treatment effects were two fold. First, one-way ANOVAS were run on behavioral counts to determine whether there were significant statistical differences between acute and chronic progesterone treatments. Secondly,

multifactorial ANOVAS were run to see if there were any statistical differences using sex, hormone, and drug as independent variables.

Results

Effects of Single Administration of Progesterone and Cocaine on Psychomotor Behaviors in Male and Female Rats.

As shown in Figure 7, ambulatory activity was affected by sex, drug, and hormone pretreatment. Cocaine increased overall ambulatory activity. There was a significant drug main effect ($F(1, 60) = 115.73$, $p = 0.000$). Cocaine significantly increased ambulatory activity compared to saline treated animals in both sexes ($p < 0.01$). Sex also affected overall cocaine-induced responses- a significant main effect for sex was also observed ($F(1, 60) = 39.995$, $p = 0.000$). Post hoc analysis revealed that females had significantly higher values of ambulatory activity in response to cocaine than did males ($p < 0.05$). Post hoc analysis revealed that females had significantly higher values of ambulatory activity in response to cocaine than did males ($p < 0.05$). A significant sex * drug interaction was observed ($F(1, 60) = 17.0542$, $p < 0.01$). Progesterone pretreatment significantly affected overall responses to cocaine resulting in a significant hormone main effect ($F(1, 60) = 35.2067$, $p = 0.000$). Specifically, progesterone pretreatment significantly decreased cocaine-induced ambulatory responses in both males and females ($p < 0.01$ for all comparisons). However, progesterone pretreatment had more robust effects in males, resulting in an inhibition of cocaine-induced ambulatory responses. However, ambulatory responses for males receiving progesterone + cocaine were not significantly different than those for males receiving saline ($p = 0.269$). Furthermore, a significant hormone * drug interaction was also seen indicating that progesterone pretreatment attenuates cocaine-induced

behaviors ($F(1, 60) = 47.114$, $p < 0.01$). A sex * hormone * drug interaction failed to reach statistical significance at the 0.06 level ($F(1, 60) = 3.421$, $p = 0.06$).

As seen in Figure 8, drug, sex, and hormone pretreatment also affected locomotor responses. Overall, cocaine increased locomotor responses in male and female rats- a drug main effect was seen ($F(1, 60) = 111.05$, $p < 0.01$). A significant sex main effect was also observed ($F(1, 60) = 17.918$, $p = <0.001$); females showed greater locomotor responses to cocaine ($p < 0.05$ for all comparisons). Progesterone pretreatment significantly affected cocaine-induced locomotor responses- Hormone main effect was seen ($F(1, 60) = 8.378$, $p < 0.01$). Post hoc analysis revealed that progesterone significantly reduced cocaine-induced locomotor responses compared to vehicle in both males and females ($p < 0.05$ for all comparisons). To this end a significant hormone * drug interaction, ($F(1, 60) = 29.612$, $p > 0.01$) was seen. Progesterone pretreatment significantly reduced cocaine-induced locomotor behaviors.

As seen in Fig 9, rearing counts were affected by drug, sex, and hormone treatment. Cocaine increased overall activity- a significant drug main effect was seen ($F(1, 60) = 109.603$, $p = 0.00$). Sex also affected rearing responses- a significant sex main effect is reported ($F(1, 60) = 4.076$, $p < 0.05$). Post hoc analysis revealed that females showed significantly more robust responses to cocaine than did males ($p < 0.05$ for all comparisons). There was also a significant hormone main effect ($F(1, 60) = 4.37$, $p <0.05$). Progesterone pretreatment significantly reduced rearing behaviors compared to vehicle treated animals in both males and females ($p < 0.025$ for all comparisons). There was also a significant hormone * drug interaction ($F(1, 60) = 6.383$, $p = 0.01$).

Effects of Progesterone on Acute and Chronic administration of cocaine in a 14 day administration schedule

As shown in Figure 10, ambulatory activity was affected by sex, length of drug treatment and hormone pretreatments. Cocaine increased overall ambulatory activates— a significant main effect for drugs was seen ($F(1, 143) = 30.887$, $p = 0.00$). Specifically, in both sexes, ambulatory activity after 1 and 14 days of cocaine was significantly higher than the respective saline controls ($p < 0.05$ for all comparisons). Sex also affected the overall responses to cocaine—a significant main effect sex was seen ($F(1, 143) = 15.1687$, $p = 0.00$). In particular, post hoc analysis reveal that regardless of the length of cocaine treatment, females had overall higher values of ambulatory activity than males ($P<0.05$ for all comparisons). Progesterone treatment also affected the overall ambulatory responses—however, progesterone main effect was significant at the 0.057 level ($F(2, 73) = 2.985$, $p = 0.057$). Furthermore, a significant interaction between drug * hormone ($F(2, 143) = 4.95$, $p = 0.008$). While in male rats, acute progesterone treatment did not alter acute-cocaine-induced behaviors, chronic progesterone treatment inhibited cocaine-induced ambulatory counts, $p = 0.169$. However in female rats, acute progesterone attenuated ambulatory activity induced by acute cocaine, $p < 0.05$.

As shown in Figure 11 similar to ambulatory activities, total locomotor activity was affected by sex, length of cocaine administration, and hormone treatment. Cocaine significantly increased overall locomotor responses—drug -main effect: ($F(2, 154) = 42.702$, $p = 0.000$). Specifically, cocaine significantly increased locomotor activity, in both sexes, compared to the respective saline controls, ($p < 0.003$). A significant sex main effect was observed ($F(1,154) = 18.352$, $p = 0.000$). Specifically, post hoc analysis showed that females displayed significantly more robust responses to acute cocaine administration than did males ($p < 0.003$). However,

although progesterone treatment did not significantly affect overall total locomotor counts, post hoc analysis showed that acute progesterone pretreatment decreased cocaine-induced locomotor counts compared to acute cocaine ($p < 0.05$).

As shown in Figure 12, rearing activity was also affected by sex, length of drug treatment, and hormone pretreatment. Specifically, cocaine increased rearing responses—significant main effect for drug: ($F(2, 138) = 32.829$, p 0.000). Regardless of sex and length of cocaine administration, cocaine significantly increased rearing activity when compared to the respective saline controls ($p < 0.05$). Although, sex also affected the overall responses to cocaine, it failed to reach a significant main effect with a p value of 0.065 ($F(1, 138) = 3.446$, $p = 0.065$). A significant main effect for hormone was seen ($F(1, 138) = 32.829$, $p = 0.000$), suggesting that progesterone pre-treatments also alter cocaine-induced rearing responses. Specifically, progesterone decreased cocaine-induced rearing responses. Furthermore, a significant drug * hormone interaction was seen ($F(2, 138) = 5.759$, $p = 0.003$). In both males and females, progesterone significantly decreased chronic and acute cocaine-induced rearing counts in males and females ($p < 0.05$ for all comparisons).

As shown in Figure 13, there were no significant differences across the stereotypic behaviors, $p > 0.05$.

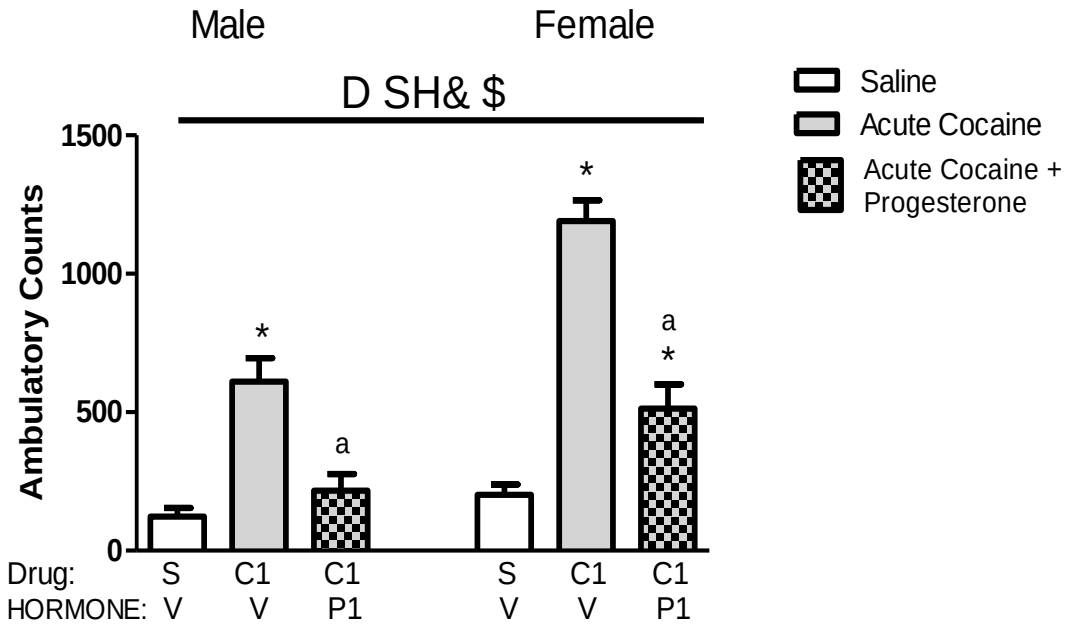

Figure 7: Sex differences in ambulatory responses to acute cocaine + progesterone administration. Data is shown as the mean ± SEM of total rearing counts. White bar represents behavioral responses for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). D indicates drug main effect; S indicates sex main effect; H indicates a hormone main effect; and & indicates drug and hormone interactions. $ indicates a sex and drug interaction. Significant differences between saline and cocaine treatments are represented by *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 9 to 11 per groups.

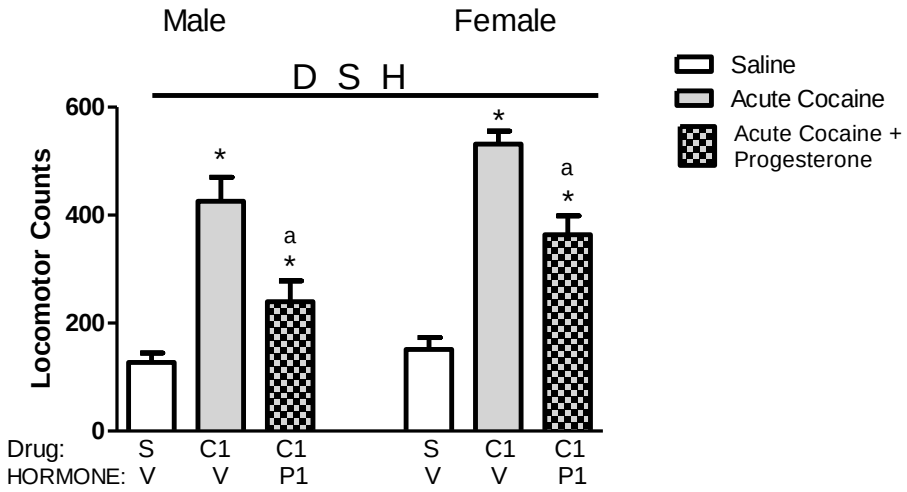

Figure 8: Sex differences in total locomotor responses to acute cocaine + progesterone administration. Data is shown as the mean ± SEM of total locomotor counts. White bar represents behavioral responses for saline-treated groups and light bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) progesterone (500 µg). D indicates drug main effect and S indicates sex main effect; H indicates a hormone main effect. Significant differences in behavioral responses between saline and cocaine treatments are represented by the *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 8 to 9 per groups.

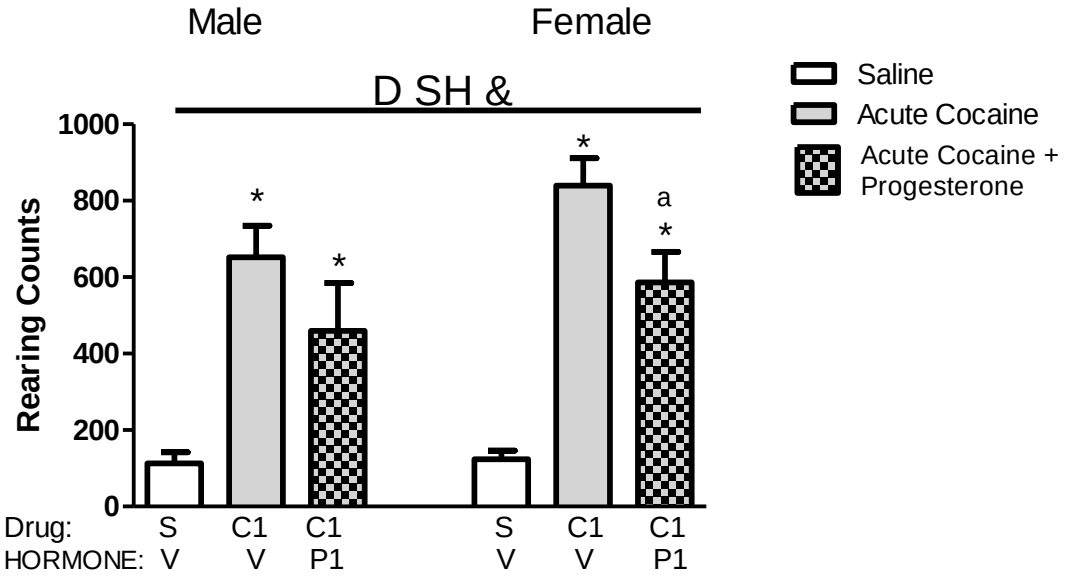

Figure 9: Sex differences in rearing responses to acute cocaine + progesterone administration. Data is shown as the mean ± SEM of total rearing counts. White bar represents behavioral responses for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). D indicates drug main effect; S indicates sex main effect; H indicates a hormone main effect; and & indicates drug and hormone interactions. Significant differences between saline and cocaine treatments are represented by *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 9 to 11 per groups.

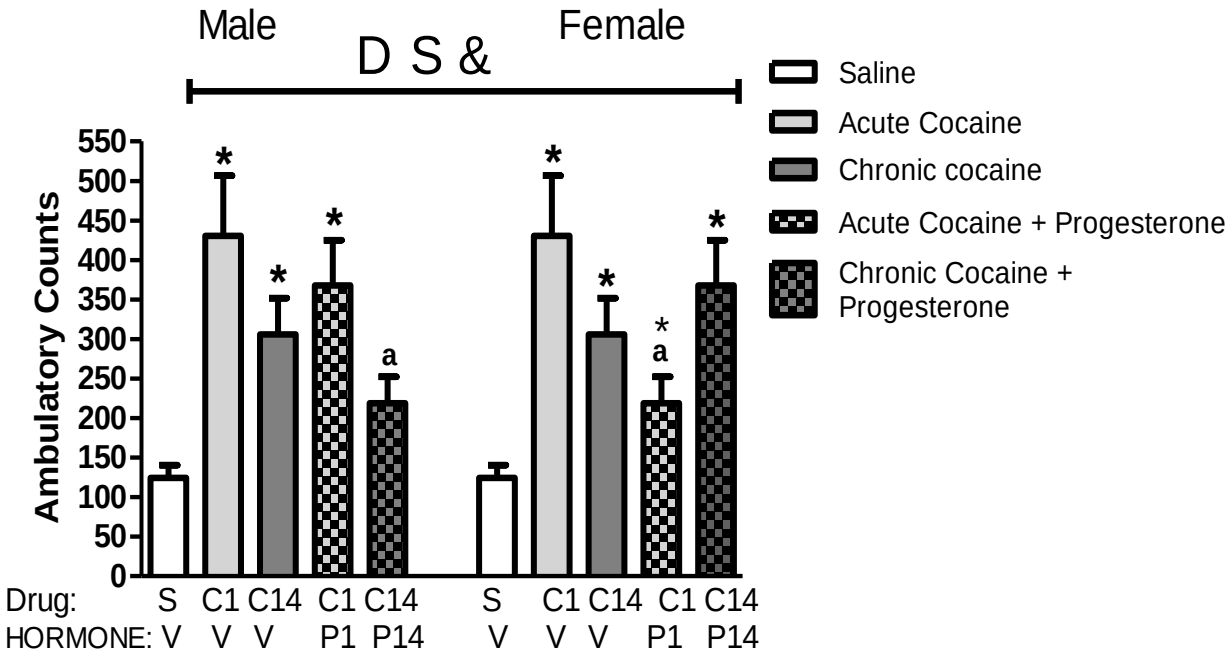

Figure 10: Sex differences in ambulatory responses to chronic and acute cocaine + progesterone administration. Data is shown as the mean ± SEM of total ambulatory counts. White bar represents behavioral responses for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). D indicates drug main effect; S indicates sex main effect; and & indicates drug and hormone interactions. Significant differences in behavioral responses between saline and cocaine treatments are represented by the *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 9 to 11 per groups.

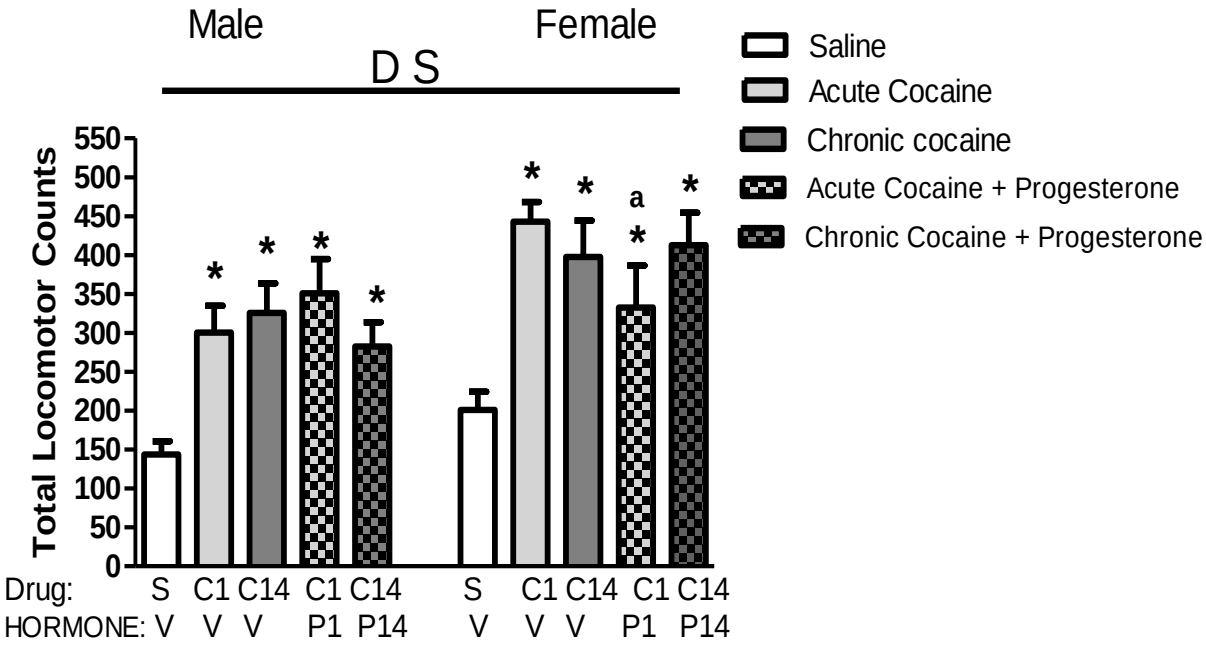

Figure 11: Sex differences in total locomotor responses to chronic and acute cocaine + progesterone administration. Data is shown as the mean ± SEM of total locomotor counts. White bar represents behavioral responses for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). D indicates drug main effect and S indicates sex main effect. Significant differences in behavioral responses between saline and cocaine treatments are represented by the *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 9 to 11 per groups.

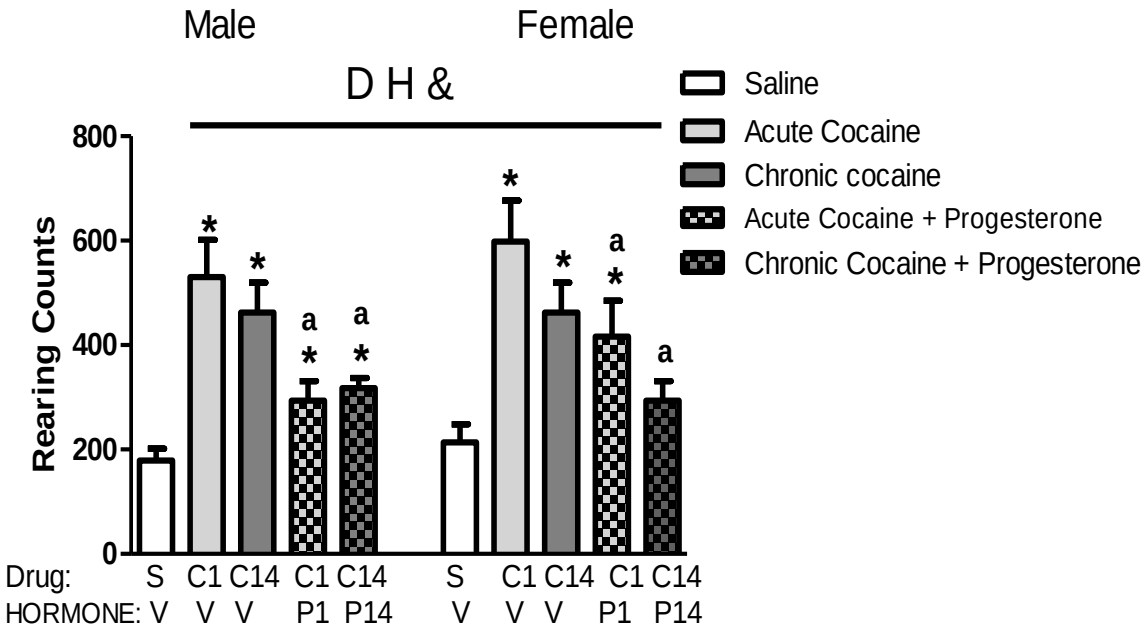

Figure 12: Sex differences in rearing responses to chronic and acute cocaine + progesterone administration. Data is shown as the mean ± SEM of total rearing counts. White bar represents behavioral responses for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). D indicates drug main effect; H indicates a hormone main effect; and & indicates drug and hormone interactions. Significant differences between saline and cocaine treatments are represented by *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 9 to 11 per groups.

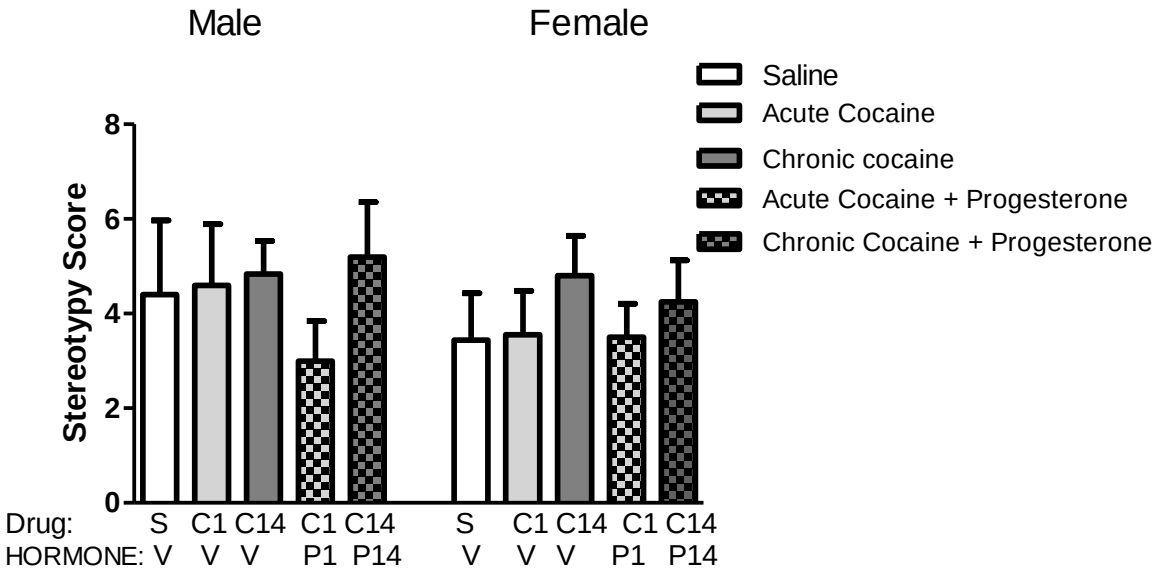

Figure 13: Sex differences in stereotypic responses to chronic and acute cocaine + progesterone administration. Data is shown as the mean ± SEM of stereotypic scores. White bar represents behavioral responses for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). N= 8 per groups.

Discussion

The present study investigated the effects of progesterone administration on the locomotor responses to cocaine in intact male and female rats. I.P. acute and repeated cocaine administration produced sex differences in overall behavioral reactions. Females exhibited greater psychomotor response following drug administration. Specifically, we observed that acute cocaine induced greater horizontal and rearing behaviors in female rats than in males. These results replicate previous findings which demonstrate that females demonstrate more robust cocaine-induced locomotor activity (Walker et al., 2001; Harrod et al., 2005; Bowman and Kuhn, 1996). Similar sexually dimorphic patterns have been reported in chronic studies (Sharma et al., 1999; Festa et al., 2003; 2004; Serchen et al., 1999). Although our animals did not develop or display cocaine sensitization, females displayed more vigorous responses to repeated cocaine administration. The lack of observed sensitization could be due to experimental design factors. For example, animals received 2 injections per day (hormone/vehicle and cocaine/saline) for 14 days. The extra handling and injections may have impeded behavioral sensitization to cocaine from developing. Circulating gonadal hormones are postulated to be the main mediators of sex differences seen in cocaine-induced responses. The evidence for hormones being culpable in activating stronger responses in females is ternary. First, the removal of gonadal hormones in female rodents via ovarectomy leads to a decrease in acute and chronic psychostimulant - induced behaviors such as rearing and total activity counts (Harrod et al., 2005; Chin et al., 2002). Secondly, sexually dimorphic responses to cocaine are not observed in early stages of development in rat pups but are seen in adolescent females indicating that sexual maturity or circulating gonadal hormones are influential in meditating behavioral responses to cocaine (Bowman et al., 1996; Lynch et al., 2007). Thirdly, the administration of exogenous hormones to OVX females reinstates increases in behavioral responses to cocaine. In particular, estradiol

increases horizontal locomotor activity, rearing, and ambulatory behaviors (Sell et al., 2000; Perroti et al., 2001). The stimulatory properties of estradiol are hypothesized to be responsible for the sex differences seen in all aspects of drug addiction including induction, maintenance, and reinstatement (Chin et al., 2002; Anker et al., 2009; Feltenstein et al., 2009; Russo et al., 2003; 2004). Furthermore, endogenous estradiol is likely responsible for the sex differences seen in this study between male and female rats. While it has also been reported that females show more intense and prolonged stereotypic responses to cocaine (Schneider and Norton, 1979; Savageau and Beatty, 1981), we did not see any statistically significant differences in stereotypic behaviors among the treatment groups. The discrepancies seen between our results and those previously published may again be a result of differences in experimental designs. While in most studies, subjects received one injection of cocaine or saline; our experimental design consisted of administering multiple injections per day, resulting in more handling, and perhaps interfering with behavioral results.

With the exception of stereotypic behaviors, progesterone treatment decreased most cocaine- induced behavioral responses in both males and female rats. In females, we observed that progesterone attenuated most behavioral responses to both acute and chronic cocaine administration. In some instances, progesterone administration inhibited cocaine's effects. Chronic progesterone pretreatment inhibited chronic cocaine from increasing rearing responses. Single administration of progesterone is also able to inhibit some cocaine-induced responses. This indicates that some of progesterone's effects can be implemented as quickly as psychostimulants can exert their effects, which may make it a good candidate for therapeutic use. Previous studies have shown that progesterone can counteract estrogen's stimulatory effects on cocaine-induced responses in GDX rats (Jackson et al., 2006). Concurrent administration of

progesterone and estrogen in OVX rats, inhibits estrogen-induced increases on cocaine self-administration (Yang et al., 2007). Our findings extend this knowledge by showing that progesterone administration can abate the excitatory interaction between estrogen and cocaine in intact females.

Progesterone's effects on cocaine-induced locomotor behaviors in GDX animals have resulted in conflicting results depending on route of administration. Psychomotor studies have revealed that i.p. administration of progesterone attenuates acute cocaine-induced locomotor responses in GDX females whereas silastic administration had no effect , indicating that route of administration is an important factor in progesterone's ability to reduce acute cocaine's stimulating effects (Niyomchai et al., 2005; Perroti et al., 2001). Our findings support and expand those by Niyomchai et al. (2005) by demonstrating that i.p. administration of progesterone decreases both acute and chronic cocaine-induced behavioral responses in intact female rats. Progesterone pretreatment inhibited acute cocaine from increasing ambulatory counts. In the chronic administration schedule, progesterone also blocked cocaine from increasing rearing counts. Behaviorally these animals did not experience the increases in locomotor activity typically induced by cocaine or other psychostimulants. In essence, progesterone pretreatment was able to block cocaine's effects.

Progesterone's attenuating effects on cocaine-induced psychomotor responses seems to over-ride endogenous hormone fluctuations. Progesterone attenuated cocaine-induced behavioral responses regardless of estrous cycle phase. Animals, in the present study, were not observed for estrous cycle changes. However, progesterone's effects on subjective and reward-dependant behaviors have been reported to be dependent and interact with endogenous endocrine profiles. This hypothesis is supported by other published findings. For example, in intact and

gonadectomized (GDX) female rodents, progesterone has been shown to reduce cocaine's rewarding and reinforcing effects by attenuating cocaine-induced conditioned place preference and the reinstatement of self -administration behaviors (Russo et al., 2003; Anker 2009, Feltenstein et al, 2007). However, progesterone's ability to attenuate associative or reward behaviors has been reported to be estrous cycle dependent. Progesterone was only effective at reducing cocaine-primed reinstatement for females during estrus suggesting an interaction between endogenous and administered hormones (Feltenstein et al.2009).

In males, progesterone attenuated cocaine-induced locomotor responses, which are novel findings. Currently, progesterone's effects on cocaine mediated responses, in males, are unclear due to confounding published reports. Progesterone has been observed to alter some of the physiological effects of cocaine in rodents. For instance, progesterone altered cocaine-induced genital reflexes and its associated sleep deprivation (Anderson et al.; 2007). However, progesterone's effects on cocaine reinforcement are still poorly understood and obscure. In clinical studies, progesterone administration has minimal effects on cocaine-induced responses in men (Evans and Foltin, 2006; Sofuoglu et al., 2007). These findings were mirrored in rodents. In rats, Anker et al. (2009) reported that while progesterone and ALLOP may attenuate cocaine reinstatement and self-administration in females, it had no effect in males. However, in mice, progesterone has been shown to block cocaine-induced CPP acquisition and expression in males (Romieu et al. , 2003). These discrepancies in progesterone's ability to mediate reward or reinforcement may be due to species differences or dosage/administration difference. Romieu et al. (2003) used a mouse model whereas Anker et al. (2009) utilized a rat model. Romieu et al. (2003) administered progesterone according to an alternating CPP paradigm whereas, Anker et al. (2009) used a reinstatement paradigm in which hormone and drug administration were

stopped before the reinstatement period. Further studies will need to investigate progesterone's effects on reward and motivation in males. Although, progesterone's effects on reward and motivation are still obscure, we wanted to look at progesterone's effects on cocaine-induced psychomotor responses. We contribute to current knowledge by demonstrating that progesterone attenuates all cocaine-induced psychomotor responses in males, regardless of treatment schedule. Progesterone had the most robust effect on ambulatory counts- inhibiting both acute and chronic cocaine's stimulating effects. In order to determine progesterone's potential as a theurapeutic aid in addiction recovery, it is important to understand whether its attenuating effects are restricted to locomotor activity or extend to reward and reinforcement. A dose response curve should be formulated to determine if the discrepancies in progesterone's ability to attenuate the motivation to seek or self-administer cocaine is due to dosage differences.

Progesterone's effects are produced by genomic and membrane receptor mediated mechanisms (Mani, 2006). Differences have been noted in progesterone's ability to attenuate cocaine-induced behaviors depending on the methods used to administer the hormone (Niyomchai et al., 2005; Perroti et al., 2001). It is postulated that i.p. administration results in acute increases in serum levels of progesterone. The differences in administration and exposure have led to the differences seen in its ability to mediate cocaine-induced responses. The differences in duration and onset of progesterone exposure may result in differences of mechanisms by which progesterone mediates its effects. An acute administration of progesterone probably mediates its effects by potentiating GABA and rapid membrane mediated mechanisms whereas, long term progesterone administration causes changes in gene expression (Mani, 2006). Changes in gene expression may lead to long term changes in responses to cocaine.

We also believe that Progesterone may also be inducing its inhibitory effects by activating receptor ion channels such as GABA for three reasons. The first reason is that progesterone and its metabolite ALLOP potentiate the $GABA_A$ and $GABA_B$ ionophore complexes which are known to mediate inhibitory responses (as reviewed in Paul and Purdy; 1992). Therefore, progesterone can modulate anxiolytic responses and cause sedation (Reddy et al., 2005; Seyle, 1941). Secondly, benzodiazapines, GABA agonists, reduce cocaine-induced increases in DA in the NAc (Meririnne et al.; 1999). Cocaine-induced increases in the mesolimbic dopamine circuitry are hypothesized to be responsible for cocaine's rewarding and reinforcing effects (as reviewed in Rocio et al., 2004). Thirdly, Gaba agonists are able to decrease cocaine-induced locomotor behaviors and the development of CPP (Meririnn et al., 1999). This and other studies, confirm these findings by showing that progesterone attenuates cocaine-induced behaviors in rodents.

Altogether, clinical and preclinical studies demonstrate that progesterone is a capable mediator of cocaine-induced behaviors in females. Progesterone decreases cocaine-induced learning, reward, reinstatement, and locomotor behaviors making it a good potential pharmacological therapy for all stages of drug addiction (Romeiu et al., 2003; Anker et al., 2007; Russo et al., 2003; Niyomchai et al., 2005). Nonetheless, it will be important for future research to elucidate the effects of progesterone on cocaine-induced reward and motivation in males. So far clinical studies have shown that progesterone has little effect on males and the data on cocaine-induced reward in rodents is inconclusive. Studies have shown that progesterone has therapeutically beneficial when treating other psycho-behavioral disorders such as anxiety, post-partum depression, hypertension, chronic obstructive pulmonary disease, and benzodiazepine

withdrawal (as reviewed in Field et al., 2010; De Wit et al., 2001; reviewed in Sofuoglu et al., 2007).

Chapter 3: Progesterone's effects on acute and chronic cocaine –induced neural plasticity

Acute cocaine exposure leads to increases in psychomotor responses whereas repeated exposure results in the development of dependence related behaviors such as tolerance or sensitization. These cocaine-induced changes in behavior are associated with biochemical and morphological adaptations (Pierce and Kalivas, 1997; Robinson and Berridge, 1993; Robinson and Becker, 1986). For example, repeated administration of amphetamine and cocaine produces increases in the complexity and vastness of the organization and density of dendritic spines in the NAc and prefrontal cortex (Robinson and Kolb, 1997; 1999; 2001; Li et al., 2004). Furthermore, as previously described in the introduction, changes in dendritic spine densities have also been associated with changes in hormonal states. For example, endogenous hormonal fluctuations during the female estrous cycle alter dendritic spine density in the hippocampus. Specifically, dendritic spines from hippocampal CA1 pyramidal neurons of proestrus rats have been reported to be more numerous than in estrus (Woolley et al., 1990; Gonzalez-Burgos et al., 2005). Reduction of spine densities have also been reported during the transition from proestrus to estrous; a stage of the cycle when progesterone levels are also reduced. Thus, indirectly suggesting that fluctuation of progesterone may in part mediate the formation of new spines in the hippocampus. However, it is unknown whether progesterone may also affect dendritic spine formation in reward associated areas.

As previously discussed, there is substantial evidence that progesterone alters cocaine-induced behavioral responses. For example, progesterone has been shown to alter cocaine-induced behavioral responses. In clinical studies, progesterone attenuates cocaine-induced subjective and physiological responses in women (Evans and Foltin, 2006; Sofuoglu et al.,

2004). Experiments utilizing CPP and reinstatement paradigms have demonstrated that progesterone administration also reduces cocaine's rewarding and reinforcing effects in both intact and GDX females (Russo et al., 2003; Anker et al., 2009; Feltenstein et al., 2007). Furthermore, progesterone weakens cocaine's stimulatory effects on locomotor behaviors (Niyomchai et al., 2005). However, progesterone's effects on cocaine-induced dendritic alterations are unknown.

We hypothesize that progesterone attenuates neuronal plasticity in areas associated with reward and/or the formation of learning associations. Regardless of the manner of cocaine administration (acute and chronic cocaine paradigms), progesterone will reduce cocaine-induced neuronal plasticity in both male and female rats. Specifically**, we hypothesize that progesterone's overall effects in attenuating psychomotor cocaine-induced neuroplasticity will have a bigger magnitude in females rather than males.** To this end, the following aims are proposed. It is the aim of this study t*o test the postulate that progesterone attenuates cocaine-induced neuronal plasticity in reward- and memory-associated areas, in males and females.* To this end, progesterone effects in dendritic spine densities in the NAcC, NAcS, and CA1 regions will be measure after acute and chronic cocaine administration in both sexes.

Methods:

Subjects:

Eight week old male and female Fischer rats (Charles River, Raleigh, NC, USA) were individually housed in standard cages with free access to food and water. Rats were maintained on a 12-hour light/dark cycle (lights on 8:00 am). All rats were weighed and handled for 5 days prior to experimental manipulations. After testing, animals were decapitated following a 20 second exposure to CO2. Animal care was in accordance with the Guide for the Care and Use of

Laboratory Animals (NIH publication 85-23, Bethesda, MD, USA) and approved by the Institutional Animal Care and Use Committee at Hunter College.

Drug and hormone administration:
Cocaine solutions were prepared daily. Cocaine hydrochloride was obtained from Sigma Chemical (St.Louis, MO). For experiments 1-3, animals received a daily i.p. injection of cocaine (15 mg/kg of body weight dissolved in 0.9% saline). Animals also received a daily subcutaneous dose of progesterone (500ug/1 cc). For the CPP experiment, female rats received 5 mg/kg of cocaine; whereas males received 15 mg/kg.

Drug administration paradigm and experimental groups for chronic cocaine administration:

Eight week old intact male and female Fischer rats (Charles River) were individually housed in standard cages, with free access to food and water. Animals were handled for one week prior to all experiments and maintained on a 12 hour light/dark cycle with lights on at 7:30 a.m. At the onset of the second week, animals were injected twice daily for 14 consecutive days. The first injection was a subcutaneous administration of either progesterone or its vehicle (sesame oil). Four hours later, cocaine or saline was administered via i.p. injection. On test day, Day 14, animals were behaviorally tested and sacrificed one hour post drug injection. This experimental design resulted in the following seven groups: Chronic Progesterone (14 days of progesterone + 14 days of saline); Acute Progesterone (13 days of vehicle/1 day of progesterone + 14 days of saline); Chronic Progesterone and Cocaine (14 days of progesterone + 14 days of cocaine); Acute Progesterone and Cocaine (13 days of vehicle, 1 day of Progesterone + 13 days of saline, 1 day of cocaine); Acute Cocaine (14 days of vehicle + 13 days of saline, 1 day of Cocaine); and Control (14 days of vehicle + 14 days of saline). See Table 1.

Brain Tissue Dissection and Golgi staining: Upon removal, brains were stained using the FD Rapid Golgi Stain Kit obtained from FD Neurotechnologies, Inc. Brains were rinsed in 0.1 M phosphate buffer, immersed in Golgi-Cox solution for 14 days in the dark. The impregnation solution was replaced after the first 24 hours of immersion. Brains were then transferred to a sucrose solution and stored at 4ºC for at least 48 hours (up to 1 week) in the dark. Once the preparatory procedure was complete, brains were frozen, sliced (100um), and mounted onto gelatin covered slides. Slides were left to air dry at room temperature for a week in the dark, and then immersed in the impregnation solutions according to the FD Rapid Golgistain Kit.

Neurons in the nucleus accumbens (NAcc) and hippocampus CA1 region were selected to be analyzed and counted. The dendritic spine density of medium spiny neurons in the Nucleus Accumbens (Shell and Core) and hippocampus (CA1) were analyzed using the Spot Advanced program, version 3.5.5 for Windows (Diagnostic Instruments, Inc., 1997-2002) and a Nikon Eclipse E400 microscope (as described in Luine, V. et al., 2006). Medium spiny neurons, in the NAcC, NAcS, were located and spines were counted according to the general methods described in Robbinson et al. (2001). Hippocampal neurons were selected and counted according to the methodology described in Gould et al. (1990). As described in Luine et al. (2006), there were three criteria for choosing eligible dendrites .First, cell bodies needed to be in one of three areas of interest; secondly, the length of the branch was unbroken; thirdly, branches analyzed were at least tertiary branches. Spines on the branches were counted under oil. The spine density/um of the dendrites were calculated and averaged for each subject.

Statistical Analysis: In the NAcc core and shell, medium spiny neurons were selected. Tertiary branches which were intact and well stain were chosen to count. First, the number of spines was

counted on the chosen branch. Next, the dendritic length was traced using the Nikon Electron Microscope's imaging and measurement tools. N= 6 for each experimental group. For each brain, 6 counts/measurements were taken and averaged. First, one-way ANOVAS were run on average dendritic counts to determine whether there were significant statistical differences between acute and chronic progesterone treatments. Secondly, multifactorial ANOVAS were run to see if there were any statistical differences using sex, hormone, and drug as independent variables.

Results

Effects of Progesterone on Acute and Chronic administration of cocaine on dendritic spine densities in the Nucleus Accumbens Core, in a 14 day administration schedule

As shown in Figure 14, both drug and hormone significantly affected dendritic spine densities in the core of the NAcC. Overall, a significant Drug main effect was observed ($F(2, 62) = 4.500$, $p = 0.015$); regardless of the sex and length of cocaine administration, cocaine increased dendritic spine ($p < 0.05$ for all comparisons). A significant main effect for Hormone was also seen ($F(1, 62) = 4.427$, $p = 0.039$); progesterone increased dendritic spine densities in both the NAcC core and shell areas ($p < 0.05$ for all comparisons. Furthermore, a significant Hormone *Sex interaction was obtained ($F(1, 62) = 5.245$, $p = 0.025$); progesterone increased dendritic spine in females more than males ($p < 0.05$ for all comparisons). Although, acute progesterone did not significantly increase dendritic spine counts compared to the cocaine only treated group in females($p > 0.05$), it did increase dendritic spine densities compared to saline groups ($p = 0.004$). Post hoc analysis showed that chronic cocaine significantly increased dendritic spine densities in males ($p < 0.05$).

Effects of Progesterone on Acute and Chronic administration of cocaine on dendritic spine densities in the Nucleus Accumbens Shell, in a 14 day administration schedule

As shown in Figure 15, similar to the NAcc, dendritic spine densities were affected by exposure to cocaine and the length of that exposure in the NAcS. A significant Drug main effect in the number of spine densities was seen ($F(2, 54) = 4.729$, $p = 0.0128$); cocaine overall increased the number of dendritic spine densities when compared to saline controls ($p<0.05$ for all comparisons). Although a main effect for Hormone failed to reach significance ($F(1, 54) = 3.253$, $p= 0.076$), post hoc analysis showed that acute progesterone pretreatment increased dendritic spine densities compared to vehicle and saline treated groups ($p < 0.05$).

Effects of Progesterone on Acute and Chronic administration of cocaine on dendritic spine densities in CA1 sub-regions of the hippocampus, in a 14 day administration schedule

Cocaine failed to produce an overall Drug main effect in the CA1 region of the Hippocampus ($F(1, 70) = 2.8354$, $p = 0.0967$), as shown in Figure 16. However, post hoc analysis revealed that chronic cocaine increased dendritic spine densities in both male and females ($p < 0.05$ for all comparisons). Progesterone also failed to produce a significant main effect ($F(1, 70) = 3.7565$, $p = 0.05663$). Post hoc analysis revealed that groups receiving progesterone demonstrated significantly greater spine densities than those receiving vehicle ($p < 0.04$ for all comparisons). A significant Hormone * Drug interaction was observed ($F(1, 70) = 10.8616$, $p = 0.002$). Only in female rats, chronic progesterone and cocaine increased dendritic spine densities when compared to control groups ($p< 0.05$).

Table 4: Dendritic spine densities (Mean ± S.E.M.) in the NAcC for experimental treatment groups receiving acute or chronic cocaine + progesterone for fourteen days. Spine counts are represented as spines/m.

Hormone	Drug	Female Mean ±S.E.M.	Male Mean ±S.E.M.
Progesterone	Saline	1.718 ± 0.08	1.745 ± 0.084
Vehicle	Saline	1.208 ± 0.108	1.661 ± 0.079
Vehicle	Acute Cocaine	1.625 ± 0.118	1.802 ± 0.118
Progesterone	Acute Cocaine	1.512 ± 0.007	1.697 ± 0.148
Vehicle	Chronic Cocaine	1.803 ± 0.114	1.698 ± 0.118
Progesterone	Chronic Cocaine	1.816 ± 0.119	1.469 ± 0.111

Table 5: Dendritic spine densities (Mean ± S.E.M.) for NAcS for males and females receiving acute or chronic cocaine + progesterone for fourteen days. Spine counts are represented as spines/m.

Hormone	Drug	Female Mean ±S.E.M.	Male Mean ±S.E.M.
Progesterone	Saline	1.635 ± 0.060	1.74 ± 0.065
Vehicle	Saline	1.242 ± 0.096	1.64 ± 0.059
Vehicle	Acute Cocaine	1.326 ± 0.056	1.327 ± 0.096
Progesterone	Acute Cocaine	1.788 ± 0.096	1.46 ± 0.86
Vehicle	Chronic Cocaine	1.857 ± 0.086	1.69 ± 0.096
Progesterone	Chronic Cocaine	1.921 ± 0.099	1.625 ± 0.071

Table 6: Dendritic spine densities (Mean ± S.E.M.) in the CA1 in male and female rats receiving acute or chronic cocaine + progesterone for fourteen days. Spine counts are represented as spines/m.

Hormone	**Drug**	**Female Mean ± S.E.M.**	**Male Mean ± S.E.M.**
Progesterone	Saline	1.318 ± 0.125	1.544 ± 0.125
Vehicle	Saline	0.788 ± 0.176	0.910 ± 0.216
Vehicle	Acute Cocaine	1.459 ± 0.180	1.176 ± 0.197
Progesterone	Acute Cocaine	1.489 ± 0.197	1.145 ± 0.198
Vehicle	Chronic Cocaine	1.571 ± 0.221	1.400 ± 0.180
Progesterone	Chronic Cocaine	1.461 ± 0.180	1.260 ± 0.201

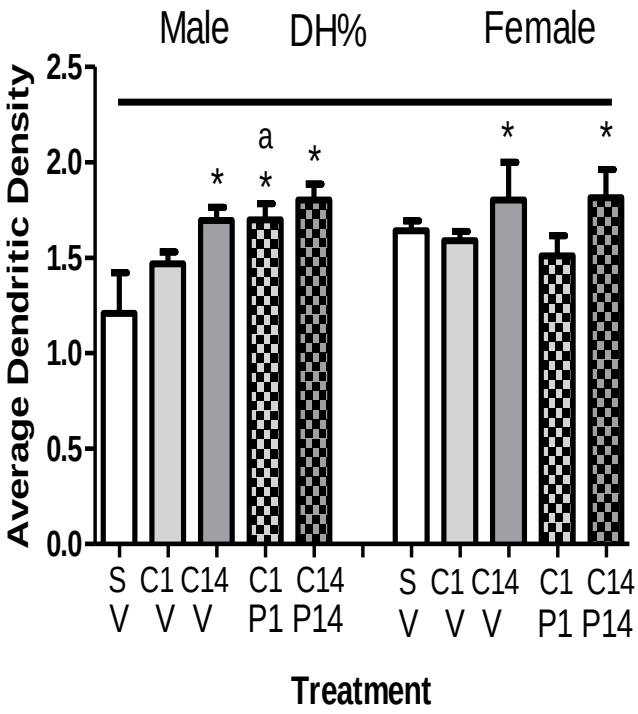

Figure 14: Sex differences in dendritic spine densities in the NACc after acute and chronic cocaine + progesterone administration. Data is shown as the mean ± SEM of average dendritic spine densities. White bar represents spine densities for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). D indicates drug main effect; and H indicates hormone main effect % indicates a hormone sex interaction. Significant differences in dendritic spine densities between saline and cocaine treatments are represented by the *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 6 per groups.

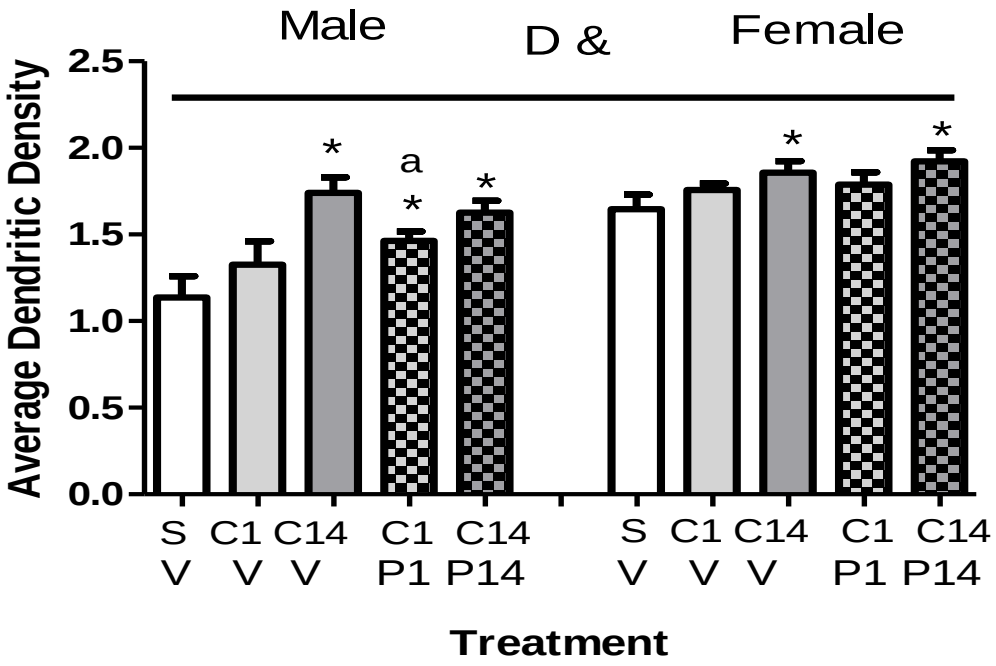

Figure 15: Sex differences in dendritic spine densities in the NAcS after acute and chronic cocaine + progesterone administration. Data is shown as the mean ± SEM of average dendritic spine densities. White bar represents spine densities for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). D indicates drug main effect; & indicates a drug and hormone interaction. Significant differences in dendritic spine densities between saline and cocaine treatments are represented by the *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 6 per groups.

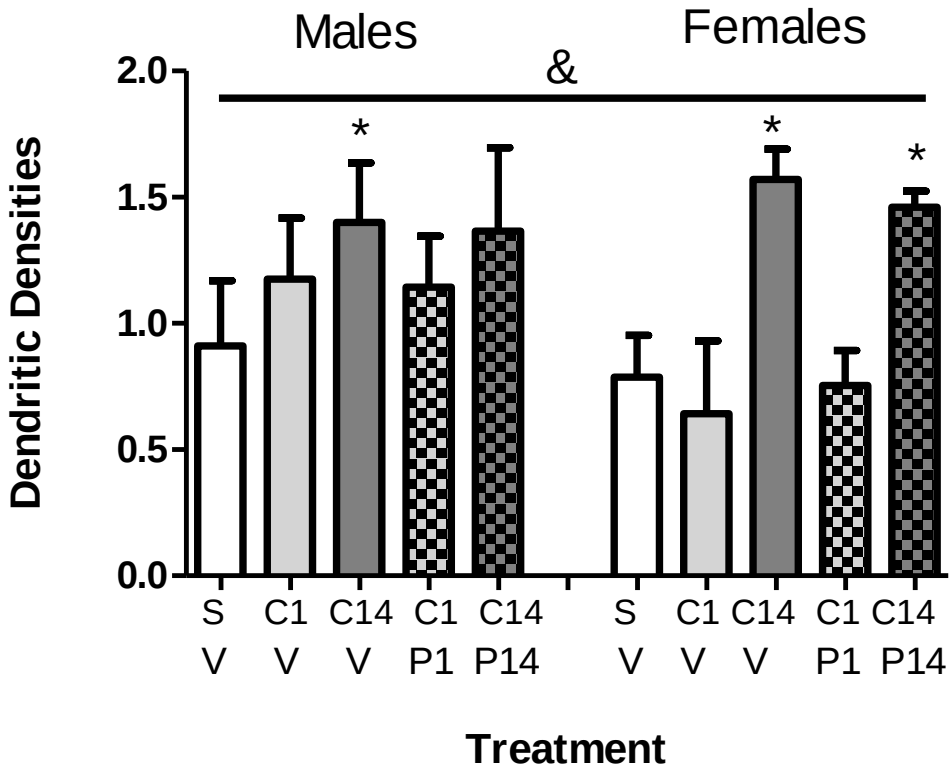

Figure 16: Sex differences in apical dendritic spine densities in CA1 after acute and chronic cocaine + progesterone administration. Data is shown as the mean ± SEM of average dendritic spine densities. White bar represents spine densities for saline-treated groups and light (acute) or dark gray (chronic) bars represent cocaine-treated groups (15 mg/kg). Bars with checker represent groups treated with either 1 day (light gray + checkers) or 14 days (dark gray + checker) progesterone (500 µg). & indicates a drug and hormone interaction. Significant differences in dendritic spine densities between saline and cocaine treatments are represented by the *, while significant differences between vehicle treatment and progesterone treatment are represented by a. N= 6 per groups.

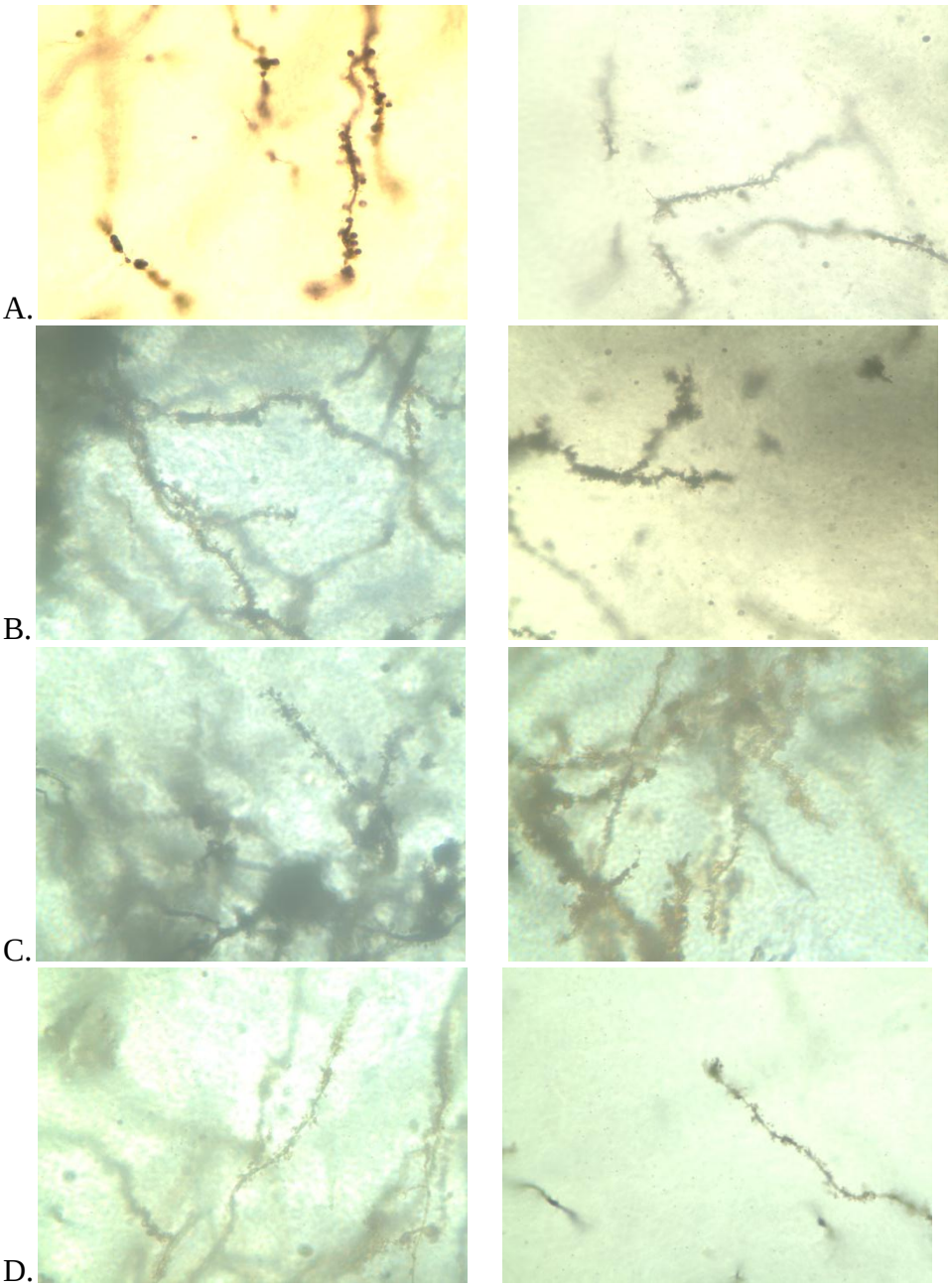

Figure 17: Dendritic spine densities in the NAcC males (left column) and females (right column) receiving (A) saline and vehicle (B) chronic cocaine (C) chronic progesterone + cocaine and (D) acute progesterone + cocaine.

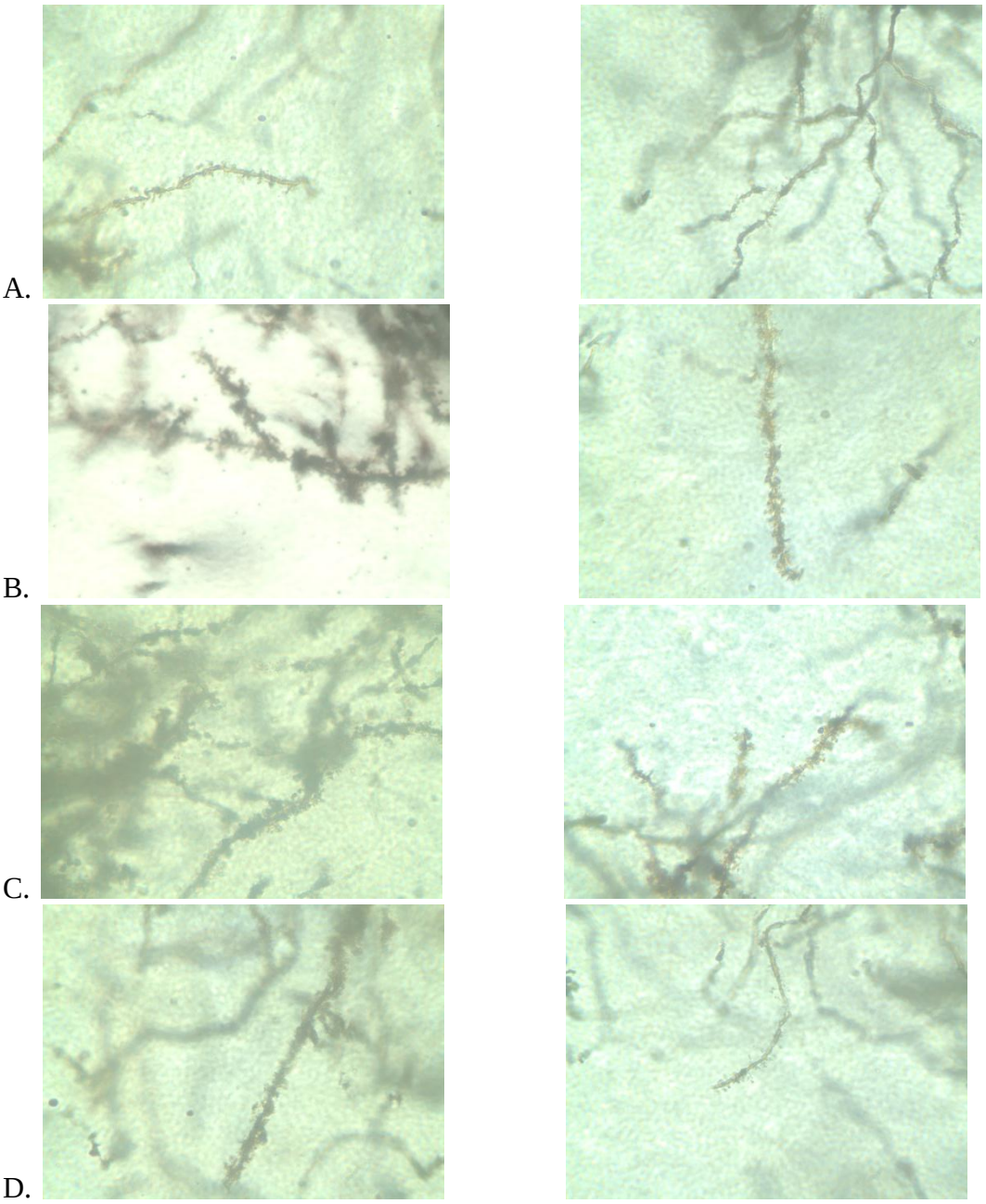

Figure 18: Dendritic spine densities in the NAcS males (left column) and females (right column) receiving (A) saline and vehicle (B) chronic cocaine (C) chronic progesterone + cocaine and (D) acute progesterone + cocaine.

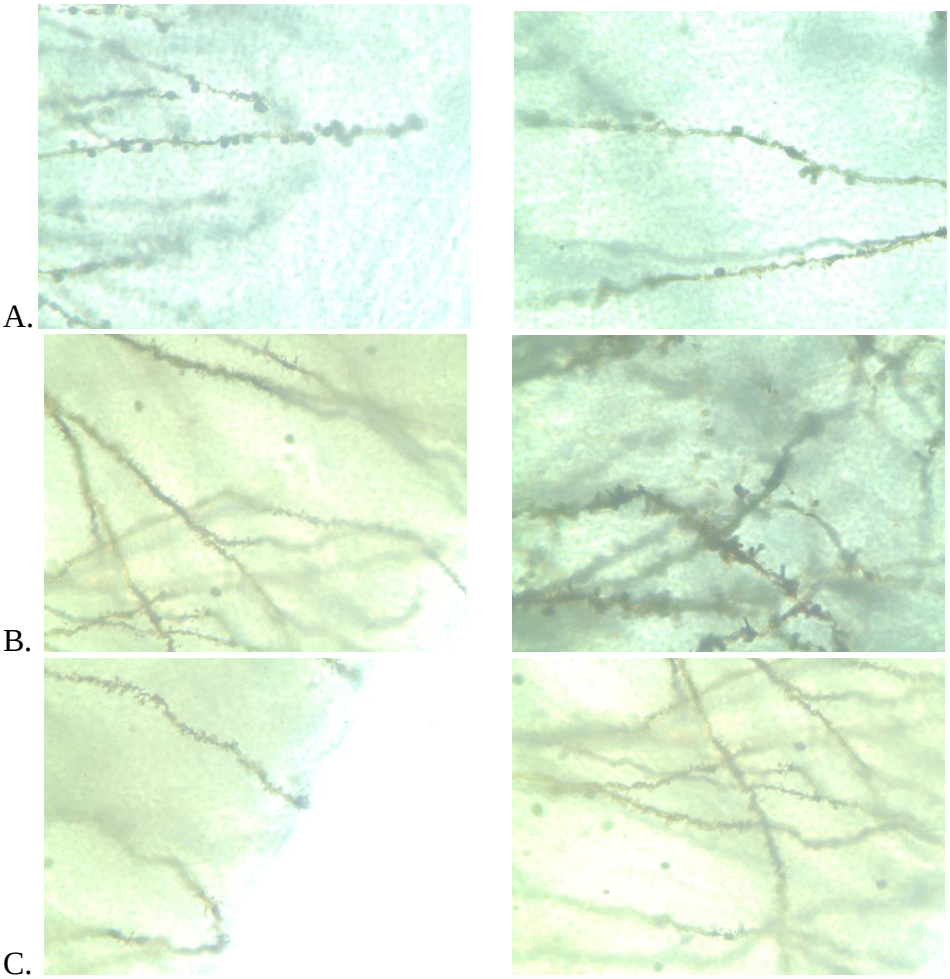

Figure 19: Dendritic spines on apical dendrites of the CA1 in males (left column) and females (right column) after (A) saline and vehicle (B) chronic cocaine and (C) progesterone + chronic cocaine administration.

Discussion

The present study aimed at investigating the effects of progesterone and cocaine on synaptic plasticity. Specifically, we used dendritic spine densities as markers of synaptic plasticity as it has been hypothesized that dendritic spines are the location of synaptic inputs (Harris and Kater, 1994). Chronic cocaine increased dendritic spine densities compared to saline and acute cocaine in the NAcS, NAcC in both males and females. These findings are in accordance with previously published observations that psychostimulants increase dendritic spine densities in the NAcS and NAcC (Robinson and Kolb, 1997; 1999; 2004; Ferrario et al., 2003; Li et al., 2003). We also report increases in dendritic spine densities in the CA1 region of the hippocampus in male and female rats. Salas-Ramirez et al. (2010) reported that prenatal cocaine exposure resulted in an increase of dendritic spine densities in the CA1 region in females but not males. These differences in observed results in males could be due to the different administration schedules. Salas-Ramirez et al. (2010) implemented an experimental design which consisted of 12 days of prenatal exposure to 30 mg/kg of cocaine, which was prepared every 3 days. The animals in this experiment were administered 15 mg/kg of freshly prepared cocaine daily. The differences in direct exposure, amount of cocaine, and method of preparation could account for the disparity sex differences seen.

Although chronic cocaine increased dendritic spine densities, acute cocaine failed to augment significant changes in density as compared to vehicle treated groups. Although Kolb et al. (2003) report seeing increases in dendritic spine densities after a single injection of amphetamine, their experimental design differed from ours in that they did not immediately sacrifice animals. Animals in this study were sacrificed about 1 hour post cocaine injection. Perhaps, any changes which were to occur needed more time to develop. Future studies should

be designed to investigate the amount of time needed to increase dendritic spine densities after a single psychostimulant administration.

Acute progesterone increased dendritic spine densities in the NAcS and NAcC in males but not females. Progesterone's effects on dendritic spine densities in males and females could be due to the differences in endogenous hormonal states in the two sexes. In females, ovarian hormones have been shown to mediate neural plasticity in portions of the mesolimbic dopamine reward system (Gould et al., 1990). Specifically, spine densities have been shown to fluctuate in the Hippocampus during the estrous cycle in female rats, with higher levels of estradiol correlating with higher synaptic densities (Woolley & McEwen, 1992; Woolley et al., 1990). McEwen and Woolley (1994) have shown that there is a decline of spine densities between proestrus and estrus, which is due to a drop in estradiol and rise in progesterone levels. Dendritic spine changes due to hormonal fluctuations have not been noted in males. However, progesterone administration in both in vivo and in vitro studies has shown to increase dendritic spine densities in cerebellar cortical areas, in immature pups. Perhaps, progesterone increases dendritic spine densities in the absence of fluctuating hormones. The interactions between progesterone administration and endogenous hormone fluctuations could also explain the statistical interaction between hormone and sex interaction seen in this study.

Acute progesterone and cocaine administered together resulted in an increase in denrtitic spine densities in males. The co-administration of progesterone and cocaine may have had an effect on dendritic spine densities while acute cocaine did not because it was administered several hours prior to sacrifice. Unlike cocaine, which was administered 30

minutes prior to sacrifice, progesterone was administered 4.5 hours before. Progesterone was able to regulate behaviors as well as alter dendritic spine densities.

Sex differences in spine density were not noted as in other studies (Salas-Ramirez et al., 2010). The reason for this could be that unlike in other studies comparing densities between the sexes, our subjects were influenced by hormonal manipulations. Progesterone administration and extensive handling may have obscured any innate sex differences in dendritic spine densities. Studies utilizing prenatal cocaine exposure have shown cocaine-induced dendritic spine densities independent of sex (Frankfurt et al., 2009).

The molecular mechanisms, by which amphetamine or cocaine produce changes in neuronal structure, are still unknown. One possibility is that psychostimulant drugs induce early genes such as cfos which could regulate the transcription of other target genes involved in D1 activation (Graybiel et al., 1990). Ujike et al (2002) also found that single dose exposure to methamphetamine induced increases in synaptophysin mRNA, strathmin mRNA, and mRNA of MKP3 in the accumbens, striatum, and hippocampus. Synaptophysin mRNA and stathmin mRNA are markers of synaptogenesis and neuritic sprouting respectively. These molecular changes were found anywhere from 1-3 hours after drug exposure. Chronic psychostimulant exposure results in increases in ARC and mRNA of MKP1 and MKP3 in the striatum and hippocampus (Ujike et al., 2002). ARC is considered to be a component of the cytoskeleton (Fosnaugh et al., 1995). In short, psychostimulants may be increasing dendritic spine densities by promoting neurite growth factors and proteins.

Although progesterone has attenuated cocaine-induced behaviors in previous studies, our study suggests that it is not exhibiting its attenuating effects by decreasing or

attenuating dendrite spine densities. However, future studies will want to look at the effects of ALLOP on dendritic spine densities. ALLOP has been reported to attenuate cocaine-induced behaviors. However, ALLOP has failed to promote dendritic growth, spinogenesis, and synaptogeniss in neonatal neurons (Sakamoto et al., 2001, 2002; Griffin et al., 2004). A possible explanation for this could be that ALLOP has been reported to mediate its actions through ion gated channel receptors rather than through intracellular steroid receptors (Sakamotoa et al., 2001; MacDonald and Olsen, 1994; Baulie et al., 1997).

Chapter 4: Progesterone and finasteride's effects on cocaine-induced conditioned place preference in males and females

Preliminary results from Chapters 2 and 3 showed that progesterone in a sex specific manner alters (1) locomotor responses to acute and chronic cocaine administration and (2) neuroanatomical changes associated with cocaine. We now aim to establish whether progesterone also alters cocaine-induced reward behaviors and if so, by which mechanisms its actions may occur.

Several new studies have shown that progestins may be important mediators of drug effects and reward (as reviewed in Quinones-Jenab and Jenab, 2010; Anker and Carroll, 2010). For example, progesterone administration attenuates many of the cocaine –induced subjective responses experienced by women (Evans and Foltin, 2006; Sofuoglu et al., 1999; Evans et al., 2002). In rodent studies, pretreatment with progesterone decreases self-administration of cocaine in females (Jackson et al., 2006; Larson et al., 2007; Anker and Carroll, 2010), and attenuates the reinstatement of cocaine self-administration (Feltenstein et al., 2009). Progesterone also alters cocaine-induced associative learning. Specifically, progesterone inhibits the acquisition and expression of cocaine induced CPP in intact female rats and male mice (Russo et al, 2008; Russo et al, 2003; Romieu et al, 2003).

Allopregnanolone (5 alpha-pregnan-3alpha-ol-20-one**,** ALLOP), a progesterone metabolite, has also been shown to inhibit learning and memory in rodents (reviewed in Maurice et al., 2006). Progesterone administration increases ALLOP levels in men and women (Soderpalm et al., 2004). ALLOP has been reported to have the same and sometimes greater effects as progesterone on cocaine induced behaviors (Anker and Carroll, 2010). In one case, ALLOP was shown to have protective effects against cocaine triggered seizures in mice (Kaminski et al., 2003). ALLOP treatment also inhibits cocaine self-administration escalation in

OVX female rats (Anker and Carroll, 2010). In intact female rats, ALLOP decreases cocaine-primed reinstatement (Anker et al., 2009). As previously mentioned, progesterone decreases cocaine-induced reinstatement in female rats but when administered with Finasteride, a compound which blocks progesterone's conversion to ALLOP, its effects are diminished (Anker et al., 2009). ALLOP was also tested in intact male and female rats for its ability to suppress the reinstatement of cocaine seeking triggered by stress. In female rodents, ALLOP decreased reinstatement elicited by stress (Anker and Carroll, 2010). ALLOP appears to only diminish cocaine-induced reward in females (Anker et al., 2009). ALLOP has not been shown to curb lever pressing for cocaine administration in intact males (Anker et al., 2009; Anker and Carroll, 2010). These findings suggest that progesterone's ability to attenuate cocaine's rewarding and motivating properties is mediated by ALLOP and are sex dependent.

We hypothesize that progesterone's metabolite ALLOP's attenuates cocaine generated motivation and reward in intact male and female rats. Inhibition of ALLOP will reverse progesterone attenuation of cocaine induced reward responses. Specifically, our study investigates whether the administration of finasteride inhibits progesterone's ability to attenuate cocaine-induced CPP in intact male and female rats. We therefore predict that finasteride will decrease progesterone's ability to attenuate cocaine-induced CPP in females only.

Methods:

Eight week old intact male and female Fischer rats (Charles River) were individually housed in standard cages, with free access to food and water. Animals were handled for one week prior to all experiments and maintained on a 12 hour light/dark cycle with lights on at 7:30 a.m. Animals were administered a series of 3 injections (hormone/vehicle, antagonist/vehicle, drug/saline). The

first injection consisted of either Progesterone or Vehicle. The second injection was Finasteride or vehicle, which was administered 3.5 hours after the hormone. Thirty minutes after the Finasteride, cocaine or saline was administered. Female rats were randomly assigned to experimental groups regardless of estrous cycle phase. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication 865-23, Bethesda, MD) and approved by the Institutional Animal Care and use Committee of Hunter College.

Drugs and administration: Cocaine hydrochloride was obtained from Sigma Chemical (St. Louis, MO). Animals received alternating i.p. injections of cocaine. Dosage was dependent on sex. As previously shown in this lab, CPP acquisition occurs at 20 mg/kg of body weight dissolved in 0.9% saline for males and 5 mg/kg of body (Russo et al, 2003). Animals received alternating subcutaneous administration of progesterone (500ug/1 cc) and Finasteride (25 mg/kg), which was also purchased from Sigma Scientific (Saint Louis, MO).

Table7: Treatment groups for CPP conditioning. Intact male and female Fischer rats were randomly separated into 6 experimental treatment groups, receiving a combination of progesterone, finasteride, and cocaine during CPP conditioning.

group number	Treatment
1	P + Fin + S
2	P + Fin + C
3	P + V + C
4	P + V + S
5	V + V + S
6	V + V + C

Cocaine-Induced CPP Procedures: Place preference cages were purchased form MED Associates (Georgia, VT). The cages consisted of 3 chambers each designed to give both a different tactile and visual experience. Two of the chambers were conditioning chambers. Each acrylic conditioning chamber was 28 cm long. One was black with a stainless steel grid rod floor. The other was white with a stainless steel mesh floor. Computer automated stainless steel doors allowed entrance to the chambers from the intermediate third chamber. The intermediate chamber was the "neutral" chamber where acclimation to the apparatus occurred. The neutral chamber was 12 cm long. It was also grey with a smooth PVC floor. During the preconditioning phase, rats were placed into the neutral chamber for a 5-minute acclimation period. After the acclimation period, the automated doors rose and animals were allowed free exploratory access to all chambers for 15 minutes. During the four conditioning days, rats in the cocaine experimental groups, received drug on days 1 and 3 in the same chamber in order to attempt to make an association between the drug and that chamber. On days 2 and 4, those animals received saline in the alternate chamber. Rats in the control conditions, received saline on all four days. On the testing day, animals received no drug and were placed into the neutral chamber (5 minutes) and then allowed access to all three chambers. Time spent, number of entrances, and explorations were recorded by a computerized photo-beam system run by the MED PC system. To determine the effects of progesterone and Finasteride on the acquisition of cocaine-induced CPP, on conditioning days, rats received Progesterone/vehicle and/or Finasteride/Vehicle four hours and 30 minutes before cocaine administration, respectively.

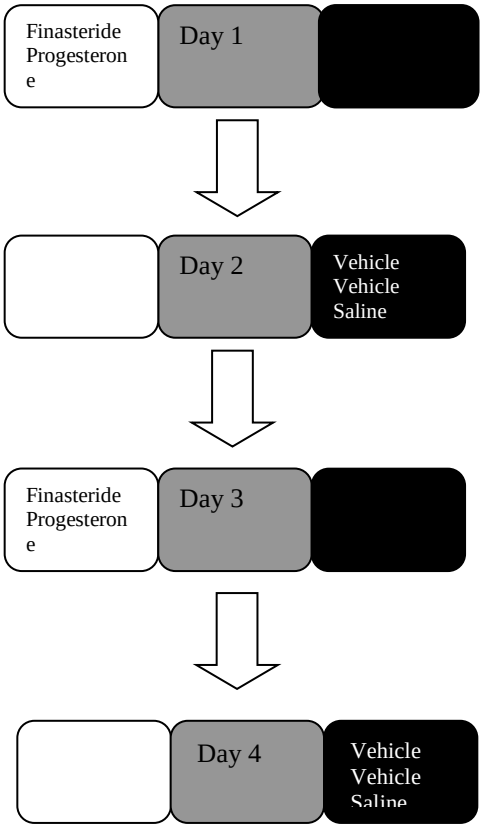

Figure 20: Schematic representation of CPP conditioning and treatments. Animals undergoing CPP conditioning were administered treatments in paired chambers on alternating days. On days 1 and 3, animals received Progesterone, Finasteride, and Cocaine (or the respective controls) in the same chamber. On even days, animals received control treatments in the opposite chambers.

Results

Progesterone's and finasteride's effects on cocaine-induced CPP

Cocaine induced CPP in both male and female rats, as seen in Figure 21. For males, the mean difference between average time spent in the paired and unpaired chambers is 17.860. T - tests showed a significant difference between the amount of time spent in the paired chamber and the unpaired chamber (t= 3.241, df= 6, p <0.01). For females, significance testing showed that t= 3.532, df= 8, p = 0.007. The mean differences were 145.2 sec. As seen in Figure 21, collectively, control animals did not demonstrate a chamber preference.

In female but not male rats, both progesterone and finasteride (p + f) induced CPP, t = 3.306, df =7, p =0.013—the mean difference of time spent in chambers was 94.44 seconds. In male rats, the difference in means, of the time spent in the chambers, was 2.750 and the t test was not significant (t= 0.7860, *df* =7, p >0.05).

Progesterone (p + c) inhibited cocaine-induced CPP acquisition in females but not males. Males spent significantly more time in the progesterone and cocaine (p + c) paired chamber than in the saline chamber, (t = 3.842, df =10, p =0.003). The mean difference was 132.2 seconds. In contrast, t test revealed that there was no significant difference between the amount of time spent in the progesterone + cocaine paired chamber and the saline one for females, (t= 1.842, df7, p > 0.05.

The effects of co-administration of progesterone and finasteride (p + f + c) on cocaine-induced CPP are shown in Figure 21. In males, the administration of finasteride did not block cocaine-induced CPP acquisition. The difference in means between time spent in the paired and unpaired side was 17.430 and the t-test showed a significant difference between means (t = 3.728, *df* = 6, p = 0.010). On the other hand, in female rats, co-administration of progesterone and finasteride pretreatment blocked cocaine-induced CPP acquisition. T test revealed no statistically significant differences between the average time spent in the paired and unpaired chambers.

In males, progesterone (p + s) induced CPP acquisition but had no effect on females, as shown in Fig 21. The difference between mean times spent in the paired and unpaired chambers is 9.500 units. Paired t-test show a significant difference between time spent in the different chambers (*t*= 4.132, *df* = 7, p = 0.004).

The effects of progesterone and finasteride on cocaine-induced explorations

In males receiving progesterone, finasteride, and cocaine (p + f + c), there was a significant difference in the average number of explorations between the cocaine paired and unpaired chambers, t = 3.728, df =6, p = 0.009. The mean difference was 17.43 explorations. As shown in Figure 22, progesterone (p + s) increased the number of explorations in the progesterone paired chamber compared to the unpaired chamber, in males. Cocaine also increased the number of explorations in the cocaine-paired chamber versus the unpaired, t= 3.241, df =6, p= 0.017. There were no significant differences in explorations of chambers in males that received progesterone and cocaine.

As shown in Figure 22, cocaine induced significantly more explorations, in females, in the paired chamber than in the unpaired chamber, t = 2.914, df =7, p = 0.0225. The mean difference was 17.88 explorations. The progesterone pretreatment (p + c) group also explored the cocaine paired chamber more than the unpaired chamber, t = 8.229, df =5, p = 0.000. The mean difference was 9.833 explorations.

Effects of progesterone and finasteride on cocaine-induced chamber entrances

As shown in Figure 23, in males, progesterone + finasteride induced a conditioned preference in which significantly more entrances into the paired chamber were made, t=2.431, df =10, $p < 0.05$.

Effects of progesterone, finasteride, and cocaine on locomotor activity

In males, cocaine failed to have a significant main effect, F (1, 28) = 4.169, p = 0.051. LSD Fischer post hoc test showed that males receiving only cocaine had significantly higher locomotor counts than any other treatment group. As shown in Figure 24, progesterone induced a significant hormone effect, overall, decreasing locomotor behaviors, F (1, 28) = 4.234, $p > 0.05$.

Post hoc analysis showed that progesterone decreased behaviors compared to vehicle, $p = 0.025$. Pretreatment with progesterone and finasteride significantly decreased locomotor behaviors compared to animals receiving cocaine only, $p = 0.015$. Administration of Progesterone also significantly decreased locomotor behaviors compared to cocaine only, $p = 0.015$.

As shown in Figure24, there was a main drug effect in females, $F(1, 28) = 6.435$, $p = 0.017$. Progesterone did not cause a significant hormone effect yet post hoc analysis, showed that animals receiving progesterone had significantly less locomotor activity than those receiving vehicle, $F(1,28) = 0.138$, $p = 0.004$. Progesterone pretreatment inhibited cocaine-induced locomotor counts from being statistically different from those of control animals. Thirdly, finasteride had a significant main effect on locomotor activity, $F(1, 28) = 23.270$, $p = 0.000$. Finasteride significantly decreased locomotor activity in females. Progesterone and finasteride administration decreased locomotor activity compared to animals receiving vehicle and saline, $p = 0.025$. Animals in the progesterone + finasteride + cocaine group showed significantly less locomotor activity than those receiving progesterone + cocaine ($p = 0.001$) and those receiving Vehicle +Vehicle +Cocaine ($p = 0.000$).

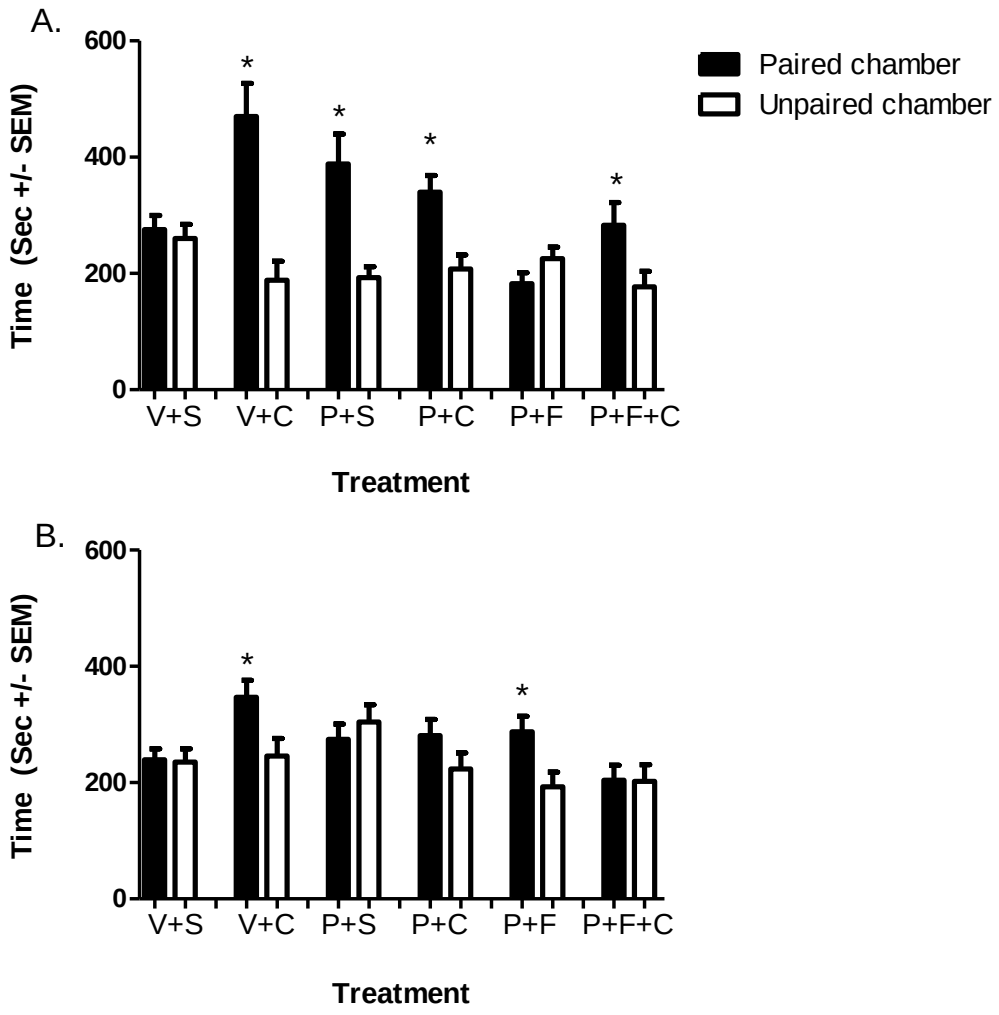

Figure 21: **The effects of progesterone and finasteride on cocaine-induced CPP in male (A) and female (B) rats**. Time spent in the saline-paired (white bars) and cocaine paired (black bars) chambers on the test day. Time spent is represented as Mean (seconds) ± S.E.M. * indicates statistically significant differences at $p < 0.05$ (N=6-10). [Abbreviations: V =Vehicle; S =Saline; C =Cocaine; P =Progesterone; F = Finasteride].

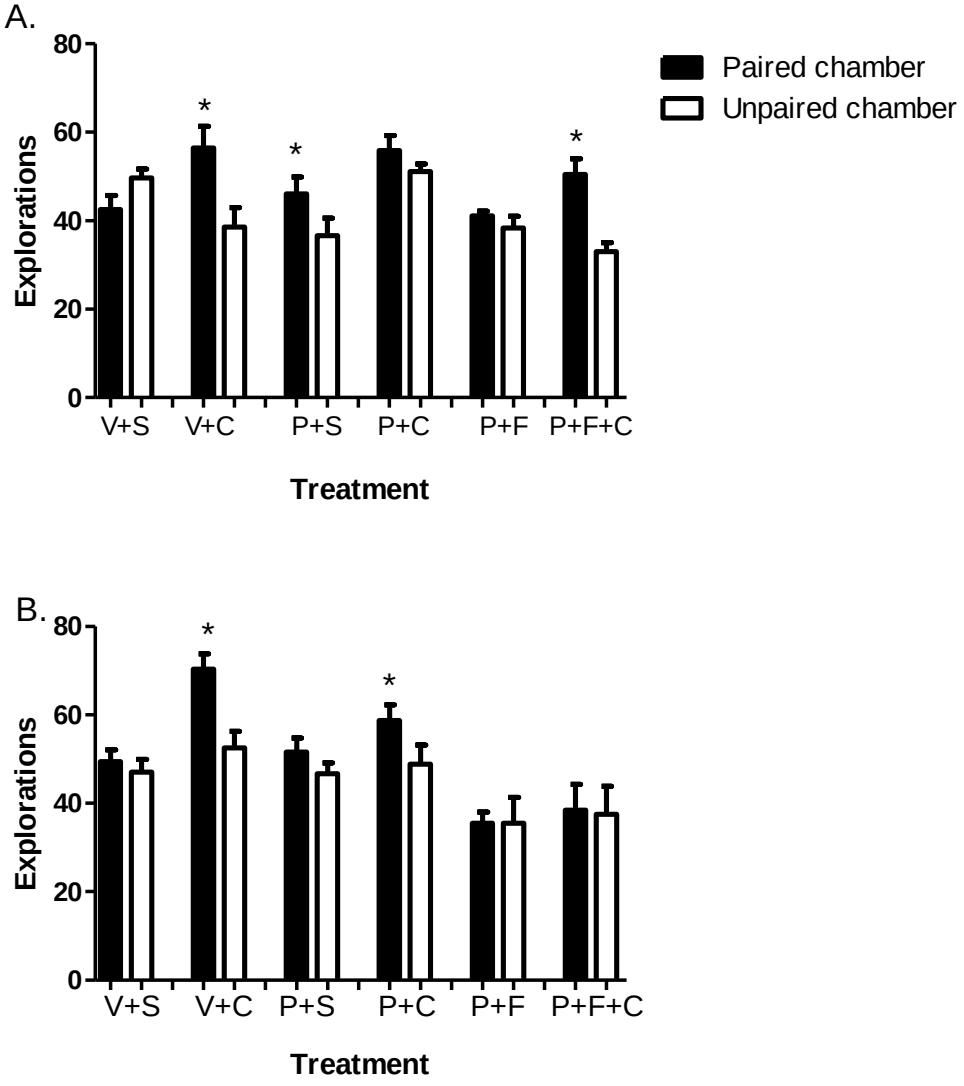

Figure 22: The effects of progesterone and finasteride on cocaine-induced CPP exploratory behaviors in male (A) and female (B) rats. The number of explorations in the saline-paired (white bars) and cocaine paired (black bars) chambers on the test day. All data is represented as Mean (explorations) ± S.E.M. * indicates statistically significant differences at $p < 0.05$ (N=6-10). [Abbreviations: V =Vehicle; S =Saline; C =Cocaine; P =Progesterone; F = Finasteride].

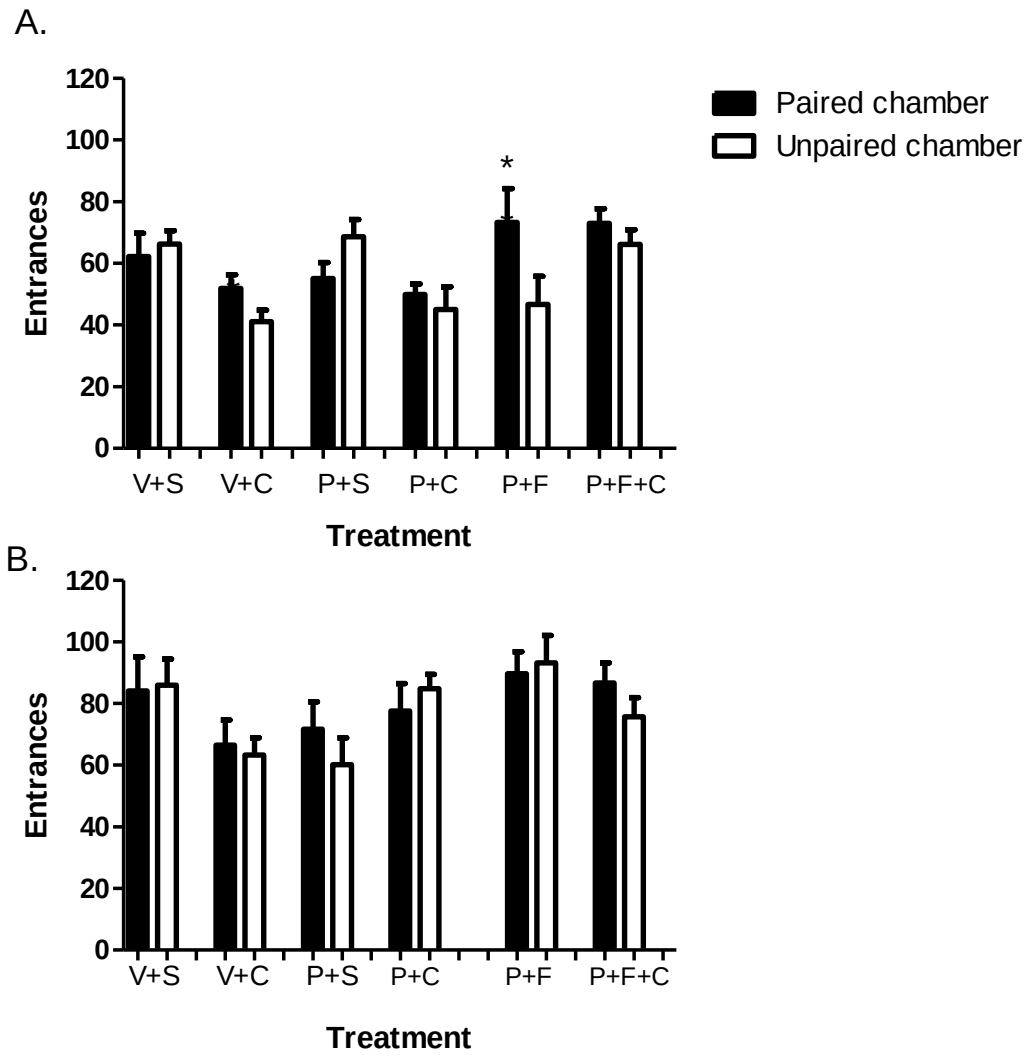

Figure 23:The effects of progesterone and finasteride on CPP entrances in males (A) and females (B). The number of entrances into the saline-paired (white bars) and cocaine paired (black bars) chambers on the test day. All data is represented as Mean (entrances) ± S.E.M. * indicates statistically significant differences at $p < 0.05$ (N=6-10). [Abbreviations: V =Vehicle; S =Saline; C =Cocaine; P =Progesterone; F = Finasteride].

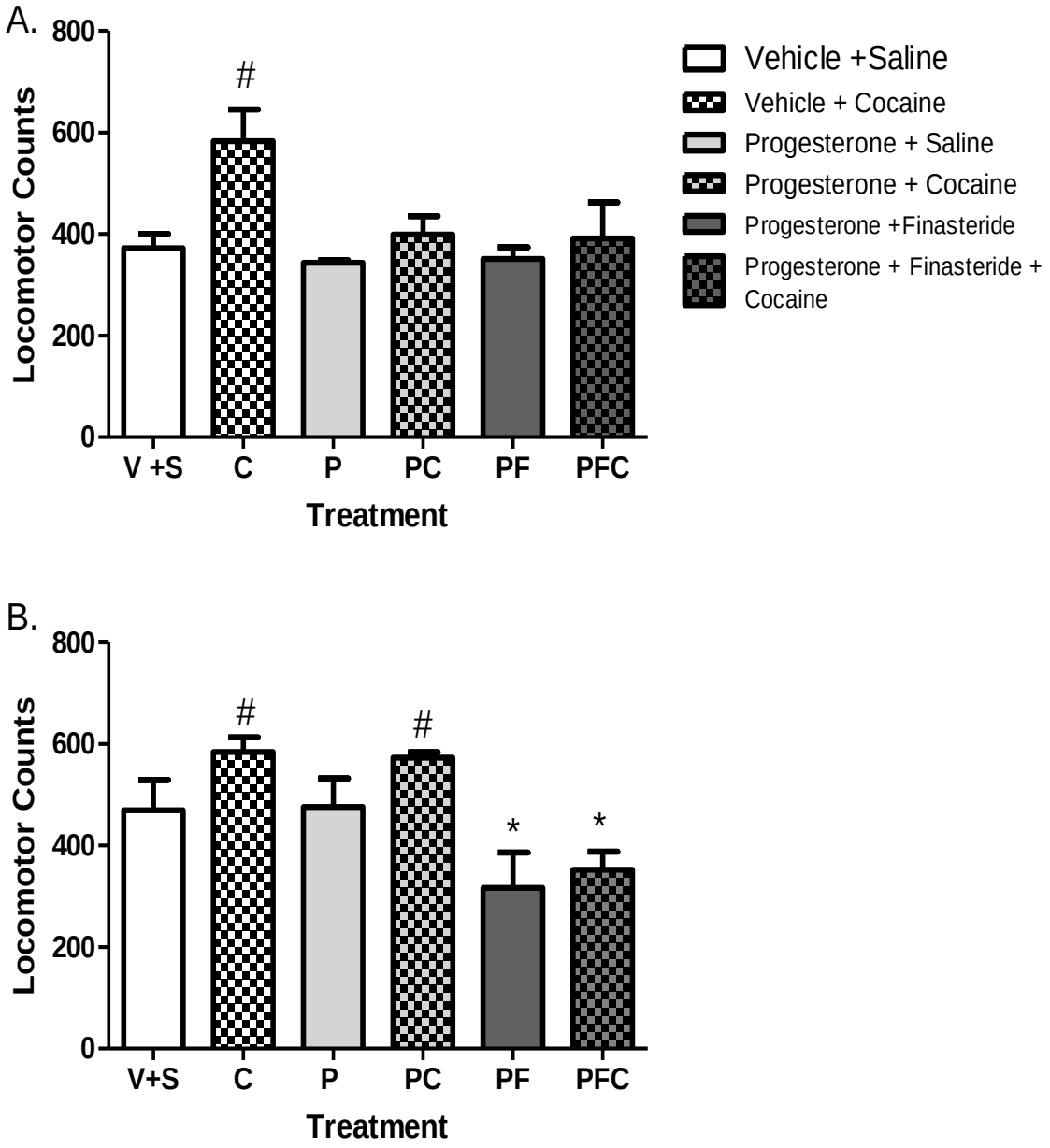

Figure 24: The effects of progesterone and finasteride on cocaine-induced locomotor responses in male (A) and female (B) rats. All data is represented as Mean (entrances) ± S.E.M. # indicates a drug effect at $p < 0.05$. * indicates a Finasteride effect at $p < 0.05$. (N=6-10). [Abbreviations: V =Vehicle; S =Saline; C =Cocaine; P =Progesterone; F = Finasteride].

Discussion

The primary goal of this study was to investigate the effects of the metabolic process of progesterone into ALLO on cocaine-induced CPP. In accordance with previously published studies, cocaine produced CPP in female and male rats (Bardo and Bevins, 2000), (Tzschentke, 1998). However, we further observed sexually dimorphic responses to progesterone administration in all treatment groups. First, progesterone produced a CPP in males but not females, suggesting sexually dependent rewarding effects. In the literature, the effects of progestins on reward are unclear; progestin-induced reward may be based on dosage and method of administration. For example, both Gonzales-Flores et al (2004) and Romieu et al. (2003) report that the administration of progestins does not induce the expression of CPP. Other studies have shown that systemic or i.p. administration of progestins can induce CPP dose dependently (Finn et al., 1997). However, Beuachamp et al., (2000) reported that different doses of progestins administered via i.c.v. produced varied CPP responses, ranging from no effect to aversion. Indeed, the discrepancies between our results and those of other studies could be due to differences in the doses of administered hormone. We administered 500ug of progesterone whereas Romeiue et al. (2003) administered between 10-40 mg/kg. Gonzales-Flores administered 13 ug/kg. The differences in our findings could also be due to species and hormonal variations. We used intact male and female rats whereas the other studies used either male mice (Romieu et al., 2003) or OVX rats (Gonzales-Flores et al., 2004). Method of progesterone administration has been shown to cause variability in its ability to mediate cocaine-induced behaviors (Niyomchai et al. 2008; Perroti et al.; 2006). Differences in route of administration could lead to differences in bioavailability and metabolism of the hormones

As previously reported by our lab, progesterone pretreatment inhibited cocaine-induced CPP in females but not males (Russo et al., 2008; 2010). ALLOP administration has been

reported to have similar sexually dimorphic effects on cocaine-induced reward in rats. ALLOP administration attenuates cocaine reinstatement in females but has no effect in males (Anker et al., 2009). However, locomotor behaviors showed the opposite trend. Progesterone pretreatment had no significant effects on cocaine-induced locomotor behaviors in females. However, in males, progesterone pretreatment significantly inhibited cocaine-induced escalations in locomotor counts. Although progesterone did not affect reward processes it did reduce the excitatory behavioral responses that are induced by cocaine.

Progesterone's effects are produced by both genomic and membrane mediated mechanisms (Engel and grant, 2001; Frye and Vongher, 1999). Progesterone interacts with different membrane receptors and ligand gated ion channels such as $GABA_a$ ionophore complex (Brann et al., 1995; Lambert et al., 1995; McEwen et al., 1991; Rupprecht and holsboer, 1999). These interactions with membrane receptors are suggested to be responsible for progesterone's rapid actions. Our administration paradigm of progesterone (4 hours prior to cocaine) has also been postulated to activate early genes such as c-fos which play a role in mediating cocaine-induced behaviors (Nestler, 2004; Russo et al., 2008; Quinones-Jenab, 2009). We suggest that progesterone may be affecting cocaine-induced reward and locomotor behaviors through membrane activated mechanisms and early gene activation.

Co-administration of progesterone and finasteride (p+f) also produced sexually dimorphic CPP behaviors. P +F administration induced a significant conditioned place preference in females but not males. Romieu et al (2003) reported that finasteride alone did not produce CPP in male rats. Our findings in male rats extend those of that lab by reporting that finasteride and progesterone do not produce CPP in male rats. However, p +f produced a sexually differentiated reward response in females resulting in CPP formation. Our findings add

to current knowledge by reporting that there are sex differences in the rewarding effects of exogenously administered progestins. In males, P+F did not induce CPP. However, P+F induced CPP formation in females implying that high levels of progesterone may have rewarding properties in intact females.

P+F produced sexually dimorphic cocaine-induced responses. Progesterone and finasteride inhibited cocaine-induced CPP formation in females but not males. Other studies utilizing finasteride and progesterone have reported that concurrent administration of the hormone and antagonist diminishes any of progesterone's attenuating effects on cocaine-induced responses such as cocaine reinstatement in female rats (Anker et al., 2009) and cocaine-induced CPP in male mice (Romieu et al., 2003). The prevailing theory is that because finasteride is able to inhibit or reverse progesterone's attenuating effects, ALLOP may play a large role in curbing cocaine's excitatory effects. We report that finasteride and progesterone did not reduce or inhibit CPP expression in male rats. In females, P+F inhibited cocaine-induced CPP formation. A possible explanation for this could be that we administered higher doses of progesterone than did Anker et al. (2009) which may have had more robust effects than the finasteride. A dose response curve of progesterone, finasteride, and cocaine should be performed to better understand finasteride's effects in OVXed and intact females. Since the females used in this study were intact, the administered P+F had to interact with endogenous hormones, which may cause variation in behavioral results. For example, Feltenstein et al. (2009) reported that progesterone's attenuating effects on cocaine-induced behaviors is tempered by endogenous hormonal environments. Exogenously administered progesterone attenuated cocaine reinstatement only in estrous staged females (Feltenstein et al., 2009). Unlike Feltenstein (2009), we did not monitor estrous cycle changes and therefore do not know if progesterone and

finasteride produced different effects on cocaine-induced behaviors dependent on hormonal profiles.

P+F treatment inhibited cocaine-induced increases in locomotor counts in both males and females. However, finsateride caused significant reductions in female locomotor behaviors in the P +F and P+F + cocaine conditions compared to the vehicle/saline experimental groups. The decrease in locomotor behaviors could have been caused by an increase in progesterone levels. Progesterone administration has been reported to produce fatigue and sedative like effects in women (Söderpalm et al., 2004).

In conclusion, progesterone's ability to mediate cocaine-induced reward behaviors such as CPP appears to be sex dependent. Varying levels of progesterone inhibit cocaine-induced CPP in females but not males. Our study adds to current knowledge by showing that finasteride does not reduce or reverse progesterone's attenuating effects on cocaine-induced CPP in females. Our experiment is also novel in that it shows that although progesterone may not affect cocaine-induced reward in males, it does decrease psychostimulant induced locomotor responses. These findings suggest that although the behavioral responses are inhibited, subjective wanting is still present in males.

Chapter 5: Conclusion

Both preclinical and clinical studies have shown sexually dimorphic patterns in cocaine-induced behavioral responses in all phases of the addiction process including induction and relapse. Gonadal hormones, particularly estrogen and progesterone, are postulated to be the primary mediators of sex differences in cocaine-induced behavioral responses. Overall, it has been hypothesized that estrogen has facilitatory effects (Becker et al., 2008; Festa et al., 2004; Lynch et al., 2002) while progesterone has reductional or attenuating effects (Evans et al., 2002; Sofuoglu et al., 2004; Anker et al., 2009; Niyomachai et al., 2006). For example, progesterone has been shown to reduce behavioral locomotor counts in OVX rats (Niyomachai et al.,2005, 2006) block cocaine-induced CPP in female rats (Russo et al., 2003; 2008); and reduce the acquisition (Jackson et al., 2006) and escalation (Larson et al., 2007) of cocaine self-administration. Our studies aimed to expand on these findings by investigating the effects of progesterone and its metabolites on cocaine-induced locomotor behaviors, neural adaptations, and learned associations in intact male and female rats. We observed that acute and chronic progesterone decreased and in some cases inhibited cocaine's effects on locomotor responses in both intact male and female rats. Our findings are novel in showing that progesterone is a potent mediator of cocaine-induced psychomotor effects in both males and females. Both acute and chronic progesterone were able to attenuate and inhibit locomotor responses to acute and chronic cocaine. These findings have two major implications. The first is that progesterone may play a large role in mediating sex differences in cocaine responses. The administration of progesterone decreases many of the hypersensitive responses to cocaine in females. The second implication is that progesterone may make a viable therapeutic agent in the treatment of cocaine addiction since progesterone was able to diminish the visible defining characteristics of the psycho-stimulant.

A major shortcoming of this experiment was in the exclusion of two experimental groups (chronic progesterone + acute cocaine and chronic cocaine + acute progesterone). The exclusion of these groups led to complexities in analyzing the data. Instead of running two way ANOVAS on the groups, we had to first decipher the schedule of progesterone's effects on behavior and then progesterone's effects. Analyzing the data would have been simpler if we had the groups to complete our data set. Also, the inclusion of those two groups would give a more complete understanding of progesterone's effects on cocaine-induced locomotor effects. Our next study will consist of looking at the effects of acute progesterone on locomotor behaviors induced by chronic cocaine administration. This will be an important study to implement because it will give us an understanding of how progesterone will work as a therapeutic agent. Although we have consistently seen that progesterone pretreatment is able to inhibit cocaine-induced locomotor responses, progesterone's effects on predetermined changes induced by chronic cocaine are still unclear. We will also look at the effects of chronic progesterone on acute cocaine-induced responses. Although we postulate that progesterone will have the same attenuating effects that we have currently observed, the effects on behaviors may be more consistent. For example, in our current experiment we found that progesterone inhibited certain behaviors such as rearing while attenuating others such as locomotor counts. Perhaps the chronic exposure may render the CNS more capable of having consistent effects.

Although progesterone did not significantly alter dendritic spine densities in females, acute progesterone +cocaine increased dendritic spine densities in the NAcS and NAcC of intact males. These findings show that progesterone is not inhibiting behavioral responses by decreasing dendritic spines as we initially hypothesized. Although our study shows that there were spine density increases, it does not tell us whether the proliferation of dendritic spine

densities is due to increases in receptor sites for cocaine modulated neurotransmitters or if they are mediated by progesterone. This may be an important factor in understanding progesterone's effects on cocaine-induced neural adaptations. Based on our findings, we cannot conclusively determine how progesterone is mediating its effects on cocaine-induced behaviors. Future studies will look at the effects of progesterone antagonist and metabolite antagonists such as RU-486 and finasteride on cocaine-induced neural adaptations.

Lastly, we hypothesized that although progesterone has been shown to block cocaine-induced CPP in females it did so mainly through its conversion process to ALLO. However, progesterone and progesterone administered with finasteride blocked cocaine-induce CPP sex dependently. Progesterone and p + f treatments were effective only in females and not males. As mentioned previously, these findings imply that female gonadal hormones, specifically progesterone, are key mediators of sex differences in responses to cocaine. Without noticeably affecting reward, progesterone administration decreased cocaine-induced locomotor counts suggesting a difference in its ability to mediate reinforcement and psychomotor stimulation.

Although different doses of cocaine were administered to males and females, we used the same dosage of progesterone in both. In most studies males and females are given the same dosage of progesterone (Russo et al., 2006, Anker et al., 2009) regardless of differences in progesterone plasma levels or progesterone distribution. Future experiments should focus on measuring the effects of different dosages of progesterone on cocaine-induced CPP in males. Much of the discrepancies in the literature on progesterone's effects may be due to differences in utilized dosages (Romieu et al., 2003, Russo et al., 2006). Therefore, we propose that a dose response curve should be performed in intact males. The lack of progesterone's effects on cocaine-induced CPP in males and its ability to attenuate cocaine responses in females may also

reveal insight into progesterone's role in mediating sex differences in psychostimulant reward and associative memory. From an evolutionary viewpoint, it is feasible that progesterone mediates reward behaviors in such a way as to reduce reward during periods of reproductive activity. Perhaps, higher doses of progesterone may be more effective in inhibiting cocaine-induced CPP in males. Since progesterone was able to mediate selective responses to cocaine, our next study will utilize higher doses of progesterone on in males, to investigate whether the cocaine's rewarding properties can be disrupted with higher doses of hormone pretreatment.

Betacyclodextrin, the vehicle used to administer finasteride decreased behaviors significantly in females. Although we did not notice any significant effects in males, the next experiments will use a more benign vehicle such as sesame oil. The Betacyclodextrin may have had effects on cocaine-induced mobility in males which went unnoticed. The findings from this study also indicate that progesterone is capable of blocking cocaine-induced behaviors without metabolizing into ALLO. Previous studies have shown that ALLO is as effective as and sometimes more effective than progesterone in reducing responses to cocaine (Anker et al., 2009, as reviewed in Quinones-Jenab and Jenab, 2009; Anker and Carroll, 2010). It has therefore been postulated that progesterone's conversion to ALLO was necessary to attenuate cocaine-induced responses. However, our findings show that ALLO is not necessary to inhibit cocaine reward in females. P +f administration blocked cocaine-induced CPP formation in females. Although p + f pretreatment blocked cocaine's rewarding effects, administered alone without cocaine it produced CPP in females. Its production of a CPP is further evidence that high levels of progesterone are important in reward mediation. Finasteride was used in this study because ALLO proved to be highly cost prohibitive. Using finasteride resulted in an indirect measure of ALLO's role in attenuating cocaine CPP. Future studies in this lab will need to look more

directly at ALLO's role in reward. Therefore, the next experiments will pretreat males and females with ALLO instead of p +f. Progesterone seems to be less effective in altering cocaine related reward responses in males. The differences in effects on cocaine-induced reward may be due to differences in progesterone receptor availability.

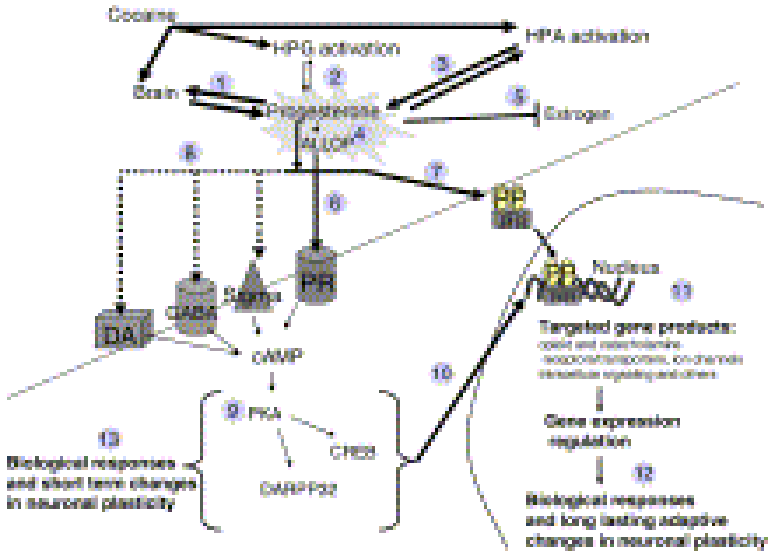

Figure 25: Proposed model of mechanisms by which progesterone alters signaling after cocaine administration. (1) Cocaine-induces changes in progesterone serum and brain levels via (2) HPG release, and/or (3) HPA activation. (4) Progesterone is metabolized to ALLOP. (5) Progesterone reduces the potentiating effects of estrogen. Progesterone and ALLOP increases lead to changes in the CNS which, in turn, will activate one or several of the following cascades: (6) Cell surface non-genomic signaling: such as (8) dopamine or serotonin secretion and receptor activation, GABA receptors (through progesterone and/or ALLOP binding site), inhibition of Sigma receptors and/or others. This, in turn, (9) activates PKA signaling pathway which (10) may affect transcription through alternative transcriptional elements such as AP-1 through activation of PKA/PKC DNA-bound transcription factors (cFos/cJun) and/or (13) lead to biological responses an short-term changes in neuronal plasticity; (11) Genomic signaling progesterone receptor will target PREs in target promoter, leading to an up-regulation or down-regulation of gene transcription and subsequent tissue responses. In turn, thickness of arrows represents the magnitude of effect. Broken arrows represent activation in which either progesterone and/or ALLOP are involved. Quinones-Jenab and Jenab (2009).

It is very likely that long term progesterone administration, as was used in our fourteen day experiment, causes changes in gene expression (Mani, 2006). Experiments utilizing progesterone have been able to demonstrate that progesterone is able to mediate changes in early

gene expression. For example, increases in progesterone serum correlates with increases in progesterone A and B protein levels and DNA-PR binding complex formation in the NAc (Wu et al., 2006). Progesterone is also able to inhibit cocaine' up-regulation of PDYN mRNA levels in striatum.

Taken together, the research shows that progesterone is capable of mediating cocaine-induced responses through membrane receptor and non-genomic mechanisms. We postulate that cocaine activates an increase in progesterone serum and brain levels from the CNS and endocrine sites such as the HPA. Progesterone is then metabolized to ALLOP. The resulting high levels of progesterone offset estrogen's potentiating effects. The escalating levels of progesterone activate membrane receptors and ligands which we hypothesize are responsible for the fast acting behavioral attenuations seen with progesterone pretreatment. For example, the increases in progesterone and ALLOP interact with the dopamine-serotonin secretion and receptor activation. Studies have shown that progesterone increases serotonin and dopamine in the VTA and alters estrogen-induced changes of dopamine levels in the NAc (Russo et al., 2003). Progesterone has also been shown to augment serotonin and its metabolite after acute cocaine administration (Perrotti et al., 2000).

The high levels of progesterone will also interact with GABA and σ_1 receptors. Progesterone and ALLOP potentiate $GABA_A$ and $GABA_B$ ionophore complexes (Brann et al., 1995; Lambert et al., 1995; McEwen, 1991; Rupprecht and Holsboer, 1999) are able to induce anxiolytic actions by activating these complexes (Reddy et al., 2005). Like progesterone, $GABA_A$ agonists (imidazenil and diazapan) have been shown to attenuate cocaine-induced behaviors such as locomotor responses and CPP (Meririnne et al., 1998).

Progesterone has also been shown to mediate responses to cocaine by interacting with σ_1 receptors (Phan et al., 2000). Like GABA, the activation of σ_1 receptors can have rapid effects on behavior. σ_1 receptors play a role in regulating cocaine induced sensitization, locomotor responses, convulsions, and CPP (Romieu et al., 2000, 2002, 2003). Progesterone has been shown to modulate many of these cocaine-induced behaviors by acting as a σ_1 antagonist. These non-genomic mechanisms lead to modulation of PKA signaling pathways which may affect transcription factors such as cFos and lead to biological responses and short term changes in neuronal plasticity (as reviewed in Quinones-Jenab and Jenab, 2009). In the experiments in this study and others from this lab, progesterone is administered four hours prior to cocaine exposure. This time frame allows for both membrane and genomic mechanisms to be activated (Russo et al., 2006, Quinones-Jenab and Jenab, 2009).

Taken together preclinical and clinical studies demonstrate that progesterone is important in mediating responses to cocaine in females. Moreover, most studies also suggest that progesterone attenuates or inhibits cocaine's subjective, reward, and psychomotor effects in females. In males, the results are more varied in the literature. We have shown consistently that progesterone attenuates cocaine-induced locomotor responses in males. Currently there are no effective therapeutic treatments for cocaine addiction. Progesterone has been shown to be a beneficial therapeutic aide in treating psycho-behavioral disorders such as anxiety (de Wit et al., 2001). Progesterone has also been beneficial in treating hypertension, chronic obstructive pulmonary disease, multiple sclerosis, and benzodiazepine withdrawal (reviewed in Sofuoglu et al., 2007; Quinones-Jenab and Jenab, 2009). Thus far, progesterone has been shown to attenuate all aspects of cocaine addiction in women marking it a candidate for therapeutic use.

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