EFFECTS OF SEX AND PRENATAL STRESS ON VULNERABILITY TO DRUGS

by

Mark B. Thomas

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in The University of Michigan
2012

Doctoral Committee:

Professor Jill B. Becker, Chair
Professor Margaret E. Gnegy
Professor Theresa M. Lee
Professor Terry E. Robinson

To my parents, who surely sacrificed many of their dreams through the years to provide me with the resources to pursue my own. It is my dad in particular that I have thought of most during the writing of this dissertation. Sadly, he has been battling terminal cancer for the majority of this process. Though I know he would never let me, I would gladly trade in all of my accomplishments for a cure.

ACKNOWLEDGEMENTS

I wish to thank Dr. Jill Becker for the invaluable guidance she has provided during my graduate studies. Without her unwavering support, none of the work described here would have been possible. My other committee members, Dr. Terry Robinson, Dr. Theresa Lee, and Dr. Margaret Gnegy, also deserve special recognition. All provided insightful comments that played a critical role in the preparation of this dissertation. Lastly, just as I am indebted to members of the committee, I also wish to thank Dr. Seema Bhatnagar for first sparking my interest in pursuing a research career. Despite moving to a different university, she has continued to play an active role in my development, and has directly contributed to some of the work presented here.

TABLE OF CONTENTS

LIST OF FIGURES

Figure

LIST OF TABLES

LIST OF APPENDICES

Appendix

CHAPTER I

INTRODUCTION

An abundance of findings indicate that some periods of physical development are hypersensitive to life experiences. When these processes have effects that are irreversible, the window of physiological plasticity is referred to as a critical period. Critical periods generally occur during episodes of rapid cell division early in life (Barker, 1998), and interference with the normal sequence of events during these times greatly reduces the likelihood of survival. A classic study by Nobel Prize recipients Torsten Wiesel and David Hubel provides a powerful example of how insults during a critical period produce consequences that are disproportionately severe. Kittens chronically deprived of visual stimuli to one eye (monocular deprivation) behaved as though they were blind when later forced to navigate using only the deprived eye. Performance with the non-deprived eye, however, was intact. Interestingly, performance with neither eye was affected when the same procedure was performed on adults. Taken together, these findings indicate that the early-life postnatal period is a critical time for cats' visual development (Wiesel and Hubel, 1963). The deprivation of stimulation during this stage, but not outside of it, resulted in a long-term and potentially debilitating condition.

As is the case with visual development in kittens, critical periods have largely been linked with events occurring early in life, when the establishment of neural connections (synaptogenesis) is most heavily concentrated. Profound neuroplasticity during this time is thought to be adaptive, in that brain circuits can be shaped by the environment in ways that promote survival and reproduction. Recently, a great deal of attention has focused on developmental processes that occur in utero. Like development during the postnatal period, insults that

compromise the integrity of the environment during this time can produce effects that are long-lasting and debilitating.

Impoverished conditions in utero have been linked with a predisposition for future disease onset in both humans and non-human animals. Though findings have been consistent across species, the preclinical setting offers some freedom from ethical and technical constraints that limit the identification of causal relationships in humans. Perhaps due to these limitations, the influence of prenatal conditions on future drug use has not been evaluated in humans. In rats, however, a consistent pattern has emerged: males whose prenatal development was compromised by repeated episodes of maternal stress (prenatal stress [PS]) are hypersensitive to drugs of abuse in adulthood. PS promotes the ability of drugs to stimulate neurochemical signaling (Kippin et al., 2008, Silvagni et al., 2008, Carboni et al., 2010), activate locomotor activity (Deminiere et al., 1992, Henry et al., 1995, Koehl et al., 2000, Kippin et al., 2008), and support the pursuit of drug reward (Deminiere et al., 1992, Yang et al., 2006, Kippin et al., 2008, Thomas et al., 2009, Gao et al., 2011). Comparatively, much less is known about effects of PS in females, even though there are profound sex differences in drug responsiveness of rodents and humans without a history of PS (No PS; Lynch et al., 2002, Carroll et al., 2004, Roth et al., 2004, Becker and Hu, 2008, Carroll and Anker, 2010). Thus, the overall aim of this dissertation is to assess whether PS in rats is a sex-dependent risk factor for susceptibility to drugs, as it is for a variety of maladaptive conditions (Weinstock, 2007, 2011).

For the remainder of this chapter, I first review evidence linking prenatal insults with long-term health abnormalities, with a predominant focus on mental health. Second, I provide an overview of critical neurodevelopmental events that occur in utero to illustrate the overlapping nature of PS and rat brain development. Next, I review a critical component of the physiological stress response, given that it is (1) activated when a pregnant female experiences stress and (2) impaired in individuals that have a history of PS. An overview of sex differences in preclinical paradigms of drug abuse/addiction vulnerability is

provided next, with a corresponding discussion of their known or suspected biological determinants. Finally, this chapter concludes with a review of findings that link drug susceptibility with a history of PS.

1. Fetal programming

1.1 "Fetal origins" hypothesis

In the 1990's, physician and epidemiologist David J. Barker argued that some human diseases are precipitated by suboptimal conditions in utero. Indeed, the consistent link between prenatal malnourishment and the development of conditions like coronary heart disease, hypertension, type 2 diabetes mellitus, and ischemia gave rise to the "fetal origins" hypothesis of adult disease (Barker, 1990, Barker et al., 1990, Hales et al., 1991, Barker et al., 1993, Barker, 1995, 1998). Nutrition was argued to be one of several non-genetic factors that "program," or irreversibly direct, the fetus to develop in a way that may be detrimental to survival outside of the womb (Welberg and Seckl, 2001). As an example, Rich-Edwards et al. (1997) report an inverse relationship between birth weight (one of several markers used to assess the quality of prenatal conditions) and non-fatal stroke incidence in women, with an 11% decrease in the risk of non-fatal stroke for every 454 gram increase in birth weight.

Of course, the quality of prenatal life is often highly correlated with that of *postnatal* life, meaning that poor nutrition after birth may be of equal or greater risk than undernourishment in utero. Interestingly, while adult risk factors for disease (e.g., cigarette smoking, alcohol consumption, physical inactivity) do add to the effects associated with undernourishment, the links between birth weight and chronic conditions persist even after controlling for these and other factors (Hales et al., 1991, Frankel et al., 1996, Rich-Edwards et al., 1997, Barker, 1998). This is also true for non-human animals, with those tested under controlled laboratory conditions offering an ideal model for identifying contributions to health that are uniquely attributable to prenatal conditions (Morgane et al., 1993).

1.2 Rodent models of prenatal undernourishment

Effects of prenatal undernourishment have largely been identified by placing pregnant rats on protein-restricted diets. Protein restriction induces a wide-ranging set of physical and behavioral deficits in the offspring, including somatic growth (Galler and Tonkiss, 1991), reproductive development (Zambrano et al., 2005, Guzmán et al., 2006), neuroendocrine functioning (Lesage et al., 2001), hypertension (Langley-Evans et al., 1994), learning (Reyes-Castro et al., 2011), behavioral adaptability (Tonkiss et al., 1990, Landon et al., 2007), motivation for natural rewards (Miles et al., 2009, Reyes-Castro et al., 2011), and neurodevelopment (Morgane et al., 1978, Morgane et al., 1993, Morgane et al., 2002) . Generally, malnourishment-induced effects are insensitive to the restoration of protein intake after birth, although said effects – with or without postnatal protein supplementation – are generally less robust than those produced by undernourishment that is restricted to the lactation period (Morgane et al., 1993). The most severe effects are observed when dietary restrictions extend across both periods of development. Thus, rat models indicate that proper nutrition in the prenatal and postnatal environment is vital for healthy development. Malnourishment across both periods induces effects that are both long-lasting and additive.

Inquiries into the neurobiological effects of prenatal undernourishment have disproportionately focused on the hippocampus (see Morgane et al., 1993, 2002 for thorough reviews). Its well-defined organization, involvement in cortical circuits, and critical contributions to the formation of enduring memories are argued to make the hippocampus an ideal region for such work. Briefly, memories are believed to stem from a process of activation-dependent strengthening of synaptic connections (long-term potentiation [LTP]) to and within hippocampal circuits. Prenatal malnourishment induces life-long deficits in hippocampal plasticity by enhancing gamma-aminobutyric acid (GABA)-mediated inhibitory activity (Austin et al., 1986, Austin et al., 1992, Morgane et al., 2002). A decrease in extra-hippocampal serotonegic inputs is thought to disinhibit GABAergic interneurons that synapse onto and exert inhibitory control over principal cells (Blatt et al., 1994), ultimately contributing to disrupted hippocampal

plasticity in rats that have been prenatally malnourished (Morgane et al., 2002). Interference with synaptic plasticity is purported to underlie reports of malnourishment-induced behavioral inflexibility (Tonkiss et al., 1990, Landon et al., 2007, Reyes-Castro et al., 2011), although more work is needed to determine whether this is directly attributable to dysfunction of the hippocampus (Morgane et al., 2002).

Though still inconclusive, some of the deficits attributed to prenatal malnourishment may in fact be a consequence of maternal stress. Undernutrition increases glucocorticoid (a steroid hormone that is increasingly secreted during times of stress; see 1.3 for more information) output in pregnant rats (Lesage et al., 2001) and women (Fowden, 1995). Administration of actual or synthetic glucocorticoids during gestation reduces birth weight in rats (Nyirenda et al., 1998), sheep (Ikegami et al., 1997, Newnham et al., 1999) and humans (Reinisch et al., 1978, French et al., 1999, Bloom et al., 2001), while creating a predisposition for health deficits also associated with prenatal undernutrition, such as hypertension (Dodic et al., 1998, Dodic et al., 2001, Dodic et al., 2002, Singh et al., 2007) and hyperglycemia (Nyirenda et al., 1998). In short, the shared symptomology between prenatal malnourishment and overexposure to glucocorticoids suggests that maternal stress may be another factor capable of programming the fetus.

1.3 Prenatal stress in humans

Maternal stress during gestation (prenatal stress [PS]) is indeed linked with risk factors (preterm birth [< 37 weeks gestation] and low birth weight [< 2500 grams]) for and the actual development of future health ailments in the developing child (Schetter, 2009, 2011, Wadhwa et al., 2011). Though long-term effects of PS are extensive, links are particularly strong with aspects of mental health (see Beydoun and Saftlas, 2008 for an overview of recent findings). To exemplify, PS is associated with increased impulsivity (Van den Bergh et al., 2005), attention deficit hyperactivity disorder (ADHD) symptoms (Van den Bergh and Marcoen, 2004, Rodriguez and Bohlin, 2005, Van den Bergh et al., 2006), impaired mental and psychomotor development (Huizink et al., 2003, Laplante et

al., 2004), and childhood problem behaviors (O'Connor et al., 2002, O'Connor et al., 2003, Gutteling et al., 2005).

As is the case with low birth weight, the ability of PS to predict these abnormalities persists even after controlling for possible confounding variables. Nevertheless, it must be noted that the correlational design of human studies does not provide a basis for making definitive causal statements about the role of PS in disease onset. Methodological concerns, such as which and when markers of PS should be recorded, have produced a literature with substantial gaps and discrepancies. With these issues in mind, non-human animal studies are increasingly seen as a valuable model of risks associated with PS. Work with animals is far less constrained by ethical concerns than comparable studies with humans, allowing for an experimental (as opposed to observational) study design. Testing under controlled laboratory conditions also facilitates the identification of traits specifically altered by PS, independent of factors that often co-occur with PS at the human level. Lastly, preclinical animal studies can link behavioral abnormalities with changes to specific neural circuits, providing some understanding of the mechanisms that mediate effects of PS.

1.4 Preclinical models of prenatal stress

PS in non-human animals generally entails repeated episodes of maternal stress during a restricted portion of the gestation period, with the length, timing, and choice of stressor depending mostly on the species being tested. Effects of PS have been documented in rats (Morley-Fletcher et al., 2003), mice (Mueller and Bale, 2008), voles (Marchlewska-Koj et al., 2003), guinea pigs (Kapoor and Matthews, 2005), farm animals (Early et al., 1991, Otten et al., 2001, Roussel et al., 2005), and monkeys (Clarke et al., 1994). Despite this variety, the consequences of PS have been best documented in rats. As a result, and because the findings presented in this dissertation were also collected from this species, the following discussion of PS will be restricted to rats, unless otherwise noted.

The original link between PS and mental health abnormalities was reported by Thompson (1957). Exposure to stress-associated cues across the

gestation period (21-22 days for rats) increased signs of emotionality in the offspring, with the effects persisting into adulthood. Comparable findings have been reported since, despite wide-ranging differences in methodological approaches. Briefly, a variety of stimuli have been utilized as maternal stressors, including restraint, footshocks, loud noises, experimenter handling, forced swimming, and forced injections (Weinstock, 2008). Some researchers have also used a mixture of stressors to reduce repeated stress-induced neuroendocrine adaptations to a homotypic stressor (see section 3.3). Additional methodological variation relates to the frequency, duration, and extent (i.e. which portion or portions of the rat gestation period) of stress exposure. Traditionally, stress is administered 1-3 times daily for a restricted portion of the gestation period. Shorter stress episodes (20-60 minutes) are typically administered multiple times per day, while the opposite is true for more extensive (e.g. 6 hours) stress episodes. Lastly, the gestational timing of stress is also thought to be an important consideration. Like Thompson (1957), some have applied stress across the entire gestation period (Weinstock et al., 1992, Weinstock et al., 1998). A small sampling has also restricted stress to the middle of the gestation period (Nishio et al., 2001, Koenig et al., 2005). Still, the predominant stress regimen has been across the last week (beginning on GD 14 or 15) of prenatal development (Weinstock, 2008). In part because it overlaps with critical periods of brain development (e.g. neurogenesis, migration, synaptogenesis, etc.; see below for discussion), stress during this period is believed to program neural tissue to develop along a maladaptive trajectory, such that emotional health is compromised throughout postnatal life (Bayer et al., 1993, Welberg and Seckl, 2001).

1.5 The influence of PS on reproductive behaviors

One particularly robust effect of PS is the disruption of prenatal masculinization processes in developing males. Ward (1972) provided the first such report after observing reduced displays of sex-typical reproductive behaviors in adulthood. Relative to sex-matched controls, PS males were much less likely to display an ejaculatory response when provided access to a female

in estrus (i.e., a sexually-receptive female). When castrated and subsequently treated with estradiol and progesterone, PS males exhibited female-like behaviors of sexual receptivity that were at intermediate levels between comparably-treated No PS males and estrus females. Taken together, these findings indicate that PS both demasculinizes and feminizes males' sexual behavior. More recent work suggests that these effects also extend to *non-sexual* behaviors (Masterpasqua et al., 1976, McGivern et al., 1986, Ward and Stehm, 1991).

Though still not conclusive, demasculinization and feminization likely stem from a disruption in males' testosterone surge that normally occurs during GD 18-19. In males with a history of PS, testosterone increases on GD 17, but then sharply declines across days 18 and 19 (Ward and Weisz, 1980, Weisz and Ward, 1980, Ward and Weisz, 1984). Because the prenatal testosterone surge is the primary, though not exclusive, mechanism responsible for sexual differentiation (see McCarthy and Arnold, 2011 for a recent review), the altered hormonal profile of PS males may explain why they fail to display a set of behaviors that are completely consistent with those of No PS males or females.

In contrast to these well-documented effects in males, the influence of PS on females remains unclear. Some reports note no differences between PS and No PS females in any aspect of reproduction or maternal care (Ward, 1974, Beckhardt and Ward, 1983). Conversely, Herrenkohl (1979) observed profound abnormalities in PS females, including irregular cycling, a decreased rate of pregnancy, failure to maintain pregnancy, a prolonged gestation period, and only a 50% litter survival rate (compared to 100% for No PS females) by postnatal day 10. A follow-up report by Herrenkohl and Scott (1984) confirmed that PS induces irregularities in estrous cycling, but not in sexual activity. More recent findings suggest that PS can produce subtle changes in reproductive behaviors, but only under certain conditions. Relative to No PS controls, females with a history of PS displayed fewer signs of sexual receptivity and proceptivity when they were randomly cycling, but not following ovariectomy (Frye and Orecki, 2002b). In addition, when they were exposed to mild stress before a mating opportunity,

hormone-primed PS females spaced out mounts and ejaculations more than No PS controls (Frye and Orecki, 2002a). In summation, results suggest that the link between PS and disrupted sexual behavior is most profound in males. In females, effects of PS are more subtle or completely non-existent.

2. Rat neural development

In the following sections I will summarize some other well-documented consequences of PS. First, however, I will provide a brief discussion of the primary developmental events that occur in utero, with an emphasis on neurodevelopmental stages that overlap with repeated stress in traditional PS models.

2.1 Embryonic period

The embryonic period of rat development extends from the time of fertilization (gestational day [GD] 0) through the completion of organogenesis (GD 15; Rice and Barone, 2000). Neurulation, or preliminary development of the neural tube, begins on GD 8 or 9 and extends through GD 11 (Rice and Barone, 2000). After neurulation, the brain is comprised of a single ventricle that is surrounded by a layer (the neuroepithelium) of proliferating, or dividing, cells (Bayer et al., 1993). Initially, neuroepithelial cells undergo many symmetric divisions, each of which generates two identical daughter cells with the same fate (Gotz and Huttner, 2005, Farkas and Huttner, 2008). Eventually, cells also undergo asymmetric divisions, in which one daughter cell is self-renewing and identical to the mother cell, while the other is a differentiated cell type that cannot self-renew. Together, symmetric and asymmetric divisions are the primary mediators of brain growth during this period; the latter, however, creates differentiated cells (neurons) that subsequently populate areas outside of the neuroepithelium.

Migration, or the movement of a neuron from the neuroepithelium to its final destination, begins as early as GD 11 (Bayer et al., 1993). Though much is unknown about how a cell's fate gets determined, it is clear that migration occurs in a temporally and spatially organized fashion (Bayer et al., 1993). This is illustrated in sections 2.3 and 2.4, in which development of the striatum is

described in detail. Links between this area, PS, and emotionality make the striatum a convenient region to explore this and other (see below) developmental processes. Consequently, Figure 1.1 provides a timetable of striatal neurogenesis, showing how the timing of neurogenesis here compares to that of other critical regions. For now, it is sufficient to note that most neurons are born between GD 13 and the end of the fetal period, with only a small percentage of neurogenesis extending into postnatal life.

2.2 Fetal period

Overlapping with or shortly following neuronal migration are the processes of differentiation and synaptogenesis. Differentiation refers to the process by which a cell expresses a terminal phenotype, as determined by intracellular and extracellular cues (Rice and Barone, 2000). Thus, differentiation is responsible for generating cells of varying characteristics, including size, shape, polarity, and receptor content. Differentiation may begin shortly after the cell becomes postmitotic and generally continues once it has reached its final destination. Following migration, cells begin to form points of contact with other cells, ultimately providing the means for extracellular communication. This process (synaptogenesis) is often stimulated by the secretion of factors that guide axons to their appropriate destinations (see Waites et al., 2005 for review). Although primarily associated with early-life development, synaptogenesis does not end in the perinatal period. Rather, the processes of synapse formation and elimination ("pruning") continue in adult animals, although to a much lesser extent than is observed in the fetal and early postnatal periods. Indeed, most synapses of the rat form and mature during the first month of postnatal life (Rice and Barone, 2000). Nevertheless, synaptic plasticity is a life-long phenomenon that is thought to make critical contributions to learning and memory (Waites et al., 2005).

Programmed cell death (PCD) is much like the synaptic pruning process mentioned above – it is primarily associated with perinatal development, though it spans from the time of embryo implantation (Pampfer and Donnay, 1999) into old age. In rats, PCD is most evident during the first 2-3 weeks of postnatal life, when the rate of synaptogenesis is also at its peak (see Kim and Sun, 2011 for a

recent review). And despite the “programmed” nature of PCD, interactions with environmental stimuli before and after this period also play a role in determining which neurons survive. Environmental enrichment and chronic stress are just two examples of postnatal conditions that promote and inhibit cell survival, respectively (Kim and Sun, 2011).

2.3 Striatal development – dorsal component

The mammalian dorsal striatum (caudate-putamen complex [CPu]) is comprised of patches (striosomes) and the matrix (Fishell and Van der Kooy, 1987). Patch and matrix compartments differ at several levels, including connections, neurochemical and enzyme production, and receptor expression profiles. As an example, patches are often defined by experimentally labeling dense clusters of mu opioid receptors (Murrin and Ferrer, 1984, Van der Kooy, 1984). In addition, the patch and matrix compartments also development according to different timelines. The earliest cells to become postmitotic and leave the proliferative zone (primarily GD 13-15) preferentially make up patches, while a second wave of migrating cells (GD 18-20) surround the patches (creating a “patchy” appearance) to form the matrix (Van der Kooy and Fishell, 1987). Patches receive DAergic input (beginning GD 14) from and subsequently project to (GD 17) the substantia nigra (SN) pars compacta several days before comparable connections are established between the matrix and the SN pars reticulata (Gerfen, 1984, 1985, Fishell and Van der Kooy, 1987, Van der Kooy and Fishell, 1987).

2.4 Striatal development – ventral component

The ventral portion of the striatum (ventral striatum) is made up of the nucleus accumbens (NAc) and olfactory tubercle. It is the NAc in particular that has garnered much attention in recent years, due in part to links with human psychiatric disorders. Based on its unique blend of connections with limbic and motor regions, the NAc is in a prime position to guide motivated behaviors. Connectivity patterns and histochemical markers have been used to identify 3 subregions of the NAc: the core, shell, and rostral pole (Zahm and Brog, 1992).

As the name implies, the rostral pole represents the rostral fourth of the

NAc. Caudal to this area, shell and core subregions become apparent. The core lies just ventral to the CPu and makes up the central and dorsal regions of the NAc. Like the CPu, the core is comprised of patch and matrix compartments. The core receives DAergic inputs from the SN pars compacta and the ventral tegmental area (VTA) and, as is true of the CPu, patch and matrix compartments project to the SN pars compacta and SN pars reticulata, respectively (Groenewegen et al., 1999). Making up the medial, ventral, and lateral portions of the NAc is the shell region. The shell also receives DAergic input from the VTA, while projecting to mesencephalic sites that include the retrorubal area, the VTA, and the SN pars compacta.

Interestingly, the NAc does not develop along a timeline that is subregion specific. Instead, development follows ventral to dorsal and lateral to medial gradients that do not strictly adhere to standard NAc divisions. Therefore, the anatomical nomenclature of Bayer (1981) will be used for the following discussion. Five zones within the NAc exhibit different peaks of neurogenic activity (Bayer, 1981) with the ventral lateral region developing the earliest. This is followed in sequence by development of the ventral intermediate, ventral medial, dorsal lateral, and dorsal medial areas. Not surprisingly, the earliest detectable NAc cells (around GD 14) appear primarily in the ventral-most (ventral lateral) region, although these represent a very small proportion of NAc cells overall (Bayer, 1981). Most NAc cells originate between GD 17 and the time of birth, when the ventral medial, dorsal lateral, and dorsal medial zones show peak rates of cellular birth. The remainder of early-life neurogenesis (about 10% of the dorsal medial area and less than 5% for each of the others) occurs between birth and the end of PD 3 (Bayer, 1981).

2.5 Striatal development – dopamine receptors

The dopamine D1 receptor is present in striatal regions as early as PD 1, at 10-20% of adult levels (Murrin and Zeng, 1990, Rao et al., 1991). Receptors are largely restricted to the patch compartment during the first week of postnatal life, and most (Giorgi et al., 1987, Zeng et al., 1988, Gelbard et al., 1989, Murrin and Zeng, 1989, Murrin and Zeng, 1990, Rao et al., 1991) but not all (Broaddus

and Bennett, 1990) reports indicate that expression increases on a linear scale during this time. After week 1, the compartmental difference begins to dissipate such that no distinction can be made by the end of week 2 (Rao et al., 1991).

Development of D1 is most rapid in the CPu, moving in a ventrolateral to dorsomedial direction (Murrin and Zeng, 1989). Overall, D1 expression increases at a near-linear rate for 3-4 weeks after birth (Murrin and Zeng, 1990, Rao et al., 1991). Expression peaks around 40 days of age, followed by a decline until PD 80 (Teicher et al., 1995, Andersen et al., 1997, Andersen and Teicher, 2000). Receptor density then returns to day 40 levels around PD 100 in the NAc, while remaining stable in the CPu (Teicher et al., 1995, Andersen et al., 1997, Andersen and Teicher, 2000). Interestingly, D1 density after PD 40 is more stable in females than in males, especially in the CPu (Andersen et al., 1997, Andersen and Teicher, 2000). Here, females show no evidence of the age-related changes in density after PD 40 that is seen in males. Although the mechanisms responsible for the sex difference are poorly understood, it is clearly not a consequence of gonadal hormone surges during the periadolescent period (Andersen et al., 2002).

D2 receptor development proceeds in a temporally and regionally different pattern from that of the D1 receptor. Unfortunately, reports on perinatal expression are predominately with males, with the exact timing of development being controversial. Most likely, discrepancies are due to differences in the specificity of probes that have been used in autoradiographic studies. Using a highly specific probe ([^{125}I]iodosulpiride), Sales et al. (1989) observed D2 receptor expression in the striatum between GD 15 and 17. A less sensitive probe ([^{3}H]spiperone) did not identify D2 receptors in the striatum until GD 18, with a robust increase occurring after PD 2 (Bruinink et al., 1983). Other reports restricted to postnatal life indicate that D2 is present at low levels (less than 10% of adult levels) on PD 1 (Murrin et al., 1985, Rao et al., 1991). Taken together, it is likely that development originates during the late fetal period, albeit at a very low density. A sex-dependent (male > female) linear or near-linear increase in density then occurs in the CPu and NAc during the first 3-4 weeks of postnatal

life, such that D2 expression reaches adult levels within 1 month of birth (Bruinink et al., 1983, Murrin et al., 1985, Murrin and Zeng, 1986, Gelbard et al., 1989, Broaddus and Bennett, 1990, Rao et al., 1991, Andersen et al., 1997). Unlike D1, D2 expression stabilizes after PD 40 in both sexes (Teicher et al., 1995, Andersen et al., 1997, Andersen and Teicher, 2000). D2 development also lags behind that of D1, with D2 density never reaching that of D1 (Zeng et al., 1988, Broaddus and Bennett, 1990, Rao et al., 1991, Teicher et al., 1995, Andersen et al., 1997, Andersen and Teicher, 2000). Finally, and also unlike the D1 receptor, early postnatal D2 expression is almost exclusively restricted to the matrix compartment (Rao et al., 1991). A profound lateral-to-medial gradient is evident, with the highest density being observed in the lateral region. Although medial density does increase with age, the regional gradient persists into adulthood (Rao et al., 1991, Teicher et al., 1995).

To sum, striatal development begins late in the second week of prenatal life and continues in a highly organized fashion into the early postnatal period. DAergic inputs from and reciprocal connections to midbrain centers develop according to region-specific timelines. DA receptors first appear around the time of birth and subsequently increase at a rapid rate in the first weeks of postnatal life. Receptor expression then undergoes age-dependent fluctuations that are sex and receptor subtype specific.

Though not yet established empirically, the overlap in timing of maternal stress and striatal development may program PS rats to be hypersensitive to drugs of abuse, whose rewarding and addictive properties are largely reliant on striatal DAergic inputs. This will be discussed in more detail in section 4. Before turning to drugs, however, a brief review of the adaptive, biological stress response is necessary, given that impairments in stress regulation are among the most well-documented effects of PS.

3. Stress Regulation

3.1 The hypothalamic-pituitary-adrenal (HPA) axis

The hypothalamic-pituitary-adrenal (HPA), or stress, axis is a negative feedback circuit that is activated in response to stress and diurnal cues. Figure

1.2 offers a schematic portrayal of the biological factors implicated in its activation and regulation. Briefly, activation is initialized with the co-release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP; Whitnall et al., 1985) from the paraventricular nucleus of the hypothalamus (PVN). CRH and AVP act at the anterior pituitary to stimulate the secretion of adrenocorticotropic hormone (ACTH) and the precursor to ACTH, proopiomelanocortin (POMC; Vale et al., 1983, Rivier and Plotsky, 1986). ACTH acts at G-protein-coupled receptors in the adrenal cortex to promote the biosynthesis and release of glucocorticoids (principally cortisol in human and nonhuman primates and corticosterone in rodents) into the bloodstream (Normand et al., 1980, Dallman et al., 1987). Glucocorticoids pass the blood-brain barrier (McEwen et al., 1968) and bind to relatively low and high affinity glucocorticoid (GR) and mineralcorticoid (MR) receptors, respectively (De Kloet et al., 1975, Reul and De Kloet, 1985).

MRs, also known as type I receptors, bind corticosterone with an affinity that is 10 times greater than that of GRs, or type II receptors (Reul and De Kloet, 1985). MRs are preferentially occupied under basal (i.e., stress free) conditions, when glucocorticoid concentrations are relatively low. Both receptors become increasingly occupied, however, during times of stress (Reul and De Kloet, 1985, De Kloet, 1991). Activation of corticoid receptors via negative feedback in the hypothalamus (Han et al., 2005) and pituitary (Krozowski and Funder, 1981, Ozawa et al., 1999) offers a means for controlling glucocorticoid output by dampening the secretion of CRH/AVP and ACTH, respectively. Additional GRs and MRs can be found in the hippocampus, amygdala, septum, medial prefrontal cortex, and the posterior division of the paraventricular thalamus (Jaferi et al., 2003, Jaferi and Bhatnagar, 2006).

Glucocorticoids are able to exert their effects via two distinct mechanisms. First, they may act as transcription factors by penetrating the cell membrane and binding to GRs and MRs in the cytosol. Once bound, the hormone and receptor form a complex that is able to enter the cell nucleus. Following nuclear penetration, the complex binds to hormone responsive elements of DNA to

influence genomic events by facilitating or inhibiting gene transcription (Joels and De Kloet, 1994). Because of the cascade of events that follow glucocorticoid-receptor binding, genomic effects are slower, but produce longer-lasting changes, than the second ("non-genomic") mechanism of HPA negative feedback. Non-genomic effects occur rapidly (within seconds) and are mediated mostly by receptors bound to the cell membrane (Evanson et al., 2010). Comparatively, much less is known about this process Nevertheless, genomic and non-genomic effects are both thought to participate in the regulation of glucocorticoid output, allowing changes to occur over a range of time dimensions.

3.2 Ontogeny of corticoid receptors

Almost all reports on the development of corticoid receptors have been restricted to the hippocampus, with the earliest studies preceding the distinction between MRs and GRs. Thus, the following discussion will also be confined to the hippocampus, with a review of work that has followed the dissociation of MRs and GRs (Reul and De Kloet, 1985, 1986). Utilized methods include autoradiography (Coirini et al., 1985, Rosenfeld et al., 1988a, Sarrieau et al., 1988, Rosenfeld et al., 1990), in situ hybridization (Van Eekelen et al., 1991, Bohn et al., 1994), receptor protein quantification (Galeeva et al., 2006, Galeeva et al., 2010), and immunohistochemistry (Rosenfeld et al., 1988c, Galeeva et al., 2006).

Analyses of MR and GR mRNA levels (in situ hybridization) suggest that both receptors are present at comparable levels late in gestation (Bohn et al., 1994), offering support for a previous finding of prenatal corticoid receptor expression (Meaney et al., 1985). At PD 1, mRNA is 25% of PD 60 levels, with MR mRNA 2-3 times greater than that of GR throughout development (Bohn et al., 1994). No sex difference in receptor mRNA is exhibited until PD 60, when females express 30% more GR than males (Bohn et al., 1994). These findings are largely consistent with those from receptor binding assays (autoradiography), which have provided a more detailed developmental timeline. MR binding is low (Sarrieau et al., 1988) or undetectable (Rosenfeld et al., 1988a) on the day of

birth, but reaches adult levels by PD 8 (although see Coirini et al., 1985 for a slightly more delayed development pattern). GR binding is detectable at birth (about 30% of adult values) and reaches adult levels within 2-3 weeks (Rosenfeld et al., 1988a). Thus, autoradiography results suggest that postnatal hippocampal GR development lags slightly behind that of MRs, but still reaches adult levels before adolescence.

Results from immunohistochemistry studies suggest that there are profound differences between subregions of the hippocampus, although most are reasonably consistent with mRNA and receptor bindings studies (Rosenfeld et al., 1988b, Lawson et al., 1991, Galeeva et al., 2006). Interestingly, using western blotting (quantifying receptor protein levels), Galeeva et al. (2010) report a developmental timeline for the MR (but not GR) that is not consistent with any other report. The MR protein was not detected until PD 9, at a much lower level than that of the GR. From there, expression increased at a linear to near-linear rate through PD 90 (Galeeva et al., 2010).

In sum, there are mostly minor discrepancies in the literature on the first appearance and subsequent developmental trajectory of MRs and GRs. Inconsistencies are most likely attributable to differences in (1) methodological approach and/or (2) regional sampling. Nevertheless, it seems clear that both receptors are present in perinatal life. Expression of both increases with age, with the slope and peak depending on hippocampal location. Lastly, while MRs are predominately found in hippocampal regions, the distribution of GRs is much more widespread (Coirini et al., 1985, Reul and De Kloet, 1985, 1986).

3.3 The influence of PS on HPA axis activity

A history of PS increases the diurnal concentration of corticosterone in rats of both sexes that are tested under basal conditions. Specifically, PS heightens the naturally-occurring rise in corticosterone during the hours preceding darkness (Koehl et al., 1999). Circadian rhythmicity may explain why a comparable effect of PS in males (Henry et al., 1994, Maccari et al., 1995, Barbazanges et al., 1996, Vallee et al., 1997) and females (Bhatnagar et al., 2005, Richardson et al., 2006) has not been reported in studies that sampled

corticosterone at only 1 time point.

Rhythmicity may also contribute to mixed reports on corticosterone output after acute stress. Most have observed no effect of PS in adult males (Henry et al., 1994, Maccari et al., 1995, Barbazanges et al., 1996, Vallee et al., 1997) or females (Bhatnagar et al., 2005, Richardson et al., 2006). Henry et al. (1994) report that PS does facilitate stress-induced corticosterone levels in neonate and weanling, but not adult, male rats, suggesting that PS effects on acute stress responsiveness may dissipate with age. Richardson et al. (2006) describe a PS-induced *attenuation* of footshock-induced corticosterone secretion in adult males, but not females. A blunting effect of PS is inconsistent with the existing literature (see above), and an explanation for the discrepancy is not immediately obvious, although other reports utilized restraint or exposure to novelty as acute stressors. Thus, it is possible that PS males respond to these purely psychological stressors differently than those which also entail physical discomfort (e.g., footshocks).

Athough considerably less attention has been devoted to PS and *chronic* stress, some evidence suggests that PS disrupts the adaptive changes that accompanying repeated stress exposure, and does so differently for males and females. Habituation, or a diminished stress response after encountering the same stressor on a repeated basis, is a phenomenon that has been well documented in male rats (Grissom and Bhatnagar, 2009), and is believed to stem from an enhancement of negative feedback processes. Interestingly, PS abolishes any evidence of habituation in males (Bhatnagar et al., 2005), suggesting that PS impairs their ability to mount the appropriate adaptations to confront chronic stress. In contrast, reports of habituation in females are less clear. No PS females showed robust habituation to repeated swim stress (Szuran et al., 2000) partial habituation to repeated (6 hours/day) restraint (Bowman et al., 2001), and no habituation to repeated mild (30 minutes/day) restraint (Bhatnagar et al., 2005). Given these discrepancies, it is difficult to interpret the findings of Bhatnagar et al. (2005), which indicate that PS has no effect on stress-induced corticosterone of females that experienced the stressor

previously.

Following the cessation of stress exposure, PS rats of both sexes exhibit a delayed recovery to basal HPA axis activity, relative to sex-matched controls (Maccari et al., 1995, Barbazanges et al., 1996, Vallee et al., 1997, Bhatnagar et al., 2005, Richardson et al., 2006, Szymanska et al., 2009). Similar to PS-blocked habituation in males, this is suggestive of a deficit in negative feedback processes, possibly stemming from a reduced complement of corticoid receptors. Indeed, PS males exhibit less MR expression in the hippocampus than no PS controls (Henry et al., 1994, Maccari et al., 1995, Barbazanges et al., 1996, Koehl et al., 1999, Tamura et al., 2011), and interventions that restore MR levels to those of control animals also abolish the delay in recovery from stress (Maccari et al., 1995, Barbazanges et al., 1996). A comparable reduction of hippocampal MRs has been found in PS females, though no receptor restoration efforts have yet been documented (Koehl et al., 1999).

3.4 Prenatal and postnatal components of PS

Despite its name, PS effects are not solely attributable to events in *prenatal* life. Rather, some reflect relationships with prenatal experiences that are indirect. As an example, maternal stress reduces the subsequent frequency of pup-directed maternal licking/grooming (LG) bouts in the first weeks of the postnatal period (Champagne and Meaney, 2006, Baker et al., 2008, Del Cerro et al., 2010). As one of the core maternal behaviors displayed by postpartum rats, LG has a well-documented role in shaping the development of a neonate's nervous system. Because these effects have been reviewed elsewhere (Meaney, 2001, Cameron et al., 2005, Szyf et al., 2005, Meaney et al., 2007) and a complete recapitulation of these links is far beyond the scope of this dissertation, the following discussion is intentionally brief.

In short, naturally-occurring and experimentally-induced differences in LG frequency are highly correlated with neurobiological components of stress and anxiety regulation in the offspring (Liu et al., 1997, Caldji et al., 1998). LG increases hippocampal GR mRNA, suggesting that the difference in acute stress-induced corticosterone secretion of rats raised by high vs. low LG dams (low LG

> high LG) is a function of impaired HPA negative feedback processes in offspring of low LG mothers (Liu et al., 1997). Because low levels of LG also increase future measures of anxiety and fearfulness (Caldji et al., 1998), variation in LG behaviors appears to make substantial contributions to long-term differences in emotionality. Since maternal stress reduces the frequency of LG, and heightened emotionality is one by-product of PS, some of the differences between PS and No PS rats may be mediated by differences in maternal care.

Indeed, some deficits associated with PS can be reversed by the supplementation of LG exposure. For example, adoption by a dam without a history of stress restored hippocampal MR expression and eliminated the decrement in HPA negative feedback (Maccari et al., 1995). Stated otherwise, PS effects on HPA axis regulation were eliminated if pups were transferred to and raised by a dam that was not exposed to stress during pregnancy. Similarly, adoption reversed PS-enhanced measures of anxiety, most likely via an increase in limbic benzodiazepine receptors (Barros et al., 2006). Finally, early postnatal handling, which has enduring effects comparable to those seen in rats raised by high LG dams (Meaney et al., 1988), prevented PS from diminishing neurogenesis (Lemaire et al., 2006). Taken together, these findings indicate that some effects of PS are mediated by maternal stress-induced decrements in the quality of maternal care.

Attempts have also been made to identify whether effects of PS can be uniquely linked with events in the *prenatal* period. Because maternal stress increases fetal corticosterone levels, much focus has centered on the possible programming effects of glucocorticoid exposure (Ward and Weisz, 1980, 1984). Indeed, Barbazanges et al. (1996) linked maternal HPA activity with PS effects by first adrenalectomizing (ADXing) or sham ADXing pregnant females prior to the maternal stress regimen. ADX, which blocks the glucocorticoid increase in response to stress, prevented PS from reducing hippocampal MRs and prolonging post-stressor HPA activity. Interestingly, in rats born to sham-operated mothers, these measures were significantly predicted by the mother's stress response (i.e., stress-induced corticosterone concentration). Lastly,

Salomon et al. (2011) found that exogenous corticosterone administration alone across GD 13-21 produced PS-like effects on anxiety, but not learning and memory. Together, these data implicate fetal glucocorticoids as programmers that contribute to some, but not all, abnormalities associated with PS.

Despite efforts to dissociate prenatal and postnatal contributions to effects of PS, the relative importance of each remains unclear. Some effects, such as a reduced complement of hippocampal MRs, can be blocked by manipulations occurring in either period (see above), suggesting that events in both stages play a role in receptor development. Unfortunately, better resolving the importance of prenatal vs. postnatal life is problematic. First, because prenatal manipulations (e.g., ADX) target only one of many components of the stress response, and said manipulation may then influence maternal care in the postnatal environment, it seems unlikely that even significant diminishment of a PS effect provides evidence for a straightforward, causal relationship between maternal glucocorticoids and the effect in question. Likewise, cross-fostering PS pups to dams without a history of stress cannot rule out the possibility that fetal development in a repeatedly-stressed mother changes the behavioral dynamics of the mother-pup relationship. Finally, adoption itself (regardless of the stress history of the mother or pups) stimulates maternal LG behaviors, such that cross-fostering to a previously stressed or non-stressed mother is sufficient to abolish effects of PS. Indeed, No PS pups cross-fostered to stressed mothers were no different from littermates adopted by non-stressed mothers (Maccari et al., 1995). In sum, findings indicate that little or no effects of PS are solely attributable to prenatal or postnatal events. Instead, most effects reflect a complex and poorly understood interaction between events that occur during *both* stages of development.

4. Drug Addiction

4.1 Definition and symptomology

Drug addiction (referred to as substance dependence in the Diagnostic and Statistical Manual ([DSM] of Mental Disorders of the American Psychiatric Association) is a psychiatric condition associated with chronic substance use.

Symptomology includes the continuation and/or escalation of drug use despite negative consequences, combined with a need and/or desire to reduce consumption (APA, 2003). Stated otherwise, addicts progressively lose control over drug use and develop an increasingly compulsive-like pattern of drug pursuit. Even after prolonged periods of sobriety, the vulnerability to relapse remains at a high level (Hunt et al., 1971, Dejong, 1994). Still, it is both interesting and important to note that drug addiction is not an inevitable consequence of sustained drug use. Indeed, only an estimated 15% of regular drug users meet the criteria for drug addiction (Anthony et al., 1994). Consequently, determining what makes an individual addiction prone or resistant is an active area of research, especially at the preclinical level. As such, this will be discussed in more detail in sections 4.4 and 4.5. First, however, is a brief summary of how the transition to addiction may be coded by changes in the brain.

4.2 Mechanisms of drug addiction

It is generally agreed that the neurobiological mechanism of addiction involves drug-induced changes in brain circuits that evolved to promote the pursuit of natural rewards (e.g., food, water, sex). Because detailing the breadth of these changes is far outside the scope of this dissertation, only a brief discussion of those associated with the most prominent theories of addiction is provided below. Neither the theories nor their proposed neurobiological mechanisms represent an exhaustive review of the literature. Rather, what follows is intended to illustrate the following point: despite considerable disagreement about the *psychological* processes responsible for drug addiction, it is neuroadaptions that are thought to shift drug pursuit from a controlled pattern of intake mediated by hedonic drug effects to use that is uncontrolled and no longer solely attributable to positive reinforcement.

The reward allostasis model suggests that addiction stems from a progressive upregulation of stress system activity during periods of withdrawal. In an effort to relieve the negative mood states that are associated with withdrawal, the addict looks to resume their previous consumption of drugs

(Koob and Le Moal, 1997, 2001). Thus, negative reinforcement is the process that motivates addicts to continue drug use, and dysregulated activity of stress-responsive peptides like CRH, neuropeptide Y, and dynorphin is the neurobiological mechanism responsible for this cycle.

In contrast, the incentive-sensitization theory attributes addiction to a sensitization of reward circuits involved in incentive motivation (Robinson and Berridge, 1993, 2003). Dendritic adaptations (Robinson and Kolb, 1997, 1999a, b, Robinson et al., 2001, Robinson et al., 2002, Li et al., 2003) and a facilitation of drug-induced DAergic signaling (Robinson and Becker, 1982, Robinson et al., 1988, Paulson and Robinson, 1995) are some of many sensitization-related changes in and around the NAc that may lead to excessive incentive salience (termed “wanting”) being conferred to drugs and drug cues. According to this model, it is stimulation of the hyperresponsive “wanting” system by a drug or drug-related cue that promotes the continuation and/or escalation of drug pursuit.

Still others theorize that addiction stems from the development of stimulus-response (S-R) habits that are driven by progressive engagement of the striatum (Everitt et al., 2001, Everitt and Robbins, 2005). In short, continued drug use is thought to shift the primary neural substrates of drug pursuit from prefrontal cortical regions to the striatum, and within the striatum, from ventral (NAc) to dorsal compartments. The ventral to dorsal shift, which is consistent with progressive neural changes in cocaine self-administering monkeys (Letchworth et al., 2001, Nader et al., 2002, Porrino et al., 2004, Beveridge et al., 2006), is proposed mechanism responsible for drug pursuit becoming compulsive-like in nature.

Finally, Jentsch and Taylor (1999) suggest that drug-associated impairments in frontostriatal functioning contribute to the maladaptive decision-making that allows drug use to continue spiraling out of control. Hypodopaminergic activity in frontal cortical regions is linked with chronic phencyclidine (PCP) use and profound cognitive impairments in monkeys (Jentsch et al., 1997a, Jentsch et al., 1997b, Jentsch et al., 1999). Similar observations have been reported in human addicts, independent of drug class

(Jentsch and Taylor, 1999). In rats, a selective lesion of the medial prefrontal cortex (mPFC) promoted acquisition, as well as drug seeking in the absence of drug-associated cues, in cocaine (but not sucrose) self-administering individuals, suggesting that drug pursuit is promoted by frontal lobe dysfunction (Weissenborn et al., 1997).

To summarize, drugs of abuse progressively change structural and functional aspects of the brain. Neuroplasticity is thought to contribute to the transition from casual drug use to uncontrollable, compulsive patterns of pursuit. Addicts progressively lose control over the ability to regulate drug consumption, and this downward spiral is mediated by one or more psychological processes that, along with their respective neurobiological substrate(s), are still a source of controversy. And, as will be discussed below, not all drug users are equally likely to become addicted. First, however, it is necessary to briefly review the most common preclinical paradigms of drug abuse and addiction susceptibility, given that much of what we know about drug effects on the brain and behavior stem from work with non-human animals.

4.3 Preclinical paradigms of drug abuse and addiction vulnerability

4.3.1 Operant self-administration

Drug self-administration is a common preclinical paradigm that uniquely models the voluntary consumption of drugs that is taken to excess in addicted humans. Weeks (1962) was the first to report that experimental animals (rats) can be trained to work for drug rewards that are delivered intravenously. Since then, self-administration training has also extended to mice and monkeys, each of which will voluntarily consume almost all drugs that are abused by humans. Nevertheless, the most common and, arguably, compelling model for predicting drug vulnerability continues to be with rats. Therefore, the following discussion will be limited to work with rats, unless otherwise noted.

The rat paradigm can be divided into operant and non-operant training regimens, with the former delivering drugs intravenously and the latter allowing for drugs – usually ethanol – to be ingested orally (Sanchis-Segura and Spanagel,2006). The following sections will briefly review both approaches,

beginning with the stages of operant training.

4.3.1.1 Acquisition

Studies that test for the acquisition of self-administration assay a drug's ability to promote the learning of a novel operant response (O'Connor et al., 2011). Typically, animals are naïve to both the test drug and self-administration procedures in general. Exploratory activities over the course of the first session inevitably result in some subject-initiated contact with a response-recording mechanism (i.e., an "active" lever or hole) that, when activated, delivers the test drug and sets off a drug-paired cue. Over time (daily sessions for 1-3 weeks, typically), drugs with reinforcing efficacy are expected to produce an increase in the active response frequency, relative to one of several possible control conditions. Active responses are most commonly compared against responses made to a physically identical stimulus ("inactive" lever or hole) that has no programmed consequences associated with activation. Alternatively, comparisons can be made against animals responding for a control substance that has no reinforcing efficacy.

In recent years, acquisition studies have provided insight into preexisting conditions that promote drug use in rats, and which may do so in humans as well. Because rapid acquisition is generally defined as frequent and stable responding for a drug, differences in the rate of acquisition may shed light on factors that increase the probability of first-time drug use escalating into a potentially uncontrollable pattern of consumption.

4.3.1.2 Maintenance

Maintenance of drug self-administration refers to the regulated drug intake of subjects that are drug experienced (i.e., they have already acquired). While acquisition indicates whether or not a drug has reinforcing properties, and whether they are experienced differently by groups or individuals, maintenance studies provide an assessment of a drug's *relative* reinforcing efficacy. For example, animals may be provided concurrent access to drug and non-drug rewards and given the opportunity to choose between them. Alternatively, one may increase the response requirements for drug delivery from a standard fixed-

ratio 1 (FR1) schedule, which is the most common schedule for acquisition experiments, with each active response delivering a drug infusion, to one that is more physically demanding. Both approaches assess the incentive value of the test drug in relation to something else (e.g. sucrose, physical exhaustion). Because escalating the response requirements is the predominant technique for studying drug maintenance, and this approach was used to generate data presented in this dissertation, self-administration with a progressive ratio (PR) schedule will be the focus for the remainder of this section (see Stafford et al., 1998 for a full review on PR studies).

Briefly, a PR schedule involves a progressive, within-session increase in the number of responses needed to receive each subsequent reward. Failure to meet a response criterion within a predetermined time window terminates the test session, at which point the subject is said to have reached its "breakpoint" or "breaking point" (BP). Typically, the BP score is expressed as the largest ratio requirement completed prior to session termination (Stafford et al., 1998). BP scores can be used to rank the reinforcing efficacy of different drugs, assess the ability of drug pretreatments to change the incentive value of the test drug, and gain insight into how motivation for drugs is influenced by preexisting differences.

4.3.1.3 Reinstatement

Even the initial reports of drug self-administration (Weeks, 1962, Thompson and Schuster, 1964) indicated that the maintenance of drug-seeking behaviors was contingent upon drug availability. Testing under conditions of non-signaled drug inaccessibility ("extinction") produced a very brief increase, followed by a progressive decline, in the rate of responses made at the drug-associated lever. Continued testing under such conditions led to drug seeking being completely abolished. Interestingly, non-contingent administration of the same drug (Gerber and Stretch, Stretch et al., 1971, Stretch and Gerber, 1973), or in some cases a different drug (Wit and Stewart, 1981, 1983, Stewart, 1984, Stewart and Vezina, 1988, Wise et al., 1990, De Vries et al., 1998, De Vries et al., 2002, De Vries et al., 2003, Simmons and Self, 2009), before the start of a test session ("priming") is able to reinstate responding to a level consistent with

that of subjects provided uninterrupted drug access, even after many extinction sessions. Subsequently, stress (Shaham and Stewart, 1995, Shaham et al., 2000) and the presentation of cues previously associated with the drug have been shown to induce produce similar priming effects (McFarland and Ettenberg, 1997, Gracy et al., 2000, Weiss et al., 2000, Crombag and Shaham, 2002, Lu et al., 2004).

The enduring vulnerability to resume drug seeking behaviors, even after many extinction sessions, is strikingly similar to one of the core features of substance abuse (APA, 2003). In short, drug addicts often relapse into drug use after days, weeks, months, or even years of voluntary sobriety. As is the case with reinstatement in rats, relapse can be triggered by episodes of intense life stress and/or encounters with stimuli associated with prior drug use (e.g. syringe, pipe, drug dealer. Consequently, reinstatement is thought to model a critical aspect of drug addiction, and at the same time provide insight into factors that promote its occurrence (for thorough reviews, see Shaham et al., 2000, Shalev et al., 2002, Shaham et al., 2003, Epstein et al., 2006, Brown and Lawrence, 2009, Knackstedt and Kalivas, 2009).

4.3.2 Non-operant self-administration of ethanol

For years, researchers have modeled human patterns of alcohol use with a procedure known as the two-bottle choice paradigm. Subjects are provided concurrent access to ethanol and water in their home cages, and vulnerability to ethanol reward is indexed as the preference for ethanol (the percentage of fluid in a given time period that comes from the ethanol bottle) and/or as the dose of ethanol (g ethanol/kg body weight) consumed across a specific time period (Crabbe et al., 2010). Unlike the intake of drugs delivered intravenously, most rodents consume ethanol in moderation (Vengeliene et al., 2009, Crabbe et al., 2010). Even those that are selectively bred to show a high preference for ethanol do not typically display clear signs of intoxication during use or withdrawal across periods of sobriety (Crabbe et al., 2010). Seemingly compulsive ethanol use does not emerge until many weeks of access (Spanagel and Hölter, 1999), although it is interesting to note that providing ethanol on an

intermittent basis hastens the development of addiction-like behaviors (Hopf et al., 2010).

4.3.3 Drug-induced sensitization

Drugs of abuse increase locomotor activity when administered to rodents at low to moderate doses. At high doses, locomotor activity then transitions into stereotyped behaviors (Flagel and Robinson, 2007). Regardless of the specific behavioral parameter being measured, activity is further increased if the animal has been exposed to a drug or drugs previously (Post and Rose, 1976, Robinson and Becker, 1986). The progressive increase in drug-induced behavioral effects with subsequent drug administrations is referred to as behavioral or psychomotor sensitization. Behavioral sensitization is linked with many neurochemical adaptations in brain circuits that mediate reward processes (Kalivas and Stewart, 1991, Kalivas et al., 1993, Pierce and Kalivas, 1997, Wolf, 1998, Vanderschuren and Kalivas, 2000), and ultimately it is the sensitization of these neural circuits (neural sensitization) that is of primary interest to those in the addiction field (Robinson and Berridge, 1993, 2003). At the behavioral level, sensitization is merely a convenient estimate of the degree to which repeated drug use alters the brain. Because circuits subserving drug-induced activity overlap with those that mediate drug reward, behavioral sensitization may provide an indirect assessment of how drug reward changes with repeated drug use. As such, it has been argued that sensitization models the neurobiological and psychological changes that promote drug addiction (Robinson and Berridge, 1993, 2003).

4.3.4 Conditioned place preference (CPP)

Outside of minor modifications, the conditioned place preference (CPP) paradigm has been largely unchanged since Rossi and Reid first reported that morphine-experienced rats preferentially spent time in an environment with which the drug was previously paired (1976). Their original study and all CPP work since have utilized Pavlovian conditioning to assess the reward potential of appetitive stimuli. While many things can act as appetitive stimuli (e.g., food, water, a sexual opportunity), drugs of abuse are the traditional ones for the CPP paradigm. Often, the CPP is used to identify preexisting conditions that heighten

or dampen a drug's conditioning properties. For more information on the CPP, see one or more of the following reviews (Bardo and Bevins, 2000, Carboni and Vacca, 2003, Carlezon, 2003, Tzschentke, 2007).

Briefly, the CPP can be divided into two periods: conditioning and testing phases. The conditioning phase involves repeated pairings of the test drug, which serves as the unconditioned stimulus [US], with one distinct, environmental context that serves as the conditioned stimulus (CS). Subjects also get repeated exposure to a different environment that is paired with a control substance (e.g., saline).

After stimuli-environmental pairings have been completed, subjects enter the testing phase. On the test day, each animal receives a saline injection before receiving free access to both environments that were used in the conditioning period. This is a timed test, and preferential time allotment in the US-paired environment vs. the saline-paired environment (or vs. the preference of animals for which saline was paired with both environments) is indicative of a US with rewarding properties.

4.4 Individual differences in drug addiction

It has long been recognized that drug use alone, even when sustained for many months or years, does not necessarily result in drug addiction. Rather, susceptibility to addiction appears to be coded by individual differences in genetics, biology, and behavior. For example, DA receptor variants differentially predict opioid dependency (Chen et al., 2011) and heroin addicts' level of drug cue-induced craving (Li et al., 2006). In addition, prospective work has linked HPA axis parameters with the incidence of relapse, suggesting that individual differences in stress regulation play a role in sustaining the addiction cycle (Sinha et al., 2006, Back et al., 2010, Fatseas et al., 2011).

Individual differences can also be observed at the preclinical level. In rats, addiction-like traits have recently been linked with each of the following preexisting traits: impulsivity (Dalley et al., 2007, Belin et al., 2008, Diergaarde et al., 2008, Anker et al., 2009, Economidou et al., 2009), novelty seeking (Belin et al., 2011), and propensity to confer incentive salience to reward-related cues

(Saunders and Robinson, 2010, 2011).

Though these findings represent a mere sampling of known relationships between individual differences and addiction, and the mechanisms responsible for the associations are largely unresolved, the overall message is clear: some individuals are more vulnerable to drug addiction than others. Thus, while drug use progressively and enduringly alters the brain, neuroplastic changes are seemingly more profound and/or become more behaviorally relevant in those predisposed to become addicted. As will be explored next, a substantial literature suggests that some variability in drug vulnerability is rooted in sex differences.

4.5 Sex differences in drug abuse and addiction

Sex differences in aspects of drug abuse susceptibility have been well documented at the clinical and preclinical levels. In humans, the risk of becoming a drug addict is higher for men than for women (Brady and Randall, 1999). It is becoming increasingly recognized, however, that this difference is largely a consequence of sociocultural factors. Indeed, sex-based stereotypes are thought to make the stigma of drug use and addiction greater in women vs. men (van Olphen et al., 2009). Stigmatization may be responsible for men having more opportunities to try drugs, especially after adolescence (Van Etten and Anthony, 2001). Interestingly, women are just as likely to initiate drug use once an opportunity becomes available (Van Etten and Anthony, 2001). After initial use, women progress to drug dependence and/or encounter debilitating consequences associated with use at a faster rate ("telescoping") than men (Piazza et al., 1989a, Randall et al., 1999, Zilberman et al., 2003, Hernandez-Avila et al., 2004). Moreover, women tend to express more severe dependence when seeking treatment (Longshore et al., 1993, McCance-Katz et al., 1999, Giacomuzzi et al., 2005). Overall, once opportunities for drug use are controlled for, women seem to be more likely than men to develop drug-related behaviors that can be considered problematic.

Clinical studies have also provided some insight into the *biological* basis of these sex differences. For example, findings that the subjective hedonic effects

of psychostimulants vary across the menstrual cycle (Justice and de Wit, 1999, 2000) suggest that drugs' reward potential is modulated by fluctuations in ovarian hormones. Still, most of our knowledge about the physiological mechanisms that contribute to sex differences comes from preclinical studies. Relative to work with humans, preclinical studies are better equipped to isolate the biological determinants of sex differences from factors that could otherwise serve as confounds. Thus, the following discussion will focus on work performed with rats in a preclinical setting.

Arguably the most convincing and intuitive evidence that females are more responsive to drugs comes from self-administration studies. As a result, this will be the focus of the following section on the *presence* of sex differences. Full reviews on sex differences in all preclinical paradigms can be found elsewhere (Lynch et al., 2002, Carroll et al., 2004, Roth et al., 2004, Becker and Hu, 2008, Carroll and Anker, 2010), although the self-administration findings are generally consistent with those of other procedures.

When allowed to self-administer drugs, females consistently consume more than males. This difference has been best documented during acquisition, when females more quickly stabilize their level of intake. A sex difference in acquisition of gonadally-intact rats has been reported for cocaine (Carroll et al., 2002, Lynch, 2008), heroin (Lynch and Carroll, 1999, Carroll et al., 2002, Cicero et al., 2003), morphine (Cicero et al., 2003), nicotine (Donny et al., 2000, Rezvani et al., 2008, Lynch, 2009), methamphetamine (Roth and Carroll, 2004), and cannabinoids (Fattore et al., 2007). This pattern continues even after the acquisition period, as evidenced by BP scores on a PR schedule. Again, females consume drugs more avidly than males, seemingly independent of drug class (Donny et al., 2000, Carroll et al., 2002, Cicero et al., 2003, Roth and Carroll, 2004, Lynch, 2008, Shahbazi et al., 2008, Lynch, 2009), suggesting that the incentive motivation for drugs is sex dependent. Lastly, females more readily resume drug seeking after extinction training (Lynch and Carroll, 2000, Anker and Carroll, 2010, Fattore et al., 2010, Feltenstein et al., 2011), indicating that there may be a sex difference in the susceptibility of recovering addicts to

relapse (although see Fuchs et al., 2005).

While clearly indicating that females are more responsive than males to drug reward, and that the sex difference is evident at all stages of self-administration, these studies do not indicate the *source* of the sex difference. For insight into how these differences are mediated, researchers use one of two general approaches. First, the source of gonadal hormones may be removed via castration and ovariectomy (OVX) in males and females, respectively. In males, the influence of testicular hormones can then be tested by exogenously restoring testosterone levels in a selection of individuals. Likewise, the individual and combined effects of ovarian hormones can be assayed by providing OVXed females with estradiol, progesterone, estradiol and progesterone together, or no exogenous hormone (OVX + vehicle). Lastly, a comparison of castrated males to OVX + vehicle females indicates whether gonadal hormones are necessary for any sex difference identified in "intact" (i.e., not receiving gonadectomy [GDX]) animals. Differences between GDXed males and females provide evidence for intrinsic sex differences that are most likely attributable to early-life "organizational" (i.e., enduring and irreversible) effects of testicular hormones in males (McCarthy and Arnold, 2011). Conversely, the absence of differences suggests that the male or female-typical profile observed in intact animals is a product of gonadal hormone-mediated "activational" (i.e. relatively short term and reversible) effects.

An alternative approach is to directly or indirectly track gonadal hormone fluctuations across the testing period. Due to the relatively stable concentration of testosterone in adulthood, this approach is generally only used with females. In females, ovarian hormones fluctuate over a 4-5 day estrous cycle (shown in Figure 1.3) that is comprised of four stages: diestrus (diestrus II), proestrus, estrus, and metestrus (diestrus I). Visual inspection of vaginal cell morphology represents a common and indirect method of estimating hormonal concentrations by determining the current estrus cycle stage. Alternatively, one may directly assay either hormone via blood collection and subsequent radioimmunoassay.

GDX studies indicate that some sex differences persist even after adult

gonadal hormone concentrations have been ablated. OVXed females acquired cocaine self-administration more rapidly, voluntarily consumed a greater drug amount (Hu et al., 2004), and showed greater cocaine-induced locomotor sensitization (Hu and Becker, 2003), when compared to castrated males. Thus, at least for cocaine, sex differences in drug responsiveness are not entirely attributable to gonadal hormones being present in adulthood. Nevertheless, gonadal hormones do have profound effects on drug sensitivity, especially in females.

Administration of estradiol to GDXed females, but not males, facilitates the acquisition and maintenance of drug self-administration (Roth et al., 2002, Hu et al., 2004, Lynch and Taylor, 2005, Jackson et al., 2006, Larson et al., 2007, Hu and Becker, 2008, Kucerova et al., 2009, Zhao and Becker, 2010), the resumption of drug seeking after forced withdrawal (Larson et al., 2005, Larson and Carroll, 2007), place conditioning (Russo et al., 2003, Silverman and Koenig, 2007), and sensitization to psychostimulant locomotor-activating effects (Peris et al., 1991a, Sircar and Kim, 1999, Perrotti et al., 2001, Hu and Becker, 2003, Yang et al., 2007, Zhen et al., 2007). Thus, estrogen does potentiate parameters of drug responsiveness, but only in females.

Interestingly, the effects of progesterone are largely the opposite of those produced by estradiol (see Quinones-Jenab and Jenab, 2010 for a recent review). When given alone or in combination with estradiol, progesterone impairs the acquisition (Jackson et al., 2006, Yang et al., 2007) and escalation (Larson et al., 2007) of cocaine self-administration, the reinstatement of drug seeking after extinction training (Anker et al., 2007), and cocaine-induced CPP scores (Russo et al., 2003, Russo et al., 2008), relative to OVX females treated with vehicle or estradiol. Because an effect of progesterone has not been found in tests of drug-induced behavioral sensitization (Peris et al., 1991a, Sircar and Kim, 1999, Perrotti et al., 2001), however, its effects may be restricted to the *subjective* drug response.

Exogenous ovarian hormone administration alters the drug response in ways that are largely consistent with what is known how susceptibility fluctuates

with the estrous cycle. Relative to females in other stages, those in estrus will work harder for drug reinforcement (Roberts et al., 1989, Lynch, 2008), preferentially self-administer the largest cocaine doses available (Lynch et al., 2000), less precisely regulate their pattern of drug intake, and more readily resume drug seeking after extinction training (Kippin et al., 2005, Feltenstein and See, 2007, Kerstetter et al., 2008, Feltenstein et al., 2009. Correlational analyses indicate that estradiol and the ratio of estradiol to progesterone are positively related to motivation for cocaine (Lynch, 2008) and nicotine, respectively, even in adolescents that were not yet cycling (Lynch, 2009). In contrast, the relationship between the motivation for nicotine and progesterone alone was negative (Lynch, 2009). Likewise, the reinstatement of cocaine seeking was greatest when progesterone concentration was low (Feltenstein and See, 2007) and estrus-facilitated reinstatement was blocked when the hormone was given exogenously (Feltenstein et al., 2009). Taken together, these findings suggest that estrogen and progesterone both affect the incentive motivation for drugs, but in opposing ways.

In contrast to the rather clear hormonal effects in females, the role of androgens in the drug responsiveness of males is more suspect. Self-administration (Caine et al., 2004, Hu et al., 2004, Jackson et al., 2006) and CPP (Russo et al., 2003) studies indicate that testicular hormones have no influence on drug reward. Still, some (Robinson, 1984, Camp and Robinson, 1988) but not all (Van Haaren and Meyer, 1991, Haney et al., 1994, Chin et al., 2002, Hu and Becker, 2003, Harrod et al., 2005a, Harrod et al., 2005b), reports indicate that castration facilitates drug-induced behavioral sensitization. When castrated subjects were then provided exogenous testosterone, the hormone facilitated sensitization to cocaine in one report (Menendez-Delmestre and Segarra, 2011), but diminished sensitization to cocaine (Chen et al., 2003) and amphetamine (Forgie and Stewart, 1994) in others. Thus, testicular hormone contributions to behavioral sensitization are not entirely clear. Why testosterone restoration produced opposing effects on sensitization to cocaine is not immediately obvious, although a careful reading of Menendez-Delmestre and Segarra (2011)

indicates that there actually was not an effect of testosterone replacement. Testosterone enhanced drug-induced activity on cocaine treatment days 5 and 13, relative to day 1, but no more so than was seen in animals not administered testosterone. Consequently, their investigation may in fact be consistent with the bulk of the literature reviewed here, which suggests that testicular hormones have negligible or weakly suppressive effects on drug susceptibility.

Many of the intrinsic and activational hormone-mediated sex differences discussed here impinge on the ability of drugs to engage the mesocorticolimbic DAergic systems. Briefly, DAergic signaling in striatal areas (especially the NAc shell) has long been linked with drug reward (for reviews, see Di Chiara, 1999, Koob and Le Moal, 2001). In males, drug-enhanced striatal DA is unaffected by castration (Becker and Ramirez, 1981a, Camp and Robinson, 1988), suggesting that testicular hormones play a negligible role in the DAergic-stimulating effects of drugs. In contrast, basal (Xiao and Becker, 1994) and amphetamine-stimulated (Becker and Ramirez, 1981a, b) DA concentrations in females are diminished by OVX. Exogenous estradiol after OVX promotes drug-stimulated DAergic signaling in vitro (Becker, 1990a) and in vivo (Becker, 1990b, Peris et al., 1991b, Becker and Rudick, 1999), while exogenous progesterone facilitates amphetamine-stimulated DA signaling in females given estradiol as a pretreatment (Dluzen and Ramirez, 1987, 1990, 1991, Becker and Rudick, 1999), but not oil (Becker and Rudick, 1999). Thus, progesterone-mediated effects on drug-induced striatal DA increases require prior exposure to estradiol.

Ovarian hormones also provide the presumptive mechanism responsible for fluctuations in striatal DA that are dependent on the estrous cycle. To exemplify, estrus potentiates the amphetamine-stimulated DA increase when compared to diestrus (Becker and Ramirez, 1981b, Becker and Cha, 1989), both in vivo and in vitro. Other aspects of DAergic activity, such as receptor density (Di Paolo et al., 1988, Levesque et al., 1989) and the number of DA uptake sites (Morissette and Di Paolo, 1993), also change across the estrous cycle.

A combination of in vivo and in vitro studies have been used to directly compare DA signaling across the sexes (see Becker and Hu, 2008 for a thorough

review). When tested with in vivo voltammetry, the increase in DA signaling after cocaine treatment was higher in intact females vs. males (Walker et al., 2000, Walker et al., 2006). However, DAergic activity in females was not estrous cycle dependent (Walker et al., 2000). Findings with in vitro microdialysis indicate that the sex difference is also observed after amphetamine exposure. The difference was not apparent, however, when females were in estrus (Becker and Ramirez, 1981b). Following gonadal hormones ablation via GDX, the amphetamine-evoked DA increase is, once again, greater in females than in males (Becker and Ramirez, 1981b, Becker, 1990a).

In summation, preclinical work indicates that females are biologically more susceptible to drugs than males. Studies with GDXed rats provide suggestive evidence that early-life masculinization contributes to this effect, and its influence can be observed once gonadal hormones are removed in adulthood. When *present*, ovarian hormones have opposing effects on drug reward. Estradiol and progesterone facilitate and reduce drug properties, respectively, such that cycling females are most responsive when the ratio of estradiol to progesterone is at its peak (i.e., during estrus). Unlike hormonal effects in females, testosterone has little or no impact on the drug response on adult males. Finally, most sex differences appear related to the ability of drugs to engage DAergic inputs to areas of the striatum.

Given the consistency of sex differences observed at the preclinical level, it is somewhat surprising that men are more likely than women to develop drug addiction. Nevertheless, sex differences in humans may be a greater reflection of differences in opportunities for and/or societal expectations concerning drug use than in biology. Preclinical work has provided clear evidence that males and females are not equally susceptible to drugs, and that differences stem primarily from biologically-based differences in the brain.

4.6 Prenatal stress and drug responsiveness

Effects of PS on the abuse potential of drugs were first reported by Deminiere et al. (1992), in which males with a history of PS acquired amphetamine self-administration faster than No PS controls. Since this original

investigation, PS has also been shown to promote males' propensity to acquire the self-administration of cocaine (Thomas et al., 2009) and facilitate the reinstatement of drug seeking after extinction training (Kippin et al., 2008). Even a milder-than-typical regimen of PS promoted morphine-induced place preference scores with the CPP paradigm (Yang et al., 2006). When tested for drug-induced locomotor activity, PS males were hyperresponsive to both acute (Deminiere et al., 1992, Koehl et al., 2000, Kippin et al., 2008) and repeated (Henry et al., 1995) treatments (although see Thomas et al., 2009 for different results with cocaine). Stated otherwise, the report by Henry et al. (1995) suggests that PS facilitates behavioral sensitization to amphetamine. Lastly, recent work indicates that PS facilitates drugs-induced stimulation of neurochemicals that are implicated in reward and addiction. Perhaps most notably, PS augmented dopamine and glutamate signaling in the shell of the NAc (Kippin et al., 2008, Silvagni et al., 2008). In other regions, effects of PS on dopamine and noradrenaline concentrations have also been reported (Kippin et al., 2008, Silvagni et al., 2008, Carboni et al., 2010).

While work relating PS to drugs and drug abuse liability in males has steadily accumulated, little is known about PS effects in females. Maternal stress across gestational days 11 to 21 altered the ability of females to metabolize 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and resulted in more severe drug-induced uncoordinated activity (Morley-Fletcher et al., 2004). A comparable PS regimen enhanced the preference for alcohol in females tested with the two-bottle choice procedure (Darnaudery et al., 2007). Because no difference was observed between PS and No PS *males* in a comparable study (Van Waes et al., 2011b), PS effects on ethanol preference may be sex dependent. This remains unknown, however, because no study has included male and female PS rats in the same study. To date, only 1 published report on drug sensitivity involved explicit tests for sex differences in PS rats. Thomas et al. (2009) found that PS dose-dependently facilitated the acquisition of cocaine self-administration in males, but not females. When tested for cocaine-induced behavioral sensitization, however, PS augmented sensitization in females, but

not males. Thus, despite little direct evidence being available, PS may have effects on drug responsiveness that are sex dependent.

5. Specific Aims

The preclinical literature clearly indicates that PS facilitates drug responsiveness in males, suggesting that early-life stress in men represents a risk factor for substance abuse. Effects of PS in females, however, are less clear, despite substantial sex-dependent effects that have been reported for other aspects of brain and behavior (Weinstock, 2007, 2011). In addition, males and females without a history of PS react to drugs in profoundly different ways, as reviewed in section 4.5. Thus, the largely untested potential for effects of PS on drug abuse liability to be sex dependent represents a surprising and unfortunate gap in the literature. The overarching aim of this dissertation is to explore this uncharted territory with a rat model that includes males *and* females.

Aim 1 (Chapter II): To test whether PS alters sex-specific DAergic signaling patterns in the striatum, with the hope that doing so will improve our understanding of whether sex-dependent effects of PS on drug susceptibility (Aims III and IV) are connected to changes in sex-typical development of striatal regions.

Rationale: Sex differences in striatal DA signaling of No PS rats have been well characterized. GDXed males express a higher steady-state concentration of extracellular DA than OVXed females, but exhibit less neurochemical and stereotyped activity after amphetamine stimulation (Castner et al., 1993). Thus, intrinsic sex differences in striatal DA and DA-mediated behavior can be observed in the absence of circulating gonadal hormones. When they *are* present, ovarian (but not testicular) hormones make additional contributions to DA signaling (Xiao and Becker, 1994). In short, the activation effects of ovarian hormones combine with a sexually-dimorphic brain to make females more responsive to drugs than males. Interestingly, PS feminizes the brain and behavior of male rats, likely by disrupting fetal androgen-mediated processes of masculinization (Ward and Weisz, 1980). Thus, PS-induced facilitation of DAergic and behavioral responsiveness to drugs may stem from incomplete

masculinization of the ascending midbrain DA system. Several studies, however, have suggested that this may not be the case. Relative to sex-matched controls, PS males had elevated concentrations of DA in the NAc when sampling was done under basal conditions (Kippin et al., 2008, Silvagni et al., 2008). Given the sex differences in No PS rats that have already been described, this might suggest that PS actually *masculinizes* the striatum. The appropriate methodology to test for masculinization vs. feminization, however, is to (1) include both sexes in the same study and (2) utilize a sampling technique that can be expected to yield a sex difference in No PS controls. Consequently, here we used *quantitative* microdialysis (no net flux method) – an approach that revealed a marked sex difference in steady-state extracellular DA concentration in a previous study (see Chapter 2 for more information) – to better characterize the basal DAergic profile of PS males, while also determining whether effects of PS extend to females.

Experimental hypothesis: PS will diminish extracellular DA levels in the NAc and dorsal striatum of males, but not females.

Aim 2 (Chapter III): To test whether PS sex-dependently enhances susceptibility to cocaine with two behavioral paradigms of drug abuse liability: behavioral sensitization and the acquisition of drug self-administration.

Rationale: PS augmented the development of sensitization (Henry et al., 1995) and the acquisition of self-administration (Deminiere et al., 1992) in males tested with amphetamine. When taken together with studies using other drugs, it seems clear that PS enhances males' sensitivity to drugs, at both the neurochemical (described in Aim 1) and behavioral levels. In females, however, far less is known about the influence of PS. In rats *without* a history of PS, robust sex differences have been reported for cocaine sensitization (Van Haaren and Meyer, 1991, Hu and Becker, 2003) and self-administration, with females showing greater susceptibility than males in both paradigms (Lynch and Carroll, 1999, Hu et al., 2004, Jackson et al., 2006, Lynch, 2008). This study tested whether PS diminishes or exacerbates these differences by producing effects that are sex dependent.

Experimental hypothesis: An interaction between PS and sex will be seen with both paradigms, such that PS augments sensitization and self-administration in males, but has little or no effect in females. The traditional sex difference (female > male) is expected for rats without a history of PS.

Aim 3 (Chapter IV): Expose PS and No PS male and female rats to a prolonged regimen of cocaine self-administration to determine if sex and PS have independent and synergistic effects on the pursuit of drugs *beyond the acquisition period*, when aspects of drug seeking and taking behaviors become compulsive-like.

Rationale: A recent finding indicates that the rate at which cocaine self-administration is acquired does not predict the severity of drug seeking and taking behaviors that develop after many weeks of testing (Belin et al., 2008). Thus, the finding that PS promotes acquisition in males, but not females (Aim 2), does not necessarily imply that PS predisposes males to develop behaviors that are consistent with those of human addicts. In addition, because most self-administration studies comparing males and females have been relatively short in duration, it is unknown whether the sex difference changes after more extensive testing, when categorically different patterns of pursuit emerge for drug-vulnerable and drug-resistant males (Belin et al., 2009, Kasanetz et al., 2010). With this study, I looked to assess how PS and sex may be implicated in drug *addiction* by exploring how these factors independently and synergistically influence the development of drug-related traits that are compulsive-like, and therefore resemble behaviors seen in addicted humans (Deroche-Gamonet et al., 2004, Vanderschuren and Everitt, 2004, Belin et al., 2008, Belin et al., 2009, Kasanetz et al., 2010, Belin et al., 2011).

Experimental hypothesis: PS will have sex-specific effects on aspects of drug pursuit across the training regimen. PS will promote acquisition in males, but not females. After weeks of training, however, the effects of PS on compulsive measures of drug taking and seeking will be more profound in females vs. males. PS females, but not males, will be overrepresented in the subpopulation that pursues cocaine at the highest level of severity.

	Fetal period (GD)					Embryonic period (GD)						Birth
	11	12	13	14	15	16	17	18	19	20	21	PDs
Spinal cord												
Cerebellum												
Thalamus												
Hypothalamus												
Striatum												
Pallidum												
Amygdala												
Septum												
Neocortex												
Piriform cortex												
Entorhinal cortex												
Subiculum												
Hippocampus												

Figure 1.1 Approximate timeline of early-life neurogenesis for a selection of rat brain regions, based on and modified from the work of Bayer et al. (1993). Shaded areas indicate periods in which cells are born, beginning as early as gestational day (GD) 11 and extending beyond birth to the first postnatal days (PDs). The prenatal stress regimen used in this dissertation entails repeated daily maternal stress episodes from GD 15-21.

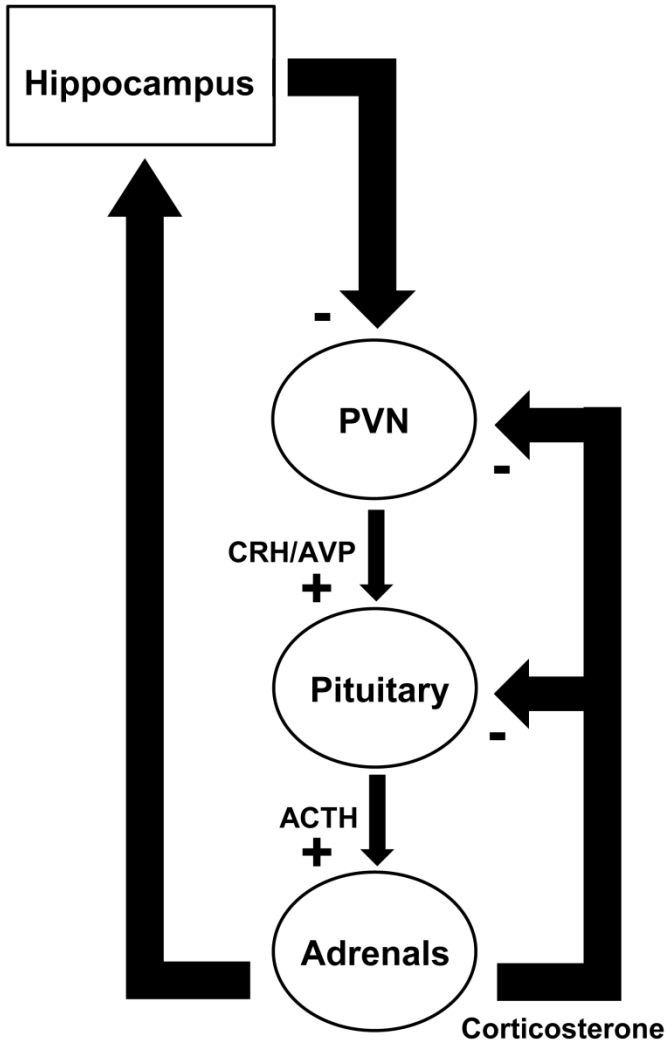

Figure 1.2 Schematic representation of the factors implicated in rat HPA axis regulation. The primary glucocorticoid stress hormone, corticosterone, regulates its own release from the adrenals via negative feedback binding sites in the brain. Activation of receptors in the PVN and pituitary decreases CRH/AVP and ACTH secretion, respectively. Additional inhibitory control over PVN CRH/AVP output is mediated by binding sites in limbic regions, such as the hippocampus.

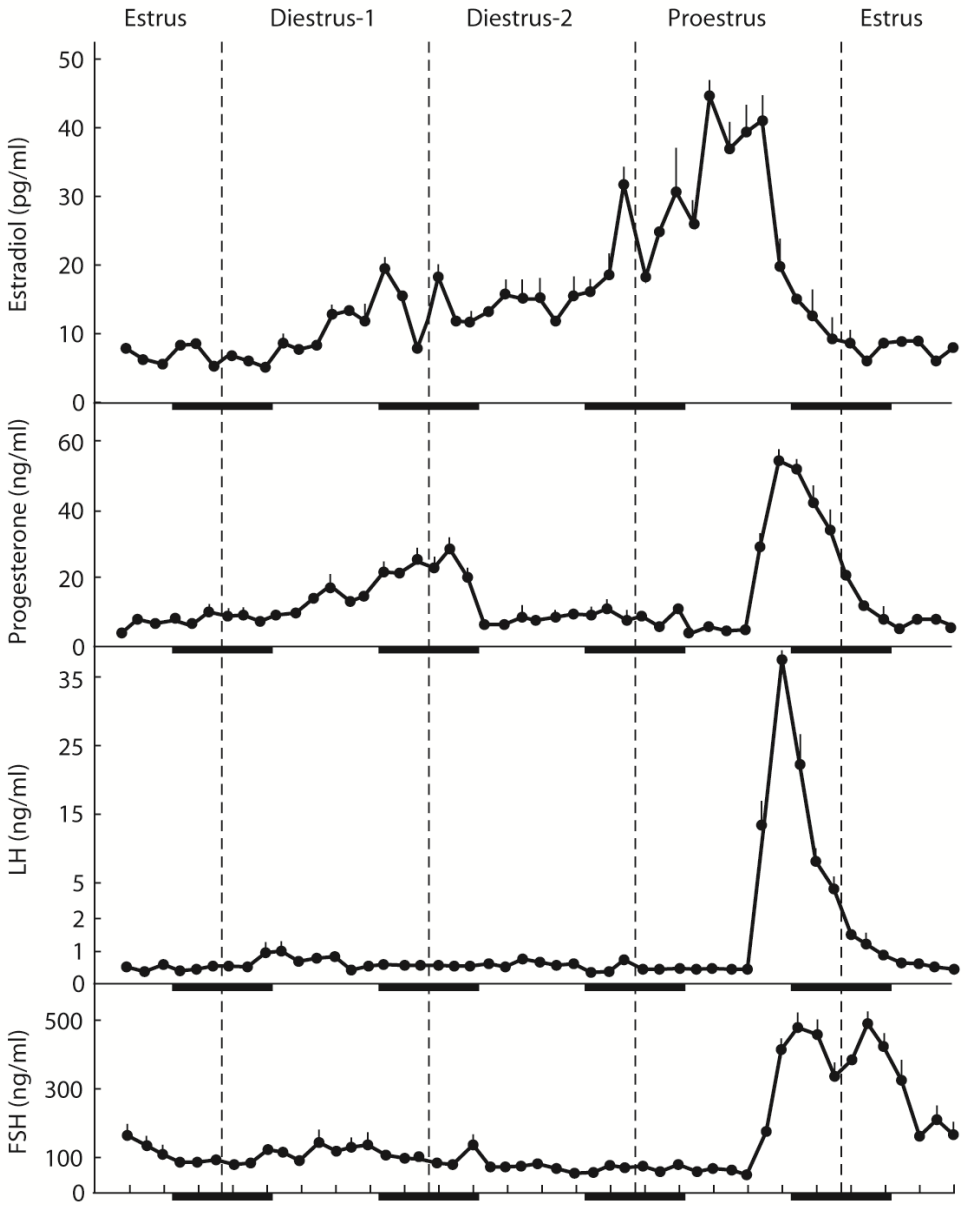

Figure 1.3 Hormonal fluctuations across the female rat's 4-day estrous cycle. Twelve-hour dark periods are indicated by black bars along the horizontal axis. Clearly, estradiol, progesterone, leutinizing hormone (LH), and follicle-stimulating hormone (FSH) peak during proestrus. The ratio of estradiol to progesterone then reaches its highest level during estrus, when rats are most responsive to drugs. This figure was obtained from Becker et al. (2008) and reprinted with permission from Oxford University Press.

CHAPTER II

SEX-SPECIFIC EFFECTS OF PRENATAL STRESS ON STEADY-STATE EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS AND DORSAL STRIATUM

Introduction

Environmental disturbances in prenatal life have long-lasting and potentially debilitating consequences for the developing young. In humans, malnourishment during this period is linked with the onset of chronic diseases later in life (Barker, 1990, Barker et al., 1990, Hales et al., 1991, Barker et al., 1993, Barker, 1995, 1998). Comparably severe effects, albeit more related to mental health, have been observed in humans and rats whose prenatal development was compromised by maternal stress (Welberg and Seckl, 2001, Maccari et al., 2003, Huizink et al., 2004, Weinstock, 2008). When administered across the final week of the rat's 3-week gestation period, maternal stress (prenatal stress [PS]) disrupts the development of male sex-typical reproductive behaviors (Ward, 1972, Masterpasqua et al., 1976), impairs regulation of hypothalamic-pituitary-adrenal axis activity (Maccari et al., 1995, Barbazanges et al., 1996, Vallee et al., 1997, Bhatnagar et al., 2005, Richardson et al., 2006, Szymanska et al., 2009), and enhances responsiveness to drugs of abuse (Deminiere et al., 1992, Henry et al., 1995, Koehl et al., 2000, Yang et al., 2006, Kippin et al., 2008, Silvagni et al., 2008, Thomas et al., 2009, Gao et al., 2011).

Because the final week of rat fetal development is a time of extreme neuroplasticity, it is tempting to speculate that these abnormalities stem from interference with neurodevelopment (Bayer, 1981, Bayer et al., 1993). To exemplify, the presumed mechanism responsible for the sex-atypical neural (Anderson et al., 1985, Fleming et al., 1992, Kerchner and Ward, 1992, Rhees et al., 1999) and behavioral (Ward, 1972, Masterpasqua et al., 1976, McGivern et

al., 1986, Ward and Stehm, 1991) development of PS males is a disruption of the testosterone surge in utero (Ward and Weisz, 1980) that masculinizes the brain and body (McCarthy and Arnold, 2011). Testosterone in PS males rises 1 day earlier, and remains elevated for a shorter duration, relative to what is seen in No PS controls (Ward and Weisz, 1980, Weisz and Ward, 1980, Ward and Weisz, 1984). While PS also alters the concentration of other hormones, such as fetal corticosterone, which increases during the application of maternal stress, it is less clear how these changes might relate to sex differences in the brain.

Just as interference with sexual differentiation is linked with incomplete masculinization, disruption of striatal development may facilitate responsiveness to drugs (Deminiere et al., 1992, Henry et al., 1995, Koehl et al., 2000, Yang et al., 2006, Kippin et al., 2008, Silvagni et al., 2008, Thomas et al., 2009, Gao et al., 2011). In short, the striatum is heavily implicated in the reward processes that promote drug abuse and addiction. Specifically, dopaminergic signaling here is critical for the initiation of drug taking, and changes over time parallel the behavioral modifications that accompany sustained use (Di Chiara, 1999, Vanderschuren and Kalivas, 2000, Koob and Le Moal, 2001, Robinson and Berridge, 2003).

As with sexual differentiation, the influence of PS on dopamine (DA)-mediated behaviors is most likely related to the overlap between maternal stress and ontogeny of the brain. In the striatum, DAergic inputs first appear after the second week of gestation and continue arriving until, approximately, the time of birth (Bayer, 1981, Fishell and Van der Kooy, 1987, Van der Kooy and Fishell, 1987). It is by this time that most cells have stopped dividing and migrated to their appropriate location (Bayer, 1981). When these highly-coordinated processes coincide with maternal stress, however, striatal integrity may be enduringly compromised. This hypothesis is supported by effects of PS that persist into adulthood, such as alterations in dendritic structure and DA receptor content (Henry et al., 1995, Berger et al., 2002, Adrover et al., 2007, Martinez-Tellez et al., 2009). PS also influences the *signaling* of DA, as indicated by elevated basal and psychostimulant-enhanced DA concentrations in the shell of

the nucleus accumbens (NAc; Kippin et al., 2008, Silvagni et al., 2008).

Nevertheless, some questions about PS and DA signaling remain unanswered. First, effects of PS on striatal DA have not been described in females, despite the considerable sex differences that have been observed in No PS controls. For example, both intact (i.e., non-gonadectomized) and castrated males express higher steady-state extracellular concentrations when tested with the no net flux [NNF] method of microdialysis than do ovariectomized (OVXed) females or intact females in diestrus (Castner et al., 1993, Xiao and Becker, 1994). When intact females transition to other stages of the estrous cycle, extracellular DA and amphetamine-stimulated DA fluctuate in a coordinated fashion (Becker and Cha, 1989, Becker, 1990a, Xiao and Becker, 1994). After OVX, estradiol has both long-term (Becker and Rudick, 1999) and rapid (Becker, 1990a, Becker, 1990b) effects when provided on a chronic and acute basis, respectively, before amphetamine stimulation. In contrast, males' striatal DA response to amphetamine is unaffected by castration or treatment with testosterone or estradiol (Becker and Ramirez, 1980, Castner et al., 1993).

Because these findings have been documented in No PS controls, however, it is unknown whether the same sex differences are evident in rats with a history of PS. Stated differently, PS may have effects that are dependent on or independent of an animal's sex. Interestingly, a report on behavioral responsiveness to cocaine suggests that PS affects the DAergic signaling of males and females in different ways (Thomas et al., 2009). To test this prediction, here we used the NNF method to compare steady-state extracellular DA concentrations in the ventral (NAc) and dorsal (caudate putamen) compartments of the striatum of PS and No PS rats of both sexes.

Briefly, the NNF method is used to determine a brain region's true extracellular concentration of a given neurotransmitter (Parsons et al., 1991). This approach involves varying the amount of transmitter that flows through the dialysis probe, using concentrations above and below expected endogenous values. Thus, there is a loss of transmitter from the probe at higher concentrations and a gain at lower concentrations. For DA, extracellular

concentrations are affected by its rate of release, reuptake, and the tortuosity of the extracellular space. When using NNF procedures under steady-state conditions, however, the primary mechanism responsible for group differences is variation in reuptake (Smith and Justice, 1994). This can be confirmed by examining the rate of DA's diffusion into and out of the probe. Diffusion away from the probe is referred to as the slope. The greater the divergence of the slope from 1.0 (i.e., the steeper the slope), the greater the rate of reuptake (Parsons et al., 1991).

Based on previously-described evidence that gonadal hormones influence DA signaling in females, but not males, only females were gonadectomized in this study. Shortly before neurochemical sampling, females were treated with 17ß-Estradiol benzoate (EB) or a control substance (oil vehicle) to test for an acute effect of estradiol; males, on the other hand, were treated exclusively with oil vehicle.

Methods

Subjects

For breeding purposes, male (275–300 g) and virgin female (200–225 g) Sprague–Dawley rats (Harlan Sprague–Dawley Indianapolis, IN) were housed under a 14/10 hour light/dark cycle (lights on at ZT0000 and off at ZT1400) in an environment maintained at 20–21 °C. Animals had *ad libitum* access to water and phytoestrogen-free rodent chow (2014 Teklad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI). All procedures were performed according to the protocol approved by the University of Michigan Committee for Use and Care of Animals.

One week after arrival, males and sexually-receptive females (based on vaginal lavage) rats were paired overnight for mating. Vaginal lavage was then performed the following morning to check for sperm. When sperm was detected (designated as gestational day 0 [GD 0]), the female was removed and individually housed. Each male was allowed to sire no more than 1 litter. A total of 3 different breeding cohorts were used to complete the study.

Beginning on GD 15, a random selection of impregnated females (n = 9)

were subjected to repeated restraint stress, which consisted of placement in a Plexiglass restrainer for 45 minutes 3 times per day (beginning at ZT0300, ZT0600, and ZT1000) through GD 21. Females not subjected to restraint (n = 10) were left undisturbed, except for weekly cage changes, throughout pregnancy. On the day of birth (postnatal day 1 [PND 1]), pups were briefly removed from the cage and weighed. Litter size was then culled to 10 pups per dam (whenever possible), with an equal or near-equal ratio of males to females. Surviving pups were placed back with the mother and left undisturbed until PND 21. On PND 21, pups were separated from their mother and housed in isosexual groups of 4-6 siblings/cage. All rats were handled at least once daily from approximately PND 33 to 50 to check for the timing of vaginal opening and prepucial separation of females and males, respectively.

To reduce the potential for "litter effects," only 1-2 males and 1-3 females from the same litter were randomly chosen to participate in the study. In addition, no more than 2 females from the same litter were assigned the same hormone treatment.

Ovariectomy (OVX)

Between PND 49 and 56, all female subjects were anesthetized by inhalation of 2.5% isoflurane and subjected to bilateral OVX using a dorsal approach (Hu and Becker, 2003). Males were also anesthetized for approximately 10 minutes in duration to control for isoflurane exposure. Because previous studies indicate that DA in dialysate is unaffected by castration (Castner et al., 1993, Xiao and Becker, 1994), however, males were not subjected to any surgical procedures.

After 7 days of recovery, all females underwent vaginal lavage testing for 10 consecutive days to confirm cessation of cycling. Males were handled to control for possible handling effects.

Cannula implantation

Between PND 68 and 78, all subjects received a buprenorphine injection (0.01 mg/kg subcutaneous [s.c.]) 30-60 minutes prior to bilateral cannula implantation surgery. Rats were then anesthetized with ketamine (40 mg/kg for

females or 55 mg/kg for males, intraperitoneal [i.p.]) and Dexdomitor (0.15 mg/kg for females or 0.2 mg/kg for males, i.p.). Guide cannulae (matching CMA/11 probes, from CMA/Microdialysis, Solna, Sweden) were inserted through the skull using standard stereotaxic techniques, aimed at the dorsal striatum (AP + 0.20 mm, ML ± 3.00 mm from Bregma, DV - 1.50 mm with 9 mm shaft guide cannulae) and contralateral NAc (AP +1.80 mm, ML ±1.50 mm from Bregma, DV -1.25 mm with 5 mm shaft guide cannulae; Paxinos and Watson, 1997). The cannulae were held in place with acrylic polymer (Lang, Wheeling, IL.) which was secured to the skull with 3-4 stainless steel jeweler screws (Small Parts, Miami Lakes, FL.). A solid stylet was placed in each cannula when not in use. Animals were allowed to recover for at least 5 days prior to microdialysis testing.

In vivo microdialysis with the NNF technique

Animals were placed into the test chamber (a clear hemispherical bowl, 12 inches in diameter, with extended sides) with continuous white noise 44-46 hours prior to neurochemical sampling. The test chamber was contained within a sound-attenuating cubicle (ENV-018M, Med Associates, St. Albans, VT). Animals had ad libitum access to food and water throughout the testing period.

After 24-36 hours of habituation to the testing environment, animals were anesthetized by inhalation of 2.5% isoflurane. Stylets were removed and microdiaysis probes were inserted (CMA11 probes from CMA/Microdialysis, Solna, Sweden; 2 mm probes for NAc and 4 mm probes for dorsal striatum) into the target brain areas through the guide cannulae. Probes were placed into the brain 16-20 hours in advance of testing to allow sufficient time for injury-related release associated with probe implantation to subside. To prevent the microdialysis probe from being subjected to torque associated with animal movement, each rat was fitted with a custom-made harness attached to a dual-channel liquid swivel (Instech Laboratories Inc., Plymouth Meeting, PA) by a flexible stainless steel cable.

The microdialysis probes were attached to syringes mounted on syringe pumps, and a freshly-made Ringer's solution (145 mM NaCl, 2.7 mM KCl, 1 mM $MgSO_4$, 1.2 mM $CaCl_2$, 1.55 mM Na_2HPO_4, 0.445 mM NaH_2PO_4) containing 150

nM ascorbic acid (pH 7.30 at room temperature [RT]) was continuously pumped through the probe at 1.5 µl/minute during the first 30-60 minutes after probe insertion. Pumping speed was then lowered to 0.3 µl/minute until 60-90 minutes before the first sample collection, when the speed was raised to 1 µl/min. At this time the perfusate was replaced by a freshly-made Ringer's solution containing 45 nM ascorbid acid (pH 7.30 at RT).

All subjects underwent NNF testing between PND 74 and 86. Sample collection was initiated around ZT0400 after 44-46 hours of habituation to the testing environment. Each set of dialysate samples (flow rate at 1 µl/min for 8 min) was collected from the NAc and dorsal striatum concurrently. All samples were collected during a five-hour span and tested within 3 hours after collection. Dialysate was collected into vials that were mounted just above the harness assembly.

Animals were assigned to one of the following 6 groups: 1) males without a history of prenatal stress (No PS males n = 10); 2) PS males (n = 9); 3) OVX No PS females treated with oil vehicle (n = 7); 4) OVX PS females treated with oil vehicle (n = 8); 5) OVX No PS females treated with 5 µg EB (n = 6); 6) OVX PS females treated with 5 µg EB (n = 8). Both male groups received oil vehicle.

According to group assignment, oil vehicle or EB was administered systemically (s.c.), 30 minutes before the first basal sample collection. After 4-5 sets of basal samples were collected, the perfusate solution was changed in a random sequence to a freshly-made Ringer's solution with 150 nM ascorbic acid containing either 5 or 15 nM DA (pH 7.30 at RT). A 40-minute equilibrium period (pumping speed at 2 µl/minute for 15 minutes, then additional 1 µl/minute for 25 minutes) was required after switching the perfusate, and then 4-5 sets of samples were collected for each DA concentration in the perfusate. The DA concentration in perfusate was tested and found to be stable (less than 7% degradation during 2 hours of perfusion).

The DA concentration of all the dialysate samples was assayed using high performance liquid chromatography (HPLC) with electrochemical detection. The system used a reverse phase column (HR-80X3.2, 3 µm particle size, 80 mm

length; ESA biosciences, Chelmsford, MA) with a mobile phase consisting of: 75 mM NaH_2PO_4, 0.2 mM EDTA, 1.0-2.0 mM 1-octanesulfonic acid sodium salt monohydrate and 15-20% methanol (apparent pH 4.0-5.6). Flow rate through the column was set to 0.7 ml/minute. DA was quantified using a coulometric detector (Coulochem II, ESA) equipped with a high sensitivity analytical cell containing dual coulometric working electrodes (ESA model #5014B).

Histology

Three to 7 days following completion of NNF testing, animals were euthanized with a fatal overdose of Fatal-Plus solution. Brains were collected and stored in 4% paraformaldehyde overnight at a temperature of 4 ºC. Brains were then transferred to 20% sucrose in 0.1 M phosphate buffer solution and stored at 4 ºC until sectioning (60 µm) with a freezing microtome. Sections were mounted onto glass slides (Cat. #1255015, Fisher Scientific), stained with cresyl violet, and coverslipped with DPX (Sigma-Aldrich, St. Louis, MO) mounting medium. Slides were then used to determine the location of probe placements. Only data from rats with microdialysis probes located inside the target brain regions were used for analyses.

Statistical analysis

All data were analyzed with mixed model analyses of variance (ANOVAs) using IBM SPSS Statistics 19 (Armonk, NY). Sex (2 levels), prenatal stress (2 levels), and hormone (2 levels) were used as between-subject factors, and brain area was the within-subject factor (2 levels). Two separate ANOVA models were used: first, only females were used to evaluate the effects of hormone (EB vs. oil), with prenatal stress and brain area also being included to test for significant interactions. After finding that EB had no effect on any parameter of DA signaling, data from oil and EB-treated female groups were pooled to together for comparisons with male groups. Again, prenatal stress and brain area were also included as factors. Litter was used as a covariate in all analyses to test for possible “litter effects,” but then removed if it failed to reach significance (considered $p < 0.05$). Post hoc comparisons were made with the Bonferroni correction to compensate for multiple comparisons.

Results

Litter characteristics

There were no differences in the characteristics of litters derived from repeatedly stressed and non-stressed mothers. As shown in Table 2.1, PS had no effect on the timing of vaginal opening in females ($F[1, 26] = 0.05$, $p = 0.83$) or preputial separation in males ($F[1, 18] = 1.48$, $p = 0.24$).

Extracellular DA concentrations: oil vs. EB-treated females

When the perfusate contained 0 nM DA, samples collected from the dorsal striatum contained more DA than those from the NAc ($F[1, 43.54] = 25.69$; $p < 0.001$). This difference was also observed after NNF testing was completed ($F[1, 45.92] = 9.32$; $p = 0.004$). As illustrated in Figure 2.1, however, there was no overall effect of EB ($F[1, 45.92] = 0.06$; $p = 0.82$) or PS ($F[1, 45.92] = 0.09$; $p = 0.77$). In addition, no interactions reached or approached significance.

Diffusion of DA from the probe: oil vs. EB-treated females

The slope of the curve that was used to determine extracellular DA concentrations is a reflection of the rate at which DA diffuses from the probe and primarily reflects DA reuptake. Analysis of the slope indicated greater diffusion from the probe in the NAc than in the dorsal striatum ($F[1, 21.96] = 10.48$; $p = 0.004$). This difference was slightly modified by hormone pretreatment (Brain area X Hormone; $F[1, 21.96] = 3.76$; $p = 0.07$). Pairwise comparisons indicated that there was no effect of EB in either region (Figure 2.2). The difference between brain regions, however, reached significance in oil-treated subjects ($p = 0.002$) but not those administered EB.

Extracellular DA concentrations: males vs. females

Again, when the perfusate contained 0 nM DA, more DA was observed in the dorsal striatum than the NAc ($F[1, 42.71] = 48.87$; $p < 0.001$). This was independent of sex and PS, even though samples from PS rats did have less DA than those from No PS controls ($F[1, 43.09 = 9.07$; $p = 0.004$).

After the completion of NNF testing, DA concentrations were again higher in the dorsal striatum than the NAc ($F[1, 41.4] = 14.45$; $p < 0.001$). Independent of this difference, concentrations were lower in rats with a history of PS ($F[1,$

42.29] = 4.37; p = 0.04). In addition, the effect of PS was stronger in males than in females (Sex X PS; F[1, 42.29] = 6.34; p = 0.02). Post hoc comparisons confirm that PS males had significantly less extracellular DA than sex-matched No PS controls (p = 0.004). In contrast, there was no effect of PS in females. Additionally, the No PS male group had DA concentrations that were modestly higher than those of No PS females (p = 0.06). No sex difference was observed in rats with a history of PS (p = 0.11). As shown in Figure 4.3, pairwise comparisons indicate that the effect of PS in males reached significance in the NAc (p = 0.04) and the dorsal striatum (p = 0.02).

Diffusion of DA from the probe: males vs. females

The slope was higher in the NAc than in the dorsal striatum (F[1, 43.16] = 25.58; p < 0.001. PS induced a slight increase in the slope, independent of brain region (F[1, 46.3] = 3.45; p = 0.07). Pairwise comparisons indicate that after PS there was a near-significant increase in the slope in the NAc of males (p = 0.09) and the dorsal striatum of females (p = 0.09; Figure 4.4).

Discussion

We report here for the first time that PS sex-specificly decreases steady-state extracellular DA concentrations in the NAc and dorsal striatum of rats tested in adulthood. Males with a history of PS had less DA in both regions, relative to sex-matched controls. In contrast, females were unaffected by PS, with or without acute EB treatment. Consequently, PS negated the sex difference in steady-state concentrations by reducing the amount of extracellular DA observed in males.

The difference in extracellular DA concentrations in PS vs. No PS males may be related to differences in DA uptake, at least in the NAc. Indeed, PS increased the rate of DA's diffusion from the probe (i.e., the slope) in this region. No such difference was observed in the dorsal striatum. Together, these findings may provide insight into how the effects of PS on extracellular concentrations are mediated. One possibility is that DA concentrations in the NAc are related to the expression of the DA D2 autoreceptor, which helps to regulate the amount of DA in extracellular space by inhibiting presynaptic release. PS increases males'

expression of this receptor, mostly in the NAc core (Henry et al., 1995, Berger et al., 2002, Adrover et al., 2007). Only Adrover et al. (2007) observed any effect of PS in the dorsal striatum, with D2 density being elevated in the medial (but not lateral) compartment of the caudate. Thus, dialysate sampling that discriminates between subcompartments of the NAc and dorsal striatum will be needed to better resolve whether the D2 receptor does indeed play a role in sex-dependent parameters of DA signaling reported here.

An alternative mechanism may involve the dopamine transporter (DAT), which removes and recycles unbound DA from the synapse. Clinical (Lavalaye et al., 2000, Mozley et al., 2001, Staley et al., 2001) and preclinical (Rivest et al., 1995, Walker et al., 2000, Walker et al., 2006, Dluzen and McDermott, 2008) findings suggest that females have higher DAT expression than males. Because PS disrupts some parameters of masculinization, it may create a DAT phenotype that is at an intermediate level between that of No PS males and females. Although more investigation is needed for confirmation, increased DAT expression in the NAc may translate into greater DA uptake and, consequently, less DA in extracellular space.

Interestingly, extracellular concentrations of DA in females were not modulated by EB, suggesting that acute estradiol exposure by itself does not mediate fluctuations that normally correspond with the estrous cycle. A previous study with the NNF method, for example, indicated that extracellular DA was higher in estrus and proestrus than in diestrus (Xiao and Becker, 1994). Although DA is increased by acute estradiol when it is followed by drug stimulation (Becker, 1990a, Becker, 1990b) the results presented here and by Xiao and Becker (1994) suggest that steady-state extracellular concentrations are influenced by *long-term* ovarian hormone fluctuations.

To sum, evidence presented here indicates that PS negates the sex difference in steady-state striatal DA concentrations by reducing the amount of DA in the ventral (NAc) and dorsal compartments of males. At least in the NAc, this may be related to a PS-induced facilitation of uptake mechanisms. In contrast to males, females were unaffected by PS. Together, these data suggest

that maternal stress interferes with the developmental processes that generate a sexually-dimorphic pattern of striatal DA signaling. Whether the changes in males that are reported here are also implicated in PS-enhanced vulnerability to drugs is an important issue that will require more work in the future.

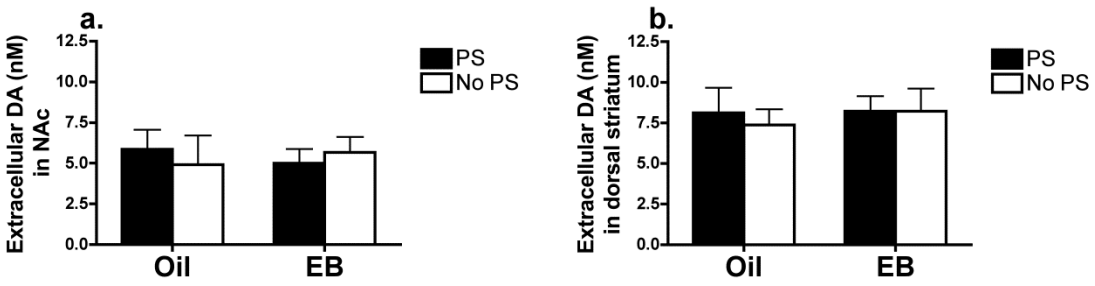

Figure 2.1 Mean (+ SEM) steady-state extracellular dopamine (DA) concentrations in the nucleus accumbens (a; NAc) and dorsal striatum (b) of females with (PS) and without (No PS) a history of prenatal stress. No effect of PS was observed in either region, regardless of whether rats were pretreated with oil or estradiol benzoate (EB).

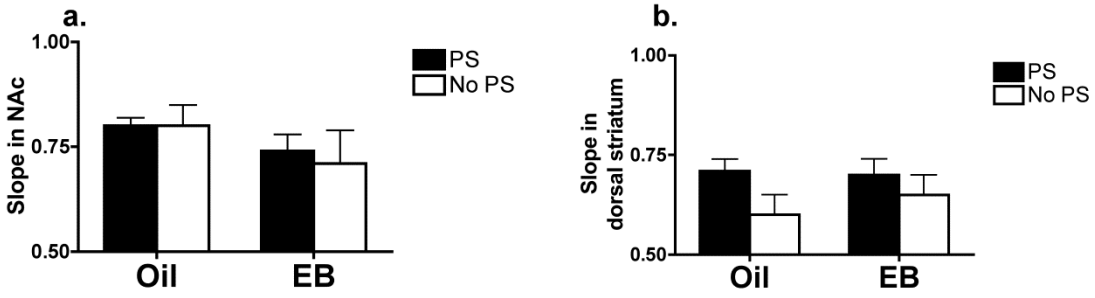

Figure 2.1 The mean (+ SEM) slope of the curve used to determine extracellular dopamine concentrations in the nucleus accumbens (a; NAc) and dorsal striatum (b) of females with (PS) and without (No PS) a history of prenatal stress. No effect of estradiol benzoate (EB) was observed when EB-treated females were compared to subjects pretreated with oil, independent of PS.

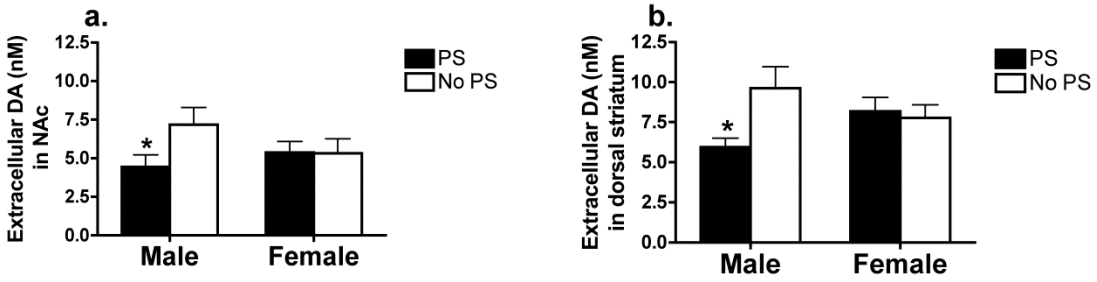

Figure 2.3 Mean (+ SEM) steady-state extracellular dopamine (DA) concentrations in male and female rats with (PS) and without (No PS) a history of prenatal stress. PS sex-specifically altered DA concentrations by reducing concentrations sampled from the nucleus accumbens (a; NAc) and dorsal striatum (b) of males (* denotes a difference between PS and No PS males; $p < 0.05$). After pooling together data from oil and estradiol benzoate-pretreated females, however, no effect of PS was found.

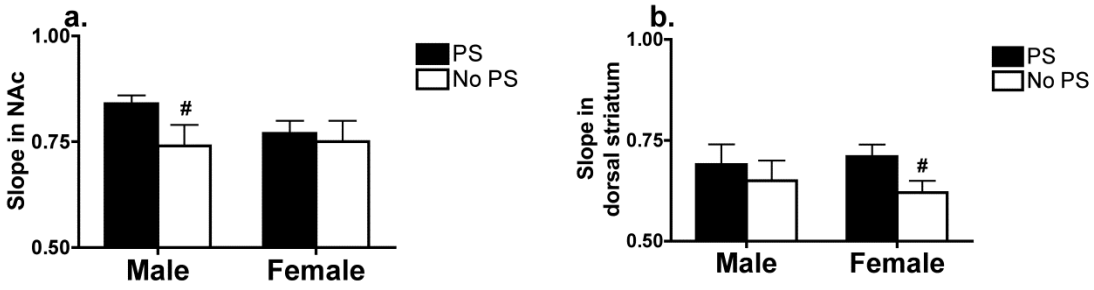

Figure 2.4 The mean (+ SEM) slope of the curve used to determine extracellular dopamine concentrations in males and females (oil and estradiol-treated subjects combined) with (PS) and without (No PS) a history of prenatal stress. PS slightly increased the slope of males and females in the the nucleus accumbens (a; NAc) and dorsal striatum (b), respectively (indicated by #; p = 0.09 for both).

	Stress	No Stress	P value
Litters	9	10	
% with > 0 naturally-occurring deaths	0	20	p = 0.47
Natural male/female ratio	1.28 ± 0.75	1.03 ± 0.33 ^	p = 0.35
Culled male/female ratio	0.91 ± 0.28	0.82 ± 0.16	p = 0.41
Natural litter size	14.78 ± 1.56	13.44 ± 3.21 ^	p = 0.28
Culled litter size	9.89 ± 0.33	8.8 ± 2.57	p = 0.23
Day 1 female size (grams)	5.77 ± 0.58	6.12 ± 0.66	p = 0.24
Day 1 male size (grams)	6.05 ± 0.52	6.37 ± 0.52	p = 0.18
Female vaginal opening day	37.33 ± 0.34	37.63 ± 0.33	p = 0.53
Male preputial separation day	43.55 ± 0.34	44.33 ± 0.56	p = 0.25

Table 2.1. A comparison of litters derived from repeatedly-stressed (Stress; n = 9) and non-stressed (No Stress; n = 10) mothers. The mean ± SEM is shown for each litter characteristic, except the percentage of litters with at least one naturally-occurring death in the perinatal period. A difference in the latter for stressed vs. non-stressed mothers was assessed with a chi-square test. All other measures were independently evaluated with ANOVAs. As indicated by the p values listed in the far right column, repeated maternal stress had no significant effect on any characteristic measured. ^ One litter was excluded from analyses because the number of dead males and females could not be determined.

CHAPTER III

SEX-SPECIFIC SUSCEPTIBILITY TO COCAINE IN RATS WITH A HISTORY OF PRENATAL STRESS

Introduction

Environmental disturbances in perinatal life can produce long-lasting physiological changes that compromise the vitality of the developing organism. In humans, maternal stress during the second trimester of pregnancy places the unborn child at greater risk for mental and physical distress (Huizink et al., 2004, Weinstock, 2008). Its most direct objective marker, low birth weight, has been linked with persistent irregularities in emotionality, sleep, cognition, and neuroendocrine functioning (Huizink et al., 2004, Weinstock, 2008). Similar maladaptations are observed in rats born to mothers that were exposed to repeated episodes of stress across the comparable stage of offspring neural development (prenatal stress [PS]; Maccari et al., 2003, Huizink et al., 2004, Weinstock, 2007, 2008). Thus, PS in rats may model an early-life factor that contributes to the onset of some diseases in humans.

In male rats, exposure to PS enhances the reinforcing and locomotor-sensitizing properties of amphetamine (Deminiere et al., 1992, Henry et al., 1995) as well as the reinstatement of cocaine-seeking after extinction training (Kippin et al., 2008). PS also augments the rewarding properties of morphine (Yang et al., 2006) and ethanol (Darnaudery et al., 2007), suggesting that early-life stress increases the abuse potential for a range of addictive drugs. Despite a well-established cross-species sex difference in sensitivity to drugs of abuse, addiction-relevant behaviors in PS models have been investigated almost exclusively with male rats (see Darnaudery et al., 2007 for an exception). As a result, it is unknown whether PS females are more vulnerable to

psychostimulants than males, as is the case for rodents and humans without a history of early-life stress (see Becker and Hu, 2008 for review).

One sex difference that has been identified in PS rats is in the ability to cope with later-life stress. Though PS males and females both exhibit a prolonged release of corticosterone (the primary glucocorticoid stress hormone following the cessation of stress in adulthood (McCormick et al., 1995), sex-specific features of corticosterone secretion have been identified both before (i.e., under basal conditions) and after acute and repeated stress regimens (Bhatnagar et al., 2005). Because glucocorticoids modulate the behavioral and neurochemical-signaling effects of drugs (Piazza and Le Moal, 1997), and drugs are themselves stressors (Goeders, 2002a, b), the sex-specific effects of PS were hypothesized to have important consequences for subsequent vulnerability to drugs of abuse. Thus, the current report offers the first between-sex comparison of gestational stress effects on drug abuse liability. PS and control male and female rats were tested for the acquisition of cocaine self-administration or the development of psychomotor sensitization to repeated cocaine injections. Based on previous reports, we hypothesized that PS would enhance sensitivity to cocaine in both paradigms, and that the magnitude of PS effects would be sex-dependent.

Methods

Subjects

For breeding purposes, male (275–300 g) and female (200–225 g) Sprague–Dawley rats (Harlan Sprague–Dawley Indianapolis, IN) were housed under a 14/10 h light/dark cycle in an environment maintained at 20–21 °C. Animals had ad libitum access to rodent chow (Purina 5001) and water. All procedures were performed in accordance to a protocol approved by the University of Michigan Use and Care of Animals Committee.

Rats were paired overnight for mating, and vaginal lavage was performed in the morning to check for sperm. When sperm was detected (designated as day 0 of pregnancy), the female was removed and individually housed. Beginning on day 15 of gestation, half of the impregnated females were subjected to repeated

restraint stress, which consisted of placement in a Plexiglass restrainer for 45 min 3 times per day (beginning at 0900, 1200, and 1600 h) until birth. Females not subjected to restraint were left undisturbed, except for weekly cage changes, throughout pregnancy. On postnatal day 2, pups were briefly (less than 2 min) removed from the cage and litters were culled to 4 males and 4 females. Surviving pups were placed back with the mother and left undisturbed until postnatal day 21. On day 21, pups were separated from their mother and housed in isosexual groups of 2–4 siblings/cage.

At 25 days of age, animals eventually used in the self-administration study with escalating doses were placed in 26.7 x 20.3 cm cages with Nalgene running wheels (9.0 cm wide and 35.4 cm diameter) to monitor circadian locomotor activity rhythms for twenty-four days. The first fourteen days animals remained on a 14/10 light/dark schedule. The following ten days animals were released into constant darkness (dark/dark) to allow free-running circadian activity. Animals were then returned to 14/10 light/dark and underwent procedures described below. The circadian data will be discussed elsewhere.

Drugs

Cocaine HCL was provided by the National Institute on Drug Abuse (Bethesda, MD) and dissolved in 0.9% sterile saline for all procedures.

Surgery

After at least a week of adjusting to reverse light/dark (14/10 h) housing conditions and a diet of phytoestrogen-free rodent chow (2014 Teklad Global 14% protein rodent maintenance diet, Harlan rat chow, Madison, WI), each rat (55–60 days old) destined for self-administration testing was implanted with an indwelling intravenous (IV) jugular catheter under anesthesia (combination of 100mg/ kg ketamine, 1.5 mg/kg xylazine, and 0.8 mg/kg acepromazine, intraperitoneal [IP] injected). Catheters were flushed with 0.2 ml heparinized saline (30 U/ml in 0.9% sterile saline) and 0.2 ml gentamicin (50 mg/ml) at the end of surgery.

Self-administration training

Seven days after catheterization, and approximately 2 hours after lights

off, animals were placed in standard operant chambers (Med Associates) and trained to self-administer cocaine at a dose of 0.2 (n = 45 rats from 7 litters; 4–6 rats/sex from each litter) or 0.3 (n = 50 rats from 15 litters; 2–3 animals/sex from each litter) mg/kg/infusion. After being connected to an infusion syringe and tethered to a swivel that allowed for free movement, rats were allowed to nose poke for IV infusions of cocaine on a fixed-ratio 1 (FR1) schedule of reinforcement. Each nose poke in the "active" hole resulted in a single infusion of 50 µl of cocaine HCL in saline, delivered over 2.8 seconds, simultaneous with a tone (85 dB) and white light illumination. For a 5 second period immediately following each infusion, nose pokes in the active hole were recorded but had no programmed consequences. All nose pokes in the "inactive" hole were recorded but had no programmed consequences. Test sessions lasted 1 hour in duration or until a total of 50 infusions was reached; each occurred daily for 5 consecutive days per week over the course of 3 weeks. Rats initially trained at the 0.2 mg/kg dose were maintained at the same dose for the duration of the experiment. This regimen was used in a previous study in this laboratory that identified sex-specific aspects of drug-taking behaviors in a rat population selected for high and low levels of novelty-induced locomotor activity (Davis et al., 2008). Conversely, rats initially trained at 0.3 mg/kg/infusion were given access to drug doses that were increased to 0.4 and 0.5 mg/kg for weeks 2 and 3, respectively — a training model used in this laboratory to successfully identify the contributions of sex and ovarian hormones to the acquisition of cocaine self-administration (Hu et al., 2004, Jackson et al., 2006). Group sizes for each training regimen are presented in the figure legends.

At the conclusion of each session, and on days in which no test session occurred, vaginal lavage was performed on all female rats; male rats were handled as a control. Vaginal cytology was used to track each female's progression through the estrous cycle. Twenty-four hours before the first weekly test session, and immediately after the fifth weekly test session, 0.1 ml of Pentothal (thiopental sodium, 20 mg/ml in sterile water) was IV infused to check for catheter patency. Rats that did not show a loss of muscle tone within 5

seconds were removed from analysis. Catheters were flushed with 0.2 ml saline before, and 0.2 ml gentamicin after, each self-administration session.

Acquisition of drug self-administration is operationally defined, typically, as stable (across $\geq$ 2 consecutive sessions) drug taking at a ratio of 2 or more infusions or active responses for every response at the inactive hole or lever. To construct an objective criterion for acquisition, the mean (± SEM) number of inactive nose pokes across the initial 5 test sessions was recorded independent of group status. For rats trained at 0.2 mg/kg/infusion, the average number of nose pokes in the inactive hole (4.59 ± 0.29) was approximately doubled to arrive at a criterion of $\geq$ 9 infusions/session (i.e., $\geq$ 1.8 mg/kg/session) for 2 consecutive sessions. Because of an increased number of inactive responses (8.53 ± 1.13) at the initial dose of 0.3 mg/kg/infusion, the criterion for these animals was set at $\geq$ 16 infusions/session (i.e., $\geq$ 4.8 mg/kg/session) for 2 consecutive sessions. At the 0.4 and 0.5 mg/kg doses, animals could reach the drug intake/session criterion by receiving 12 and 10 drug infusions, respectively. The first of the two sessions in which the cocaine intake/session criterion was reached was recorded as the day of acquisition, and animals that failed to acquire after participating in at least 10 test sessions were assigned a value coinciding with their final session number (e.g., acquisition day 15 for a rat failing to acquire after 15 test sessions). When data were unavailable for a single session – usually due to an animal becoming untethered – that missed day was not incorporated into the individual's acquisition score.

Cocaine sensitization

Animals (n = 66 rats from 9 litters; 4 animals/sex from each litter) not participating in self-administration sessions were instead tested for locomotor activity after acute and repeated injections of cocaine or saline. On 3 separate occasions prior to the first session of locomotor recording, these drug-naïve rats were removed from their cages in the animal colony room and habituated to the environment in which later testing occurred. Each was placed in a circular plastic tub (60 cm high, 50 cm in diameter) with an insert in the center that formed a corridor along the perimeter of the tub in which the animal was free to move.

After 30 min, each was given a 1 ml/kg IP injection of saline and placed back in the tub for 60 min. After this period, all were transported back to their home cages in the colony room. Litters were then evenly divided – when possible, and on a random basis – between males and females destined for repeated cocaine or saline injections. Thus, 2 rats/sex/litter were subsequently exposed to cocaine. Group sizes used for analyses are provided in figure legends.

After reaching 60–65 days of age, rats were again transferred from the animal colony room to the testing environment for the first session of locomotor recording ("session 1"). Following a 30-minute habituation period, animals were given a series of 3 IP injections, with each being separated by 60 minutes in time. Cocaine-treated rats received doses that escalated with time (5, 10, and 20 mg/kg cocaine) while saline-treated rats were administered a constant dose of 1 ml/kg saline. Rats were immediately returned to the tub after each injection, and transferred back to the animal colony room 60 minutes after the final injection. A digital camera was suspended above each tub to record behavioral activity and transmit the results to a computer for later analysis (ANY-Maze, Stoelting Co.).

These procedures were repeated in full 3 weeks later ("session 12") after the completion of 10 intervening sessions in which the following modified protocol was used without any recording of locomotor activity. Following the 30-minute habituation period, animals that received injections of cocaine during session 1 were administered only a single IP injection of cocaine (20 mg/kg); those given saline during session 1 received a single injection of saline (1 ml/kg). After the injection, each was individually placed in a tub for 60 minutes. Between sessions 3 and 4 and sessions 7 and 8, all rats were left undisturbed in their home environment for a 72-hour period. Infrequent technical complications prevented the activity of some rats from being recorded during one of the two locomotor-recording sessions; results that were obtained from these animals were excluded from all analyses. Body weights were recorded 24 hours prior to the first session of each week, and each rat was tested during the light cycle (lights on at 0730 h) at either 1000 or 1400 h.

Statistical analysis

Self-administration

Analyses of variance (ANOVAs) were conducted separately for the constant (0.2/mg/kg/infusion) and escalating-dose (0.3–0.5 mg/kg/infusion) regimens of self-administration to test for differences in cocaine intake/session/week, total drug intake across the three-week test period, session of acquisition, and the number of active and inactive nose pokes/session/week. Cocaine intake and nose pokes in the active and inactive holes per session during week 1 were also compared across training regimens (i.e., rats trained with the 0.2 mg/kg dose vs. those initially trained at the 0.3 mg/kg dose) to evaluate the effect of initial drug dose on self-administration behaviors. Finally, the Kaplan–Meier survival analysis and the Breslow–Gehan–Wilcoxon statistic were used to evaluate the effect of PS on the rate of acquisition for males and females tested under both dosing regimens.

Cocaine sensitization

The total distance traveled (with the center of the animal used as the reference point for tracking) during habituation, and after each injection, during sessions 1 and 12 of the experiment were initially divided into 10-minute bins. The peak locomotor response for each animal during all 4 segments (habituation and after each injection) during the locomotor-recording sessions was used as the primary dependent measure for analysis. Except for the 30-minute habituation period on day 1 – to test for possible pre-existing differences between groups subsequently exposed to cocaine or saline – data from saline-treated rats were entered into separate statistical models from those given cocaine. Mixed-factors ANOVA, with the within-subject factor of dose (3 levels, representing the peak response following each of the 3 injections), was used to evaluate possible differences in the acute (i.e., during session 1) response to cocaine and saline.

Repeated cocaine exposure, especially in combination with high testing doses, results in psychomotor sensitization that may be masked by the quantification of ambulation alone (Flagel and Robinson, 2007). Inspection of the dose–effect function for cocaine-treated animals indicated that locomotor sensitization was visible at the lowest doses (5 and 10 mg/kg), but not after the

20 mg/kg dose (i.e., locomotor activity was no greater during session 12 compared to session 1). Thus, we assessed locomotor sensitization by using the cumulative peak response after only the first two injections.

Locomotion often transitions into a preponderance of stereotyped head movements when rats are tested at high cocaine doses (Flagel and Robinson, 2007). Thus, we sought to capture this qualitative shift in activity after the highest dose by calculating the peak ratio of distance traveled by an animal's head (i.e., total distance traveled with the head as the reference point for tracking) to that of the animal's body. To account for the transition from ambulation to stereotypic head movements, we compared the peak head: body distance traveled during session 1 and session 12 as a secondary measure of sensitization. Both metrics of sensitization were individually entered into mixed-factors ANOVA designs, with locomotor-recording session (2 levels) as the within-subject factor and sex (2 levels) and prenatal stress (2 levels) as between-subjects factors. Significant interactions were followed up with two-way ANOVAs, with session (2 levels) as the repeated measure. Chi-square tests were used to identify the percentage of individuals in each group attaining a 10, 50, and 125% increase in head: body distance from session 1 to session 12.

To test for possible litter effects, litter was included as a covariate in ANOVAs, except when the following two conditions were both met: (1) a significant main effect or interaction was being followed up and (2) the effect of litter itself failed to reach statistical significance. For all experiments, Bonferroni post hoc comparisons were performed to compensate for multiple comparisons, and statistical significance was set at $p < 0.05$.

Results

Self-administration of 0.2 mg/kg/infusion cocaine

Males self-administered significantly more cocaine/session (Figure 3.1; $F[5, 41] = 9.38$; $p < 0.01$) and made more responses at the active hole ($F[5, 41] = 9.81$; $p < 0.01$) than females during week 2 (sessions 6–10), but not weeks 1 or 3, at the low dose of 0.2 mg/kg. When drug intake was also measured across the entire three-week period, males received more cocaine than females (F[5,

41] = 5.33; $p < 0.03$). No group differences in responses at the inactive hole were observed during any week, and no between or within-group differences were found when the drug-taking behaviors of females were compared across the stages of the reproductive cycle.

The amount of cocaine/session ($F[3, 15] = 4.76$; $p < 0.05$) and number of nose pokes in the active hole ($F[3, 15] = 5.61$; $p < 0.04$) for No PS rats was greater for males, relative to females, during week 2. The sex difference in drug intake was more modest during week 3 ($F[3, 15] = 3.23$; $p = 0.09$), with no difference in responses at the active hole. In PS rats, males received slightly more cocaine/session ($F[3, 25]= 3.76$; $p = 0.06$) and tended to make more responses at the active hole ($F[3, 25] = 3.64$; $p = 0.07$) when compared to females during week 2. No effect of prenatal stress was observed in drug intake or the number of nose pokes in the active or inactive holes.

Survival analysis indicated that prenatal stress had no effect on the rate of acquisition in males ($\chi^2 = 0.01$, df = 1, $p = 0.92$) or females ($\chi^2 = 0.47$; df = 1; $p = 0.49$), and no group differences were found in the number of sessions needed to acquire self-administration (Figure 3.2).

Self-administration of progressively increasing cocaine doses

During the first week of training (0.3 mg/kg/infusion) for rats on the escalating-doses regimen of self-administration, there was a trend for animals to receive more cocaine/session ($F[9, 87] = 3.5$; $p = 0.07$), and respond more at the active ($F[9, 86] = 3.06$; $p = 0.09$) and inactive ($F[9, 87] = 8.4$; $p < 0.01$) holes, when compared to those tested with the low dose of 0.2 mg/kg. PS animals responded more at the active hole (Prenatal Stress x Dose interaction; $F[9, 86] = 6.09$; $p < 0.02$), and self-administered slightly more drug/session, than No PS rats at the 0.3, but not 0.2, mg/kg dose (Prenatal Stress x Dose interaction; $F[9, 87] = 3.59$; $p = 0.06$). PS rats also responded slightly less at the inactive lever overall ($F[9, 87] = 3.33$; $p = 0.07$). Post hoc comparisons between groups started at different training doses indicate that PS males ($p < 0.01$) and PS females ($p < 0.001$) self-administered more cocaine during week 1 at the 0.3 mg/kg dose than those that began at the 0.2 mg/kg dose. Conversely, nosignificant effect of initial

training dose was found for No PS males or females.

In males, PS rats self-administered more cocaine/session than No PS individuals during week 3 (0.5 mg/kg dose; $F[3,12] = 5.34$; $p < 0.04$; Figure 3.3a), and tended to do so during week 1 (0.3 mg/kg dose; $F[3,19] = 3.86$; $p = 0.06$). Furthermore, PS males received more cocaine/session/week ($F[2, 11) = 6. 27$; $p < 0.03$) and total cocaine across the study ($F[3, 10] = 5.73$; $p < 0.04$) relative to sex-matched No PS controls. In contrast, no difference in drug intake was observed between PS and No PS females during any of the 3 weeks or across the entire study (Figure 3.3b). Overall, the amount of cocaine/session that was self-administered increased with each escalation in drug dose ($F[5, 34] = 4.7$; $p < 0.04$). No between or within-group differences in the drug-taking behaviors of females were observed as a function of estrous cyclicity.

As illustrated in Figure 3.4, PS males acquired self-administration faster than sex-matched No PS controls ($F[3, 18] = 4.62$; $p < 0.05$); this was not the case for females ($F[3, 24] = 0.01$; $p = 0.93$). Survival analysis (Figure 3.5) indicated that PS altered the rate of acquisition exclusively in males, ($\chi^2 = 4.62$; $df = 1$; $p < 0.04$; Figure 3.5a), such that more PS subjects (66.7%) acquired in 3 sessions or less than No PS controls (16.7%; $\chi^2 = 6.04$; $df = 1$; $p < 0.02$).

Cocaine sensitization

No preexisting differences in activity were observed between or within groups exposed to cocaine or saline during the 30-minute habituation period during session 1 of locomotor behavior recording. As expected, the distance traveled by cocaine-treated rats increased with each escalation of drug dose ($F[5, 30] = 21.11$; $p < 0.001$; Figure 3.6). The increase was more pronounced in females than males ($F[5, 30] = 6.07$; $p < 0.03$), and females given cocaine ($F[5, 30] = 8.44$; $p < 0.01$) and saline ($F[5, 25] = 4.82$; $p < 0.04$) traveled farther than identically-treated males.

The summed locomotor response after the first two injections (5 and 10 mg/kg) indicated that cocaine-treated rats were more active during session 12 compared to session 1 ($F[5, 30] = 5.25$; $p < 0.03$). Relative to males, females exhibited a greater increase in ambulation across sessions ($F[5, 30] = 13.01$; $p <$

0.01; Figure 3.7), with the steepest increase occurring in females with a history of PS (Session x Sex x PS; $F[5, 30] = 5.54$; $p < 0.03$; Figure 3.7c). PS females traveled farther overall ($F[1, 16] = 5.75$; $p < 0.03$), and showed a modestly larger increase in activity over time ($F[2, 16] = 4.22$; $p = 0.06$), than No PS controls. Alternatively, no such effect of PS on overall activity ($F[1, 15]=0.31$; $p=0.59$) or the change in locomotor activity across sessions ($F[2, 15]=1.34$; $p=0.26$) was observed in cocaine-treated males (Figure 3.7a). In PS rats, females traveled farther overall ($F[1, 15] = 12.61$; $p < 0.01$), and exhibited a greater increase from session 1 to session 12 ($F[2, 15] = 14.3$; $p < 0.01$), when compared to males. In No PS animals, females traveled farther overall ($F[1, 16] = 6.86$; $p < 0.02$), but were no different in their activity change over time, relative to males ($F[2, 16] = 1.31$; $p=0.27$). Post hoc comparisons indicated that PS ($p < 0.001$) and No PS ($p < 0.04$) females were more active during session 12 than during session 1, with a difference between the groups (PS > No PS) during session 12 ($p < 0.03$), but not session 1. In males, only the No PS group showed a near-significant increase in activity during session 12 relative to session 1 ($p = 0.05$). However, PS and No PS males were no different from each other across either test session.

In contrast to cocaine treatment, no change in locomotion from sessions 1 to 12 was observed in rats exposed to saline ($F[5, 25] = 0.95$; $p = 0.34$; Figure 3.7b, d). Females traveled farther than males overall ($F[5, 25] = 6.27$; $p < 0.02$) but exhibited no more change in activity across sessions ($F[5, 25] = 0.04$; $p = 0.84$).

PS males showed a change in ambulation over time that was different than that of females (Session x Sex x PS; $F[5, 25] = 4.3$; $p < 0.05$). In females, the PS and No PS groups were no different in their total distance traveled or their change in activity over time. Conversely, in males the activity of PS rats changed more over time (Session x PS; $F[2, 13] = 8.65$; $p < 0.02$), but was no different overall ($F[1, 13] = 0.22$; $p = 0.64$), when compared to the No PS group. Post hoc comparisons revealed no within or between-group differences in locomotor activity.

No significant change in the head: body distance from session 1 to

session 12 was observed after the final injection of cocaine (20 mg/kg) or saline. However, chi-square analysis indicated that more PS females (50%) increased their peak ratio in response to cocaine by at least 125% when compared to No PS sex-matched controls (0%; $\chi^2 = 6.43$; $p < 0.02$; Figure 3.8b). No effect of PS was observed in males (Figure 3.8a) or rats exposed to saline.

Discussion

The present investigation shows, for the first time, that after PS males and females are hyper-responsive to different properties of cocaine. Following PS the percentage of males that acquired cocaine self-administration within 3 test sessions was increased, and the average time needed to reach acquisition status was reduced, when training was initiated at a dose of 0.3 mg/kg and progressively increased across test sessions. Furthermore, PS males (but not females) self-administered more cocaine across this training regimen than sex-matched NPS groups. At a low dose of cocaine (0.2 mg/kg/infusion), PS had no effect on the acquisition of self-administration in either sex, but males overall received more drug/session than females. When tested for cocaine-induced locomotor activity, PS rats were no more responsive to the acute drug effects than NPS individuals. After repeated exposure, however, females with a history of PS were hyper-responsive to the drug's psychomotor-activating effects relative to No PS females. In contrast, no such effect of PS was observed in males. Thus, gestational stress enhanced psychomotor sensitization in females, but not males. Finally, females overall developed more robust locomotor sensitization than their male littermates. Taken together, these results suggest that sex differences interact with PS to modulate the sensitivity of rats to the reinforcing and psychomotor-sensitizing effects of cocaine.

The heightened drug-taking proclivity of males, relative to females, that we observed at the low dose (0.2 mg/kg) of cocaine was in the opposite direction of a trend within the literature and results obtained from this laboratory (Becker and Hu, 2008). As an example, 90–100 day old females acquired faster, and self-administered more cocaine/session overall, than age-matched males when tested at the 0.2 mg/kg dose in an extended-access (6-hour sessions) paradigm

(Lynch and Carroll, 1999). The shorter duration of test sessions used here (1-hour sessions) may have contributed to our discrepant results. In a previous study in this laboratory that included 1-hour sessions, ovariectomized females with and without estradiol reinstatement readily self-administered cocaine at the dose of 0.3, but not 0.2, mg/kg (pilot data; Hu et al., 2004). In contrast, males appear less sensitive to drug dose (training at 0.2 vs. 0.3 mg/kg/infusion) under these conditions (Hu et al., 2004, Jackson et al., 2006; Figs. 1 and 3a). Additional procedural differences between our study and others in the literature, such as the responses required for drug infusions (nose pokes vs. lever presses) and the age at first testing (60–65 days vs. 90 days and older), may have also contributed to our findings. Resolving the importance of these variables will undoubtedly be critical to further model the role of sex differences in the path to substance abuse.

Clearly, dose is a powerful determinant of the rate at which animals will self-administer drugs (Carroll and Lac, 1997, Goeders, 2002a). In the only other assessment of cocaine self-administration in PS rats (males), in which training doses of 0.25, 0.5, and 1mg/kg were used for separate groups, PS had no effect on the acquisition of or drug intake during self-administration at any dose (Kippin et al., 2008). Thus, PS facilitated acquisition at an initial training dose of 0.3 mg/kg/infusion (reported here), but not at slightly higher or lower doses. These data suggest that cocaine may engage reward and/or stress circuitry differently for PS and NPS males at some doses. Interestingly, the acquisition stage of cocaine self-administration is particularly sensitive to circulating corticosterone, such that basal levels predict which rats will self-administer low doses (Goeders and Guerin, 1996). Indeed, some have hypothesized that a critical corticosterone threshold must be reached for low-dose acquisition to be achieved (Goeders, 2002a). The elevated basal concentration of corticosterone found in males with a history of PS, but not females (Bhatnagar et al., 2005), may have contributed to our dose and sex-dependent results. Doses (mg/kg/infusion) within the range of 0.3 and 0.5 may consistently breach the hypothesized threshold in males previously subjected to gestational stress. Conversely, higher

and lower doses may be insufficient to differentiate the drug-taking proclivities of PS and No PS males. Of course, such an interpretation is necessarily specific to the acquisition stage, as PS augments drug taking at the 0.5 mg/kg dose in males that have already acquired (Figure 3.3a), but not for those introduced to the dose at the beginning of training (Kippin et al., 2008). Alternatively, modest procedural differences between laboratories and/or the influence of individual differences on drug-taking behaviors (Piazza et al., 1989b, Belin et al., 2008, Davis et al., 2008) may have contributed to our conflicting results.

In contrast to our findings, others have observed that the acute locomotor response of PS males to psychostimulants is greater relative to males without a history of PS (Deminiere et al., 1992, Koehl et al., 2000, Kippin et al., 2008). Indeed, combined with emerging evidence of PS-induced neuroadaptations, some of which mirror the neuroplastic events associated with drug sensitization, one might hypothesize that PS rats are pre-sensitized to drugs of abuse (Henry et al., 1995, Kippin et al., 2008, Silvagni et al., 2008). Failure to replicate such a finding with cocaine, however, together with the results obtained by Henry et al (1995) with amphetamine, casts doubt on this possibility. PS rats are more responsive to novel testing environments themselves (Deminiere et al., 1992, Kippin et al., 2008), making it difficult to parse out the level of locomotor activity attributable to the drug without extensive efforts to habituate subjects to testing conditions. Our pre-test (i.e., before session 1 of locomotor recording) habituation regimen was similar to that of (Henry et al., 1995), while others have used less rigorous habituation strategies that began on the first day of testing (Deminiere et al., 1992, Kippin et al., 2008 ; although see Koehl et al., 2008 for an exception with nicotine infused IV). PS failed to augment locomotor activity before (30-minute habituation period during session 1) or after initial drug exposure in our investigation, suggesting that pre-test conditions attenuated any hyper-responsiveness to the test setting and provided a more accurate assessment of acute cocaine sensitivity. Still, that drug-naïve PS males express neuroadaptations in limbic regions provides evidence for preexisting differences, relative to No PS controls, that may underlie their heightened vulnerability to drug

reward (Deminiere et al., 1992, Henry et al., 1995, Kippin et al., 2008).

Psychostimulant sensitization in PS rats (males only) has been assessed in a single prior study, in which sensitization to amphetamine was greatest in those with a history of PS (Henry et a., 1995). The effect appears largely attributable to a more rapid development of sensitization, suggesting that our discrepant finding may be due to the greater duration between recording sessions in our design (2 sessions separated by 10 intermittent sessions of drug exposure) compared to theirs (6 sessions, each separated by 1 drug-free day). Whether PS influences the rate of sensitization to cocaine, as opposed to the final magnitude of sensitization, reported here, remains unclear. At the very least, our findings indicate that PS furthers an already heightened vulnerability to cocaine sensitization in females (Hu and Becker, 2003). Sensitization has been linked with the ability of drugs to reorganize neural circuits that normally mediate the pursuit of natural rewards (Vanderschuren and Kalivas, 2000, Robinson and Berridge, 2003). As some of these changes are thought to underlie components of drug addiction (Robinson and Berridge, 2003), the enduring effects of gestational stress may be related to the recent, marked increase in cocaine abuse in America's adolescent and young adult female populations (reviewed in Becker and Hu, 2008). In conclusion, sensitivity to drugs can be shaped by early-life events. PS is a sex-specific risk factor for addiction-relevant behaviors in adult rats, suggesting that sub-optimal prenatal conditions contribute to the etiology of substance abuse in humans. Undoubtedly, much work at the clinical and pre-clinical levels is required to better understand the implications of our findings. Nevertheless, as some deleterious effects of PS can be attenuated by increased levels of physical stimulation (Vallee et al., 1996, Lemaire et al., 2006) and enriched social housing conditions (Yang et al., 2006), PS models may offer exciting insight into clinical strategies to prevent the abuse of drugs.

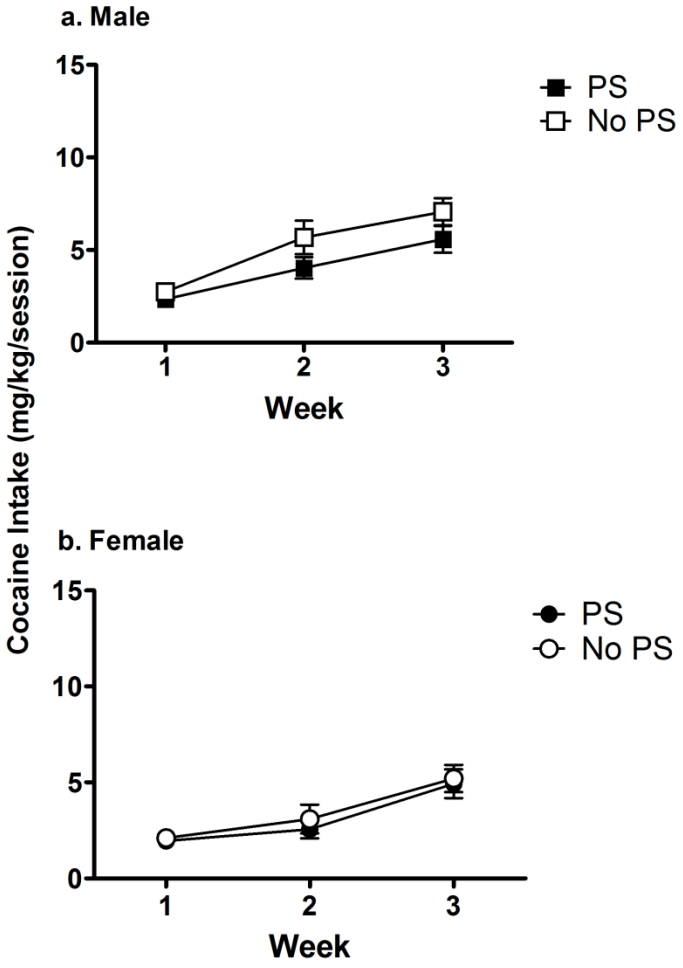

Figure 3.1 Mean (± SEM) amount of cocaine (mg/kg/session/week) self-administered at a constant low dose (0.2 mg/kg/infusion) by male (a) and female (b) rats with (PS; male n = 14; female n = 15) and without (No PS; male n = 9; female n = 9) a history of prenatal stress.

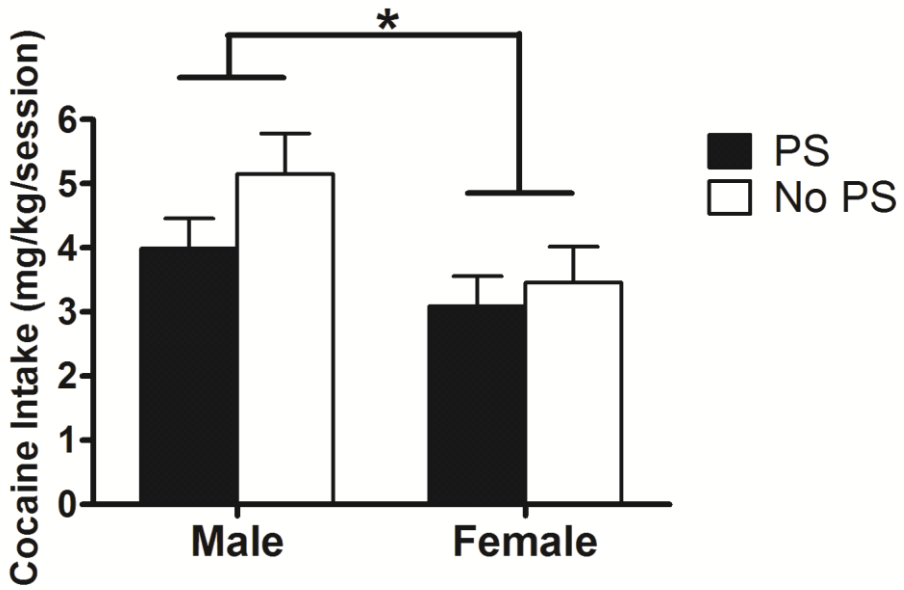

Figure 3.2 Mean (+ SEM) amount of cocaine (0.2 mg/kg dose) per session self-administered across the entire 3-week study. Males (n = 23) self-administered significantly more than females (n = 24), independent of prenatal stress (PS). * p < 0.05, difference between males and females.

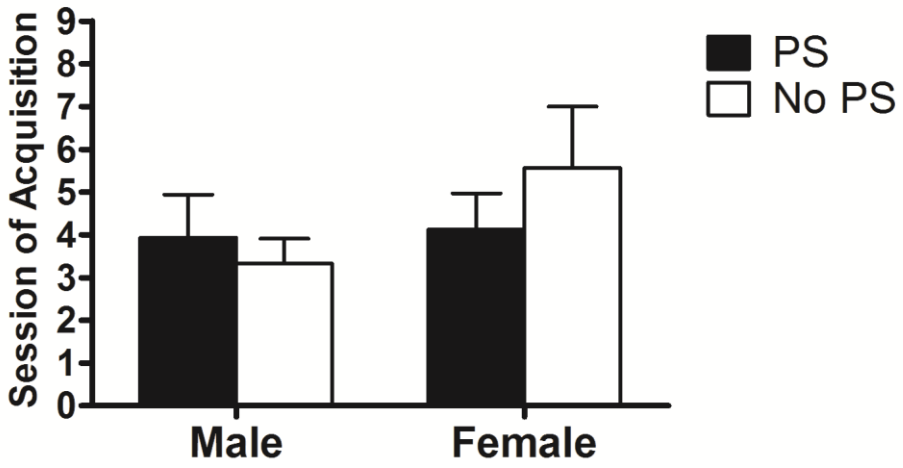

Figure 3.3 Mean (+ SEM) number of 1-hour sessions of self-administration needed for male and female rats with (PS; male n = 14; female n = 15) and without (No PS male n = 9; female n = 9) a history of prenatal stress to acquire self-administration at a constant low dose (0.2 mg/kg/infusion) of cocaine.

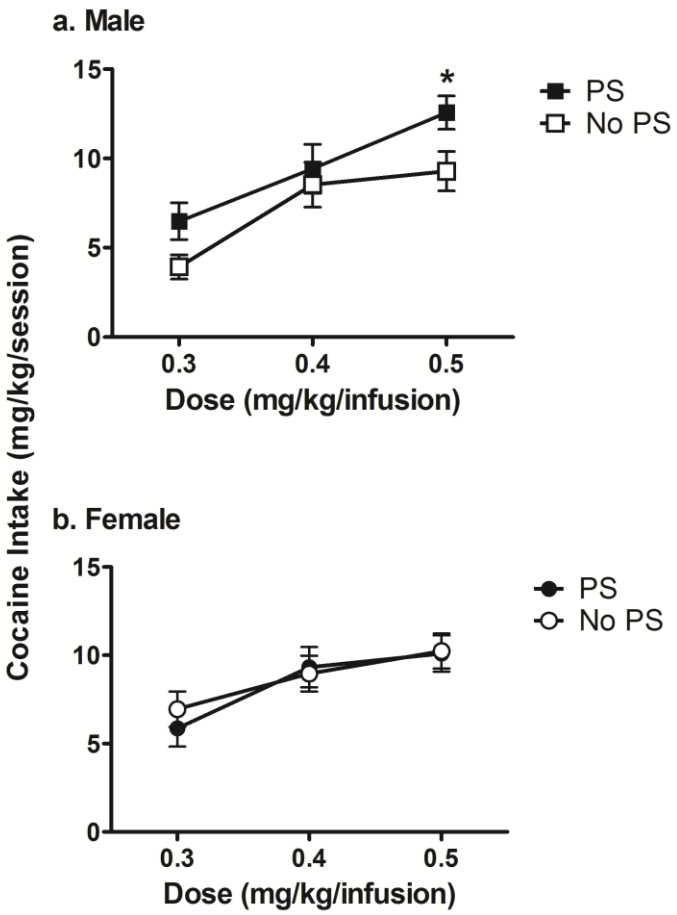

Figure 3.4 Mean (± SEM) amount of cocaine (mg/kg/session) self-administered at all three drug doses. a) Cocaine intake for males with (PS; n = 8 at 0.5 mg/kg dose) and without (No PS; n = 7 at 0.5 mg/kg dose) exposure to prenatal stress. b) Drug intake for PS (n = 13) and No PS (n = 14) females. *p < 0.05, PS male > No PS male at the 0.5 mg/kg dose.

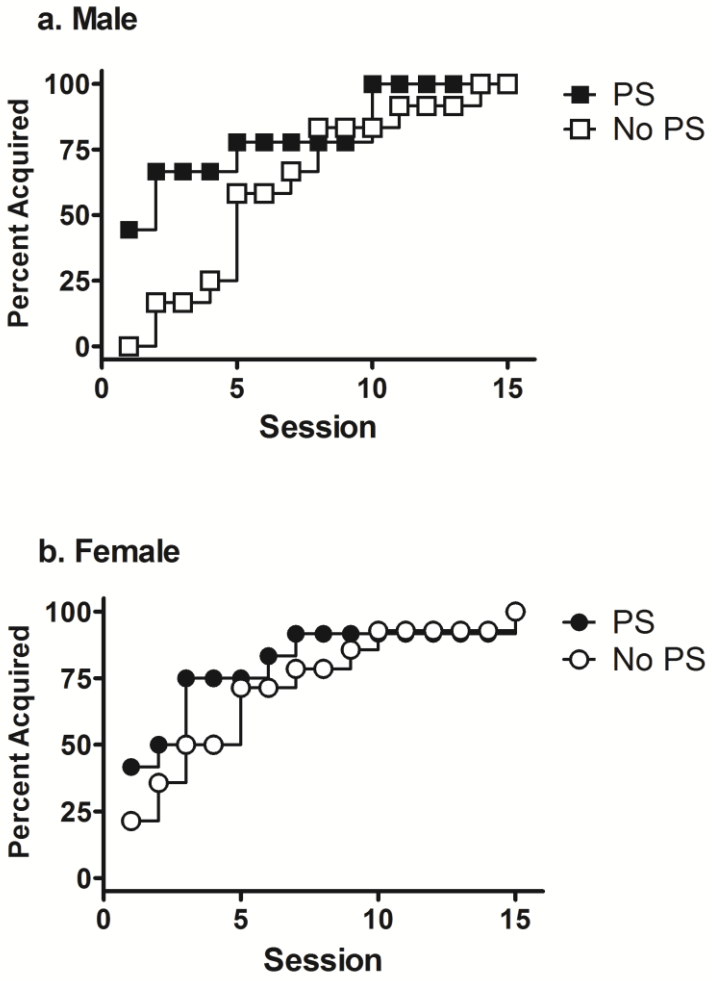

Figure 3.5 The rate of acquisition on the escalating-doses regimen of self-administration. a) Males with a history of prenatal stress (PS; n = 9) acquired at a faster rate than No PS controls (n = 13). b) The rate of acquisition was no different for PS (n = 13) and No PS (n = 14) females.

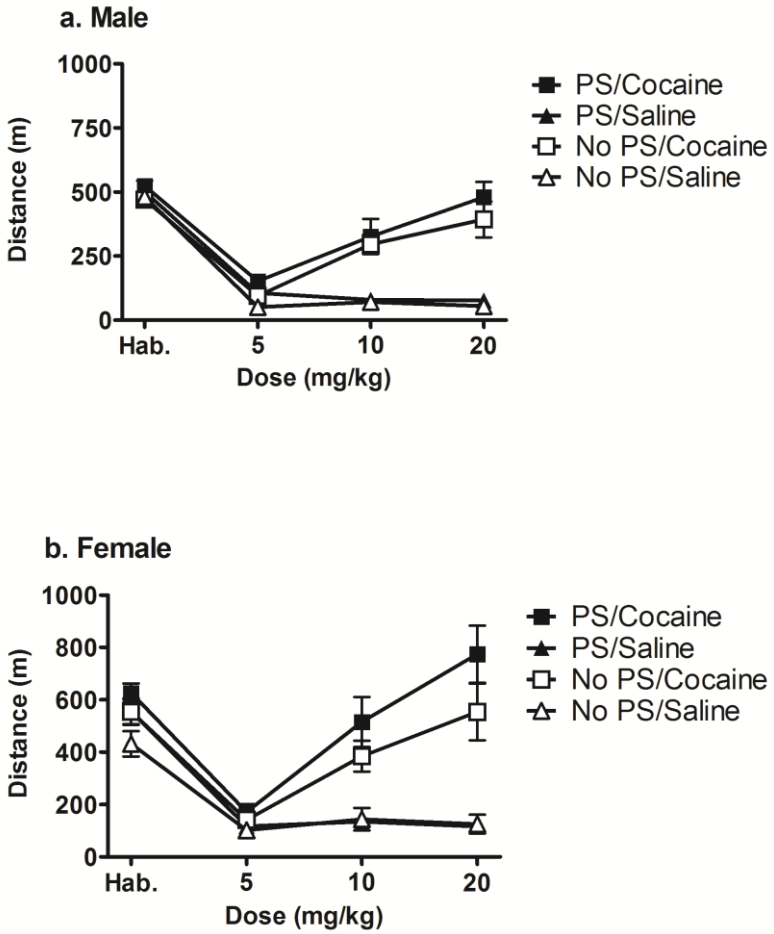

Figure 3.6 The mean peak (± SEM) in distance traveled during session 1 of locomotor recording. a) Distance traveled by male rats with (PS; cocaine n = 9; saline n = 6) and without (No PS; cocaine n = 9; saline n = 9) a history of prenatal stress. b) Distance traveled by PS (cocaine n = 8; saline n = 6) and No PS (cocaine n = 10; saline n = 9) females during a 30-minute habituation period (Hab.) and after each injection of cocaine or saline (1 ml/kg).

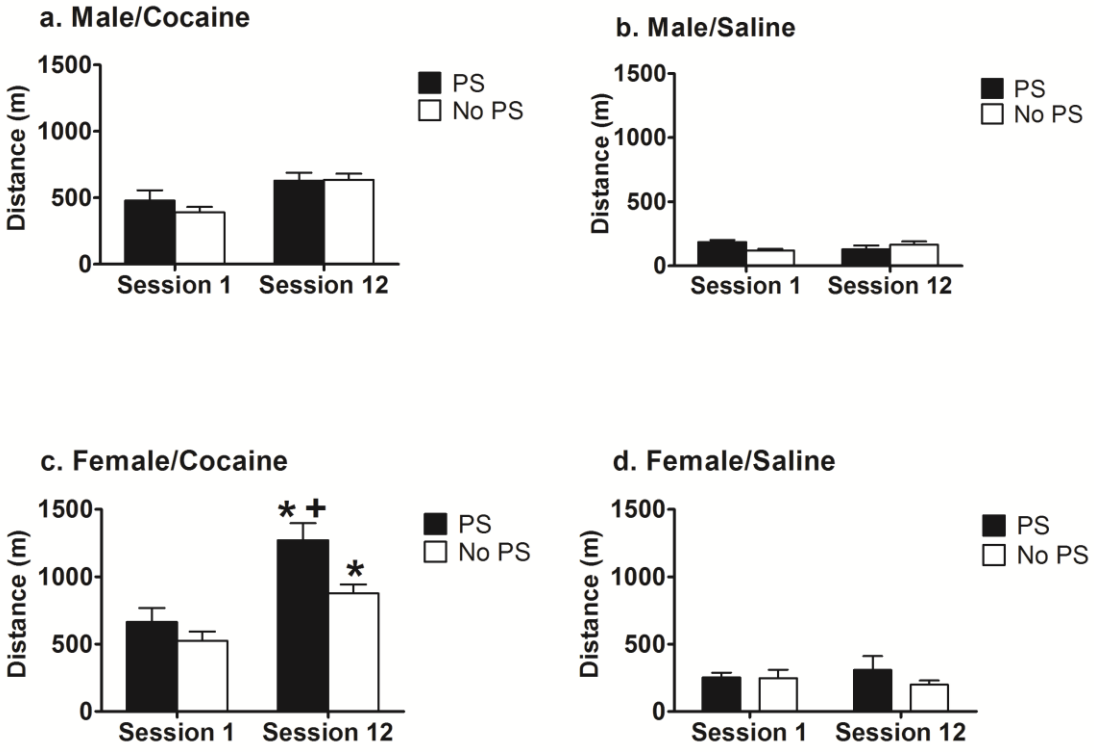

Figure 3.7 The summed peaks in distance traveled after the first 2 injections of cocaine (5 and 10 mg/kg; a, c) or saline (1 ml/kg; b, d) during sessions 1 and 12 for rats subjected to prenatal stress (PS) or no prenatal stress (No PS). a) Distance traveled for cocaine-treated PS (n = 9) and No PS (n = 9) males. b) Distance traveled in response to saline for PS (n = 6) and No PS (n=9) males. c) Response to cocaine for PS (n = 8) and No PS (n = 10) females. d) Distance traveled for saline-treated PS (n = 6) and No PS (n = 9) females. Data are expressed as mean + SEM. * indicates session 12 > session 1, + denotes PS > No PS ($p < 0.05$).

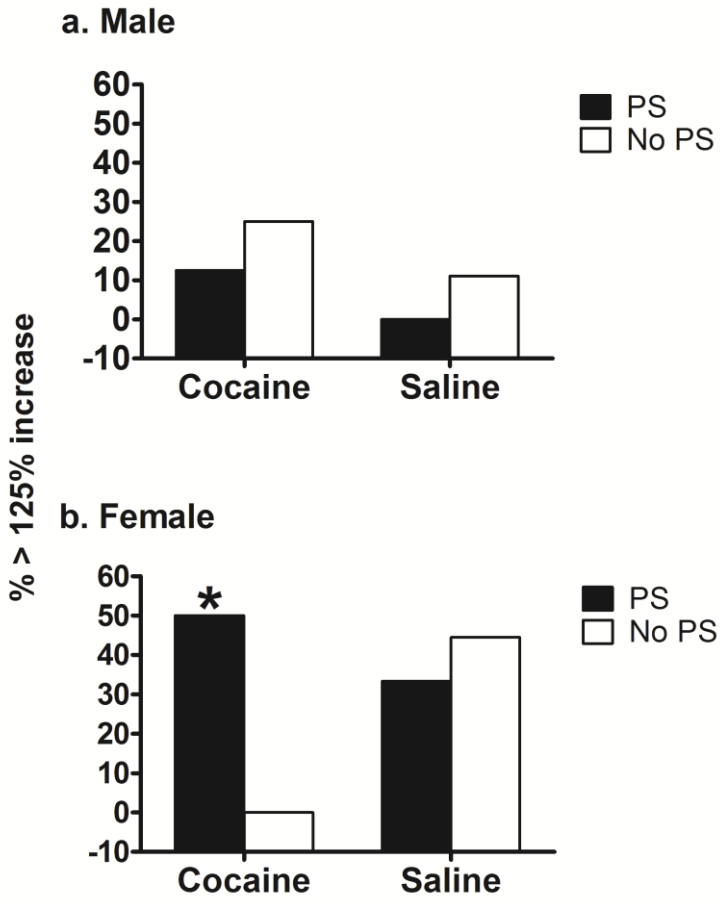

Figure 3.8 The percentage of each group exceeding a 125% increase in stereotyped head movements from session 1 to 12 in response to the final injection of cocaine (20 mg/kg) or saline (1 ml/kg). a) The percentage of males exposed to prenatal stress (PS; n = 8) or no prenatal stress (No PS; n = 9) with at least a 125% increase in the peak ratio of distance traveled by the head to that of the center of the body (head: body distance) after cocaine treatment. b) The percentage of females (PS n = 8; No PS n = 10) with at least a 125% increase in head: body distance from day 1 to day 12 after cocaine. *p < 0.05, difference between PS and No PS females exposed to cocaine.

CHAPTER IV

SEX DIFFERENCES IN PRENATAL STRESS EFFECTS ON COCAINE PURSUIT

Introduction

Interference with in utero development can have long-lasting consequences for the health of the organism. Perhaps most notably, deficits in prenatal nutrition are linked with chronic, debilitating conditions in both rodents (Morgane et al., 1978, Morgane et al., 1993) and humans (Barker, 1990, 1998). Similarly severe effects, albeit more related to mental health, are observed when development is compromised by maternal stress (Welberg and Seckl, 2001). Mothers that experience intense stress and/or anxiety during pregnancy, for example, give birth to children that are at increased risk of developing attention deficit hyperactivity disorder (Van den Bergh and Marcoen, 2004, Rodriguez and Bohlin, 2005, Van den Bergh et al., 2006). Based on work in the preclinical setting, maternal stress may also predispose individuals to develop problematic patterns of drug use.

Indeed, maternal stress during the final week of the rat gestation period (prenatal stress [PS]) creates a life-long hypersensitivity to psychostimulants. PS augments drug-induced dopaminergic signaling in the nucleus accumbens, providing a plausible mechanism for enhanced responsiveness at the behavioral level (Kippin et al., 2008, Silvagni et al., 2008). PS promotes the locomotor-activating effects of drugs given acutely (Deminiere et al., 1992, Koehl et al., 2000, Kippin et al., 2008) and on a repeated basis (Henry et al., 1995), with the latter indicating that PS facilitates the progressive increase in drug-induced activity with repeated administrations known as behavioral sensitization. Given that sensitization is thought to model the neuroplastic changes that contribute to

drug addiction (Robinson and Berridge, 1993, 2003), PS may represent a risk factor for drug use and abuse later in life.

Consistent with this hypothesis, PS facilitates the pursuit of drugs when animals are tested with the self-administration paradigm. PS promotes the rate of acquisition for cocaine and amphetamine (Deminiere et al., 1992, Thomas et al., 2009) and augments the reinstatement of cocaine seeking after extinction training (Kippin et al., 2008). Thus, when allowed to administer drugs voluntarily, PS rats are faster to stabilize their level, and are more prone to exhibit relapse-like behaviors after forced withdrawal, when compared to No PS controls.

Nevertheless, significant gaps in the preclinical literature temper the translation of these findings to humans. First, virtually all PS studies have excluded female subjects, even though profound sex differences in drug responsiveness have been documented in humans and non-human animals (for reviews, see Lynch et al., 2002, Carroll et al., 2004, Roth et al., 2004, Becker and Hu, 2008, Carroll and Anker, 2010). Briefly, rodent studies indicate that females are more sensitive to psychostimulants than males, and that the difference is not dependent on gonadal hormones being present in adulthood (Hu and Becker, 2003, Hu et al., 2004, Jackson et al., 2006). The sex differences can be *modulated*, however, by ovarian hormone effects in females. Estradiol potentiates the drug response, for example, when administered exogenously to females that have been ovariectomized (OVXed; Roth et al., 2002, Hu and Becker, 2003, Hu et al., 2004, Jackson et al., 2006). Consistent with these findings, effects in females that are naturally cycling are more pronounced in estrus than in diestrus, with the ratio of estradiol to progesterone being higher in the former than the latter (Roberts et al., 1989, Lynch et al., 2000).

Despite these well-documented effects, very little is known about how they are changed by PS. Although one report indicates that PS renders females more sensitive to the motor-altering effects of 3,4-methylenedioxymethamphetamine (MDMA; Morley-Fletcher et al., 2004), it is unknown whether the effects are sex dependent. Moreover, it is unclear if and/or how motor alteration is related to MDMA's abuse potential. To date, only one published report directly assesses

the relationship between sex, PS, and drug responsiveness. Thomas et al. (2009) observed sex-dependent effects of PS in 2 models of cocaine abuse: the acquisition of self-administration and the expression of behavioral sensitization. Interestingly, PS facilitated acquisition only in males, but augmented sensitization exclusively in females. Although it is surprising that PS females' propensity to sensitize did not translate to enhanced drug taking in the self-administration paradigm, this may indicate that effects in females only emerge after *chronic* drug exposure.

To better understand the influence of PS in females, this investigation utilized a lengthened self-administration regimen to test for an effect of PS *outside of the acquisition period*. Importantly, this follows in the footsteps of recent work suggesting that compulsive-like pursuit of cocaine develops only after many weeks of testing (Deroche-Gamonet et al., 2004, Vanderschuren and Everitt, 2004). Consequently, this investigation was expected to provide insight into PS as a potential risk factor for drug addiction. Both males and females were included to test for sex differences independent of and acting in synergy with PS, as in Thomas et al. (2009)

Methods

Subjects

Male (275-300 grams) and virgin female (200-225 grams) Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN) for breeding purposes and placed in isosexual groups of 3-4 upon arrival. Animals were housed in a temperature-controlled environment on a 14/10 hour light/dark cycle, with ad libitum access to food (2014 Teklad Global 14% protein rodent maintenance diet, Harlan rat chow, Madison, WI) and water. All procedures were performed in accordance to a protocol approved by the University of Michigan Use and Care of Animals Committee.

Based on vaginal lavage, each sexually-receptive female was housed overnight with a randomly-selected male. Successful mating was confirmed the following morning by vaginal lavage inspection for the presence of sperm. The morning of sperm detection was designated as day 0 of pregnancy. Mated

females were then individually housed for the entirety of the gestation period.

From gestational days 15-21, a selection of females (n = 13) were exposed to repeated daily restraint stress. Restraint stress consisted of removal from the animal colony room and placement in a Plexiglass restrainer for 45 minutes, 3 times per day. Restraint episodes were evenly distributed across the light cycle (0900, 1200, and 1600 hours). Except for cage changes, females not chosen for restraint stress (n = 12) were left undisturbed for the duration of pregnancy.

Within 24 hours of birth (postnatal day 1), pups were briefly separated from their mother and handled by the experimenter for sex identification. Litters were then culled to 10, with an equal or near-equal ratio of males to females (when possible). To reduce the potential for "litter effects," only 1-2 rats/sex/litter were randomly chosen to participate in self-administration. Litter characteristics are presented in Table 4.1.

Drugs

Cocaine HCL was provided by the National Institute on Drug Abuse (Bethesda, MD) and dissolved in 0.9% sterile saline for all procedures.

Surgical procedures

Between approximately 65 and 75 days of age, rats destined for self-administration testing were implanted with an indwelling intravenous catheter under isoflurane (2.5% in oxygen) anesthesia. Catheters were flushed with 0.2 ml heparinized saline (30 U/ml in 0.9% sterile saline) and 0.2 ml gentamicin (3 mg/kg) at the end of surgery. Gentamicin (3 mg/kg) was used to flush catheters on a daily basis for the entirety of the experiment. Following catheterization, rats were housed under a reverse light/dark (14/10 hour) cycle for the remainder of the experiment.

Self-administration testing

Self-administration training began 1 week after catheterization. Animals were tested once daily for 5 consecutive days per week across a 7-week period. All testing took place in standard operant chambers (25 x 27 x 30 cm) located within ventilated sound-attenuating cubicles (Med Associates, Inc., Georgia, VT)

during the dark period of the light/dark cycle. Shortly before the start of each test session, rats were connected to an infusion syringe and tethered to a swivel. The swivel was mounted to a counterbalanced arm to allow unrestricted movement throughout the chamber. Illumination of the house light then signaled the beginning of each session.

A drug dose of 0.8 mg/kg/infusion was used for all test sessions. Cocaine (50 µl/infusion) was delivered over the course of 2.8 seconds after the required number of nose pokes in the active hole was completed. The active hole was illuminated with white light during this time and remained so during the subsequent 40-second "time-out" period, during which time nose pokes in both holes were without programmed consequences. In all situations, nose pokes in the inactive hole were recorded, but had no programmed consequences.

The test regimen utilized a mixture of fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement. The duration of almost all sessions was determined by the time needed to reach the session's predetermined infusion criterion (INCR). Only after reaching the INCR was an animal removed from the chamber and returned to their home cage.

Self-administration training began on a FR1 schedule with an INCR of only 5. The INCR and response requirements were then steadily increased over the first week, in preparation for the PR schedule used in session 10. An FR1 schedule with an INCR of 10 and 15 was used for sessions 2 and 3, respectively. Subjects were then moved to an FR2 schedule for sessions 4 and 5, with an INCR of 15 and 20, respectively. The INCR then remained at 20 for sessions 6-9. Rats were tested with an FR3 schedule in session 6 and an FR5 schedule for sessions 7-9.

Following the acquisition period (sessions 1-9), most testing was done with an FR5 schedule and an INCR of 27. Each FR5 session was divided into 3 signaled drug-accessible ("drug") periods, separated by 2 signaled drug-inaccessible ("no drug") periods. "No drug" periods were initiated after the 40-second time-out periods that followed infusions 9 and 18, with the house light being turned off for 15 minutes each time. Nose pokes in the active and inactive

holes were recorded during “no drug” periods, but were without programmed consequences. Illumination of the house light was used to signal the conclusion of each “no drug” period. The house light remained on until the start of the next “no drug” period or until the end of the time-out period following the 27th infusion. Upon reaching the INCR, animals were removed from the test chambers and returned to their home cages.

Interspersed among regular FR5 sessions were tests for specific “addiction-like” traits, as in other reports (Deroche-Gamonet et al., 2004, Belin et al., 2008, Belin et al., 2009, Kasanetz et al., 2010, Belin et al., 2011). Each trait was assayed relatively early and late (weeks 2-3 and 6-7, respectively) in the testing regimen.

Motivation for cocaine was assayed during sessions 10 and 30 with a PR schedule. The responses needed to receive each infusion increased according to the following progression (2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, 1347, 1646, 2012, 2459). The maximal number of responses performed to receive a single infusion constituted the individual’s “breaking point” (BP). PR sessions terminated after 6 hours or after 1 hour elapsed since the last infusion, whichever came first.

The effect of negative consequences (“punishment”) associated with drug use was assessed with an FR1 schedule during sessions 14 and 35, with each lasting 2 hours in duration. Each drug infusion was paired with a mild electric footshock (0.4 mA, 1 s). Because reliable drug-taking behaviors were not observed during these sessions, data from punishment sessions were not included in subsequent analyses.

Drug seeking in the absence of reinforcement was measured by averaging “no drug” active nose pokes during FR5 sessions 12-13 and 33-34.

The rate of cocaine intake was assessed by averaging the inter-infusion intervals (III) during “drug” periods in sessions 13 and 34.

The BP score, drug taking in the absence of reinforcement, and III obtained late in the training regimen were used to determine the final severity of drug pursuit for each individual.

Statistical analysis

Acquisition of drug taking was assessed with a mixed model analysis of variance (ANOVA), with dependent measures being the average (1) latency to obtain the first infusion and (2) III across test sessions 1-9. Sex (2 levels) and prenatal stress (2 levels) were used as between-subject factors, while session (9 levels) was the within-subject factor.

Each addiction-like trait assayed late in training was analyzed with ANOVAs that also had sex and prenatal stress as factors. BP and "no drug" active poke scores, however, were first subjected to natural log and square root transformations, respectively, to normalize the distributions.

Post hoc inspection of plotted data suggested that scores were not distributed in the same way for all groups. Specifically, a slightly higher percentage of PS females scored at or above descriptive parameters (i.e., mean, median, 1 standard deviation from the mean) of the overall rat population, relative to individuals from other groups. Thus, binary logistic regression was used to test for main effects of sex, prenatal stress, and a sex X prenatal stress interaction, on the probability of scoring at or above these cutoffs. Chi-square tests were then used to compare probability values of PS females to each group individually or all other rats pooled together.

To create a *composite* score of propensity to pursue cocaine, each of the 3 addiction-like trait values were independently normalized. Normalization entailed subtracting the population mean from each individual score, and then dividing by the population standard deviation (for III the subtraction process was reversed, such that each rat's III was independently subtracted from the overall mean III). All trait values were then summed for each animal and subjected to ANOVA.

Finally, scores for all 3 traits were independently ranked, and rats scoring in the highest 35% were considered positive for that trait and assigned an arbitrary value of 1. Rats scoring outside of the upper 35% were considered negative for that trait and assigned an arbitrary value of 0. Summing newly assigned values then yielded a continuum of persistence in pursuing cocaine,

with individuals being positive for 0, 1, 2, or 3 addiction-like criteria. Sex and PS effects on the number of criteria met after the 7th week were then analyzed with ordinal logistic regression. Correlational analyses of addiction-like criteria satisfied after 3 and 7 weeks of testing were then used to determine whether the final severity of drug pursuit was predicted by behaviors observed weeks earlier.

For all ANOVAs, significant effects were followed up with Bonferroni post hoc tests. P values < 0.05 were considered statistically significant.

Results

Litter characteristics

Table 4.1 displays a comparison of litter characteristics of pups born to mothers that were vs. were not subjected to repeated stress during pregnancy. ANOVAs and chi-square tests indicated that stress had no influence on any parameter measured.

Acquisition

As Illustrated in Figure 4.1, the average III changed from session 1 to 9 ($F[8, 100.06] = 16.14$; $p < 0.001$). The III was lower for females than for males ($F[1, 222.08] = 4.96$; $p = 0.03$), although the change in III over time was not sex dependent.

Females also required less time than males to receive their first infusion during this period ($F[1, 160.39] = 4.05$; $p < 0.05$). PS slightly increased the latency to first infusion ($F[1, 160.39] = 3.76$; $p = 0.05$), independent of sex. Overall, latency decreased across time ($F[8, 127.55] = 4.26$; $p < 0.001$).

Rate of drug intake

ANOVA indicated that females self-administered cocaine at a faster rate than males ($F[1, 70] = 10.18$; $p = 0.002$; Figure 4.2). No effect of PS, or an interaction between sex and PS, was found.

Drug seeking during periods of signaled drug inaccessibility

Figure 4.3a displays a scatterplot of “no drug” active nose poke scores for all rats that participated in self-administration. Visual inspection of scores suggested that the distribution of drug seeking by PS females was different from that of the other groups. Indeed, a greater percentage of PS females scored at

least 1 standard deviation above the overall population mean, relative to all other rats (Figure 4.3b $\chi^2 = 4.23$; $p = 0.04$).

A square root transformation was used to normalize the distribution of drug-seeking scores and equalize group variances. ANOVA on transformed results revealed no effect of sex or PS. In addition, no interaction between the two was observed.

Lastly, correlational analyses were used to assess whether drug seeking was predicted by III. The following correlation coefficients indicate that no significant relationship was evident in any of the 4 groups: PS Female $r = -0.16$, $p = 0.5$; No PS Female $r = -0.12$, $p = 0.64$; PS Male $r = 0.18$, $p = 0.5$; No PS Male $r = -0.03$, $p = 0.9$).

Breaking point

Figure 4.4a displays a scatterplot of BP scores from the entire rat population. Binary logistic regression indicated that the likelihood of scoring at or above the overall median BP value was subject to an interaction that approached significance (Sex X PS; Wald $\chi^2 = 3.56$; $p = 0.059$). Subsequent chi-square tests indicated that more PS females met this criterion than individuals from any other group (all $p < 0.05$; Figure 4.4b).

ANOVA on transformed BP values indicated that females attained higher BP scores than males ($F[1, 70] = 4.6$; $p < 0.05$; Figure 4.5), and PS produced a modest overall facilitation of motivation for cocaine ($F[1, 70] = 3.69$; $p = 0.06$). No significant sex X PS interaction was observed.

Composite drug pursuit score

The overall score of females exceeded that of males ($F[1, 70] = 7.35$; $p < 0.01$; Figure 4.6). No effect of PS, or an interaction between sex and PS, was observed. A significantly greater percentage of the PS Female group scored at or above the overall median value when compared to all other groups pooled together ($\chi^2 = 4.39$; $p = 0.036$; Figure 4.6 inset).

Addiction-like criteria satisfied

Logistic ordinal regression indicated that females met more addiction-like criteria than males (Wald $\chi^2 = 9.64$; $p < 0.01$) and PS rats satisfied more criteria

than controls (Wald $\chi^2 = 4.22$; $p < 0.05$). In addition, the effect of PS was stronger in females than in males (Sex X PS; Wald $\chi^2 = 4.54$; $p < 0.05$; Figure 4.7).

Consistent with previous findings (Belin et al., 2009, Belin et al., 2011), rats that developed a "bursting" pattern of drug intake (6 or more infusions earned within a 5-minute time period) by the 7th week of testing satisfied more criteria than those that did not (Wald $\chi^2 = 15.35$; $p < 0.001$; Figure 4.8). Follow-up analyses indicated that the development of "bursting" itself was not group dependent.

Interestingly, the number of criteria met was also predicted by several litter characteristics. The ratio of male to female pups observed on day 1 of birth (before culling) was a modest overall predictor (Wald $\chi^2 = 3.08$; $p = 0.08$), with a stronger relationship in No PS vs. PS rats (Sex Ratio X PS; Wald $\chi^2 = 7.49$; $p < 0.01$). For No PS individuals, a significant, negative correlation was observed (Spearman's rho = - 0.43, $p < 0.01$). In the PS population, the relationship failed to reach or approach significance (Spearman's rho = - 0.02, $p = 0.9$).

In addition, the number of addiction-like criteria met was sex-dependently predicted by the number of naturally-occurring perinatal deaths in the litter (Deaths X Sex; Wald $\chi^2 = 6.42$; $p = 0.01$), even though the overall effect of littermate death failed to reach significance. In females, deaths were associated with less criteria being met (Spearman's rho = - 0.36; $p = 0.03$), even after controlling for subsequent litter size and the ratio of males to females before and after culling (partial r = - 0.34; $p < 0.05$). No significant relationship was observed in males (Spearman's rho = 0.21; $p = 0.21$) after performing identical analyses (partial r = 0.14; $p = 0.44$).

Finally, correlational analyses were used to determine how the number of addiction-like traits that were satisfied changed over time. The sum of addiction-like criteria satisfied after the 3rd week did not predict the number of traits met after week 7 for either of the female groups (PS: Spearman's rho = 0.22, $p = 0.34$; No PS: Spearman's rho = 0.23, $p = 0.36$). Nor was a correlation evident in PS males (Spearman's rho = 0.34, $p = 0.18$). Only in males without a history of

PS did the relationship between scores early and late in testing reach significance (Spearman's rho = 0.53, p = 0.03).

Discussion

The present findings indicate that sex differences and PS have independent and synergistic effects on the development of compulsive-like patterns of drug pursuit. Overall, females expressed a more severe profile of cocaine use, and were identified as positive for more addiction-like traits, when compared to males. As predicted, females were even more vulnerable to cocaine if they had a history of PS. PS consistently shifted the distribution of females' scores to more severe proportions, relative to the rest of the population. To exemplify, PS increased the percentage of females (but not males) expressing a BP score above the overall population median. In addition, females were more affected than males by the PS-induced increase in the total number of addiction-like symptoms expressed at the end of testing. As a result, PS females were moderately underrepresented in the most drug-resistant (0-criteria rats) subpopulation, and represented 60% of the most drug-vulnerable (3-criteria rats) subpopulation. Taken together, sex differences and PS clearly contribute to differences in cocaine susceptibility. Females were more vulnerable than males, and rats with a history of PS met more addiction-like criteria than No PS controls. Interestingly, these factors also synergized to disproportionately promote compulsive-like drug pursuit developing in females with a history of PS.

It is well established that there are robust sex differences in responsiveness to psychostimulants. Indeed, females are more avid in their drug pursuit than males during all stages of self-administration (Lynch et al., 2002, Carroll et al., 2004, Roth et al., 2004, Becker and Hu, 2008, Carroll and Anker, 2010). An element that is unique to this report, however, is an evaluation of how the sexes differ on *multiple* dimensions of behavior. Indeed, although the use of several criteria to assess drug susceptibility has gained popularity in recent years, in part because it resembles the diagnostic process utilized by clinicians, all such work to this point has been done in males (Deroche-Gamonet et al., 2004, Belin et al., 2008, Belin et al., 2009, Kasanetz et al., 2010, Belin et al.,

2011). Consequently, it was unknown whether females are more likely than males to develop a collection of addiction-related traits, just as they express more susceptibility to drug properties that are assayed in isolation. Here we indicate that females are, indeed, more likely to co-express severe patterns of drug pursuit and presumably develop an addiction-like phenotype.

Another important nuance to the design used here is the tight control over cocaine access both within and across test sessions. The length of "drug" periods was determined by the time needed to reach a predetermined INCR, preventing drug seeking during "no drug" periods from being confounded by differences in drug intake. Although there was variability in the *rate* at which cocaine was administered, correlational analyses indicated that drug seeking was not predicted by III. Consequently, variation in drug seeking was independent of differences in III.

The control of drug access *across* test sessions also served an important purpose. Because females typically acquire drug taking faster than males, for example, sex differences observed outside of the acquisition period are often confounded by differences in drug history. Stated otherwise, without strict limits on drug access, it is unclear whether observed sex differences are a consequence of (1) intrinsic, biologically-based differences that are independent of previous drug exposure or (2) sex-dependent drug intake during the preceding stage of testing, presumably mediated via drug-associated neuroadaptations. Due to this study's design, the second possibility can be eliminated as a cause of any sex difference reported here. Thus, females' greater propensity to develop compulsive-like drug habits is not reliant on a sex difference in drug intake earlier in testing.

Despite novel aspects of this study's design, patterns of drug pursuit were still consistent with those observed in similar reports. To exemplify, only a small fraction of the population ever developed a "bursting" pattern of intake. "Bursting," a marker of intense drug use across short intervals of time, distinguished the most drug-vulnerable from drug-resistant individuals in a previous report (Belin et al., 2009). Likewise, here we found that evidence of

“bursting” strongly predicted the number of addiction-like criteria satisfied by the end of testing. Consequently, although the test regimen used here was shorter than in similar experiments, it was of sufficient length for the emergence of a categorical difference between subjects that were vulnerable and resistant.

To sum, this study identifies risk factors for drug use developing compulsive-like features. Sex and PS join other preexisting differences, such as impulsivity, as modulating factors in the transition of drug use to seemingly uncontrollable levels (Dalley et al., 2007, Belin et al., 2008, Economidou et al., 2009). Both factors have independently been implicated in aspects of drug responsiveness in other reports. An important nuance incorporated into this study, however, was including both in the same experiment to test for synergistic effects. In a previous study, it was unclear why PS promoted sensitization to and the self-administration of cocaine selectively in females and males, respectively (Thomas et al., 2009). The lengthened self-administration regimen used here was intended to test whether PS facilitates the drug pursuit of females with a more extensive drug and test history. In addition, we sought to test whether there is an overall sex difference in susceptibility to developing an addiction-like phenotype, as suggested by the existing literature. Indeed, after weeks of testing, females expressed a more severe profile of drug pursuit than their male littermates. Likewise, PS rats met more addiction-like criteria than No PS controls. Interestingly, the enhancing effect of PS was stronger in females than in males. Thus, PS increases the already-heightened vulnerability of females to pursue drugs, perhaps by augmenting the neuroplastic changes associated with chronic drug use. Given the correspondence between behavioral traits measured here and the core symptoms of drug addiction, PS may represent a risk factor for drug use reaching uncontrollable levels, especially in women.

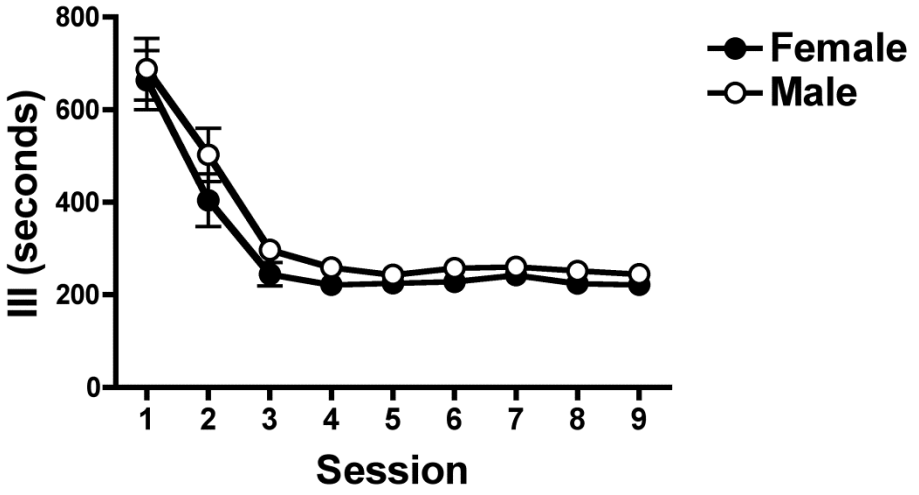

Figure 4.1 The inter-infusion interval (III; mean ± SEM) of male and female rats for each of the first 9 self-administration sessions. Overall, the III was lower in females vs. males, independent of stress history.

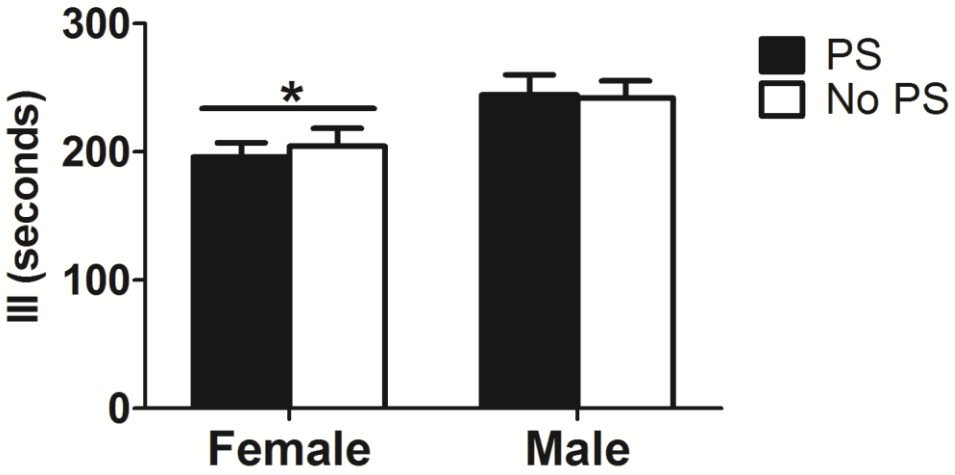

Figure 4.2 The mean (+ SEM) inter-infusion interval (III) of male and female rats with (PS) and without (No PS) a history of prenatal stress during session 34 of self-administration testing. Females self-administered cocaine at a faster rate than males (denoted by *), independent of PS. No overall or sex-dependent effect of PS was observed.

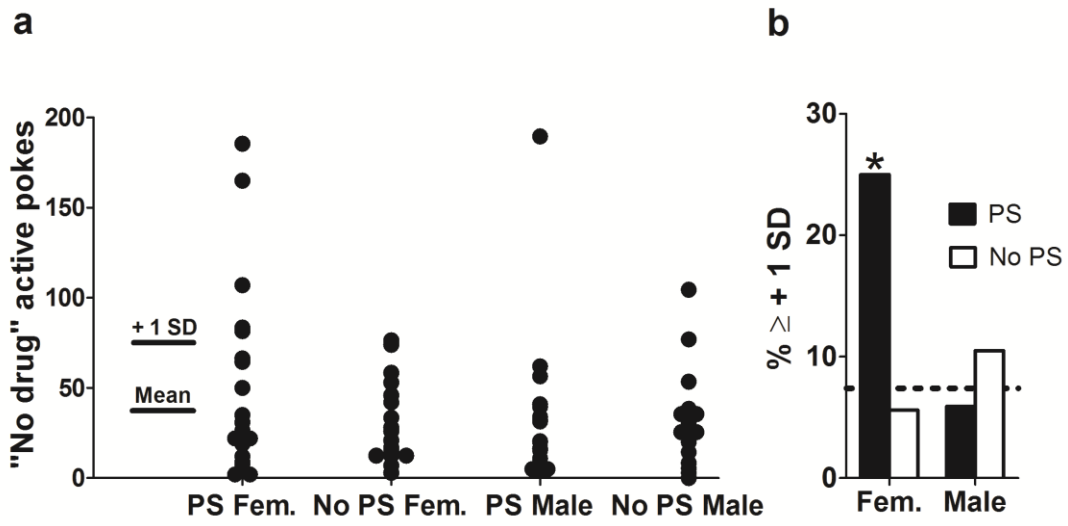

Figure 4.3 Drug seeking in the absence of reinforcement, as measured by active nose pokes during periods of signaled drug inaccessibility ("no drug" active pokes). a) The distribution of scores for males and females with (PS) and without (No PS) a history of prenatal stress. Identical scores within each group are stacked along the horizontal axis. The overall population mean and 1 standard deviation above the mean (+ 1 SD) are indicated by horizontal lines on the far left side. Visual inspection of the data points suggested that PS females were overrepresented in the subpopulation of rats showing the most profound drug-seeking activity. b) We confirmed this by using 1 standard deviation above the mean as a cutoff value. As indicated by the *, a significantly higher percentage of PS females reached or exceeded the cutoff when compared to all other rats in the population (7.4%; denoted by the dashed line), irrespective of group membership.

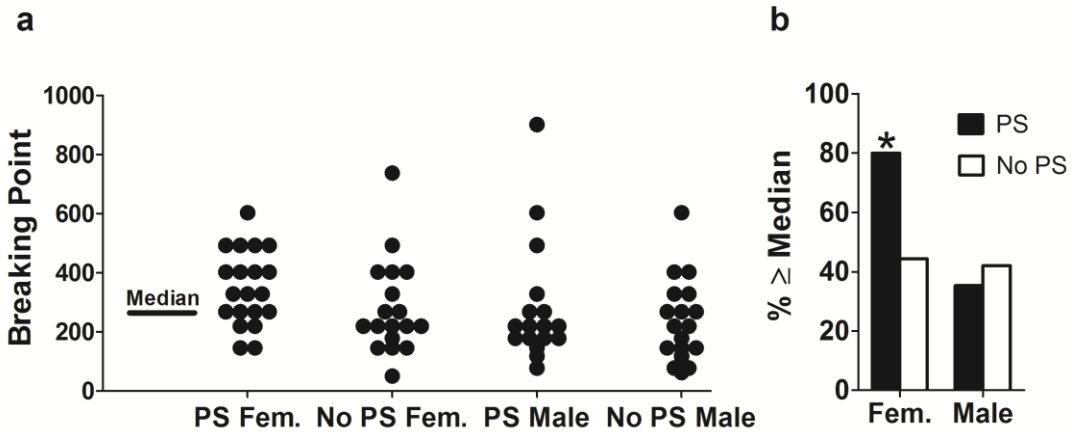

Figure 4.4 Motivation for cocaine, as determined by breaking point (BP) scores on a progressive ratio (PR) schedule. a) The distribution of BP scores for males and females with (PS) and without (No PS) a history of prenatal stress. Identical scores within each group are stacked along the horizontal axis. The overall median from the entire population is indicated by the horizontal line on the far left side. Visual inspection of group distributions suggested that the PS Female group was shifted upward, relative to the other groups. b) Indeed, a greater percentage of PS females scored at or above the overall median value than that which was observed in each of the other groups (significant difference denoted by *).

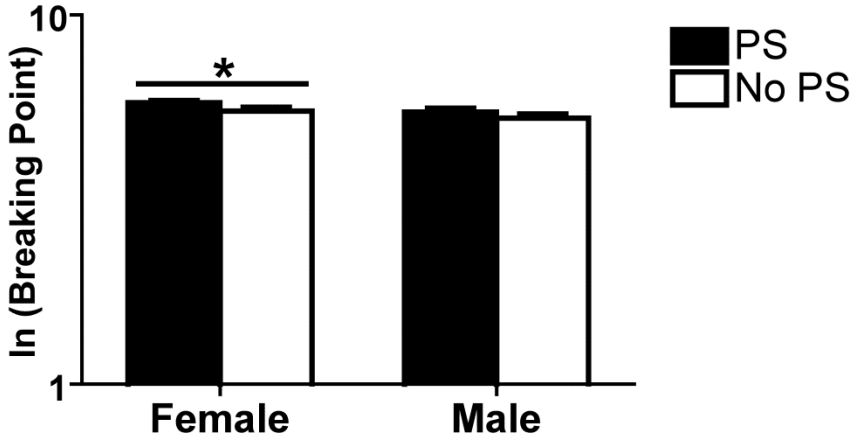

Figure 4.5 The mean (+ SEM) breaking point (BP) score of males and females with (PS) and without (No PS) a history of prenatal stress, following a natural log (ln) transformation. Females expressed higher BP scores than males (indicated by *) and PS rats tended to reach higher BP values than No PS controls (p = 0.06). However, the influence of PS was not sex dependent.

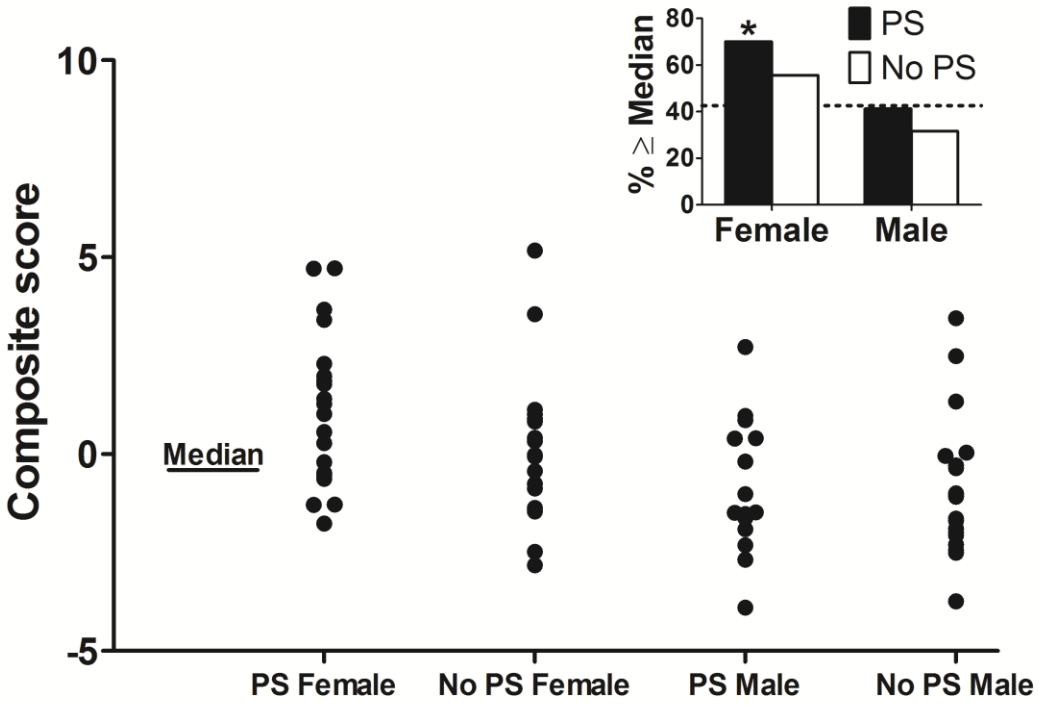

Figure 4.6 Overall severity of drug pursuit, as indicated by the sum of normalized scores for 3 addiction-like traits. The scatterplot shows the distribution of summed scores for males and females with (PS) and without (No PS) a history of prenatal stress. Identical values within each group are stacked along the horizontal axis, and the overall population median is indicated by the horizontal line on the far left side. The distribution of scores in the PS female group appeared to be shifted upward, relative to those of the other 3 groups. As shown in the inset, a significantly greater percentage of PS females met or exceeded the overall median value (denoted by *) when compared to all other rats in the population (42.6%; indicated by the dashed line), irrespective of group membership.

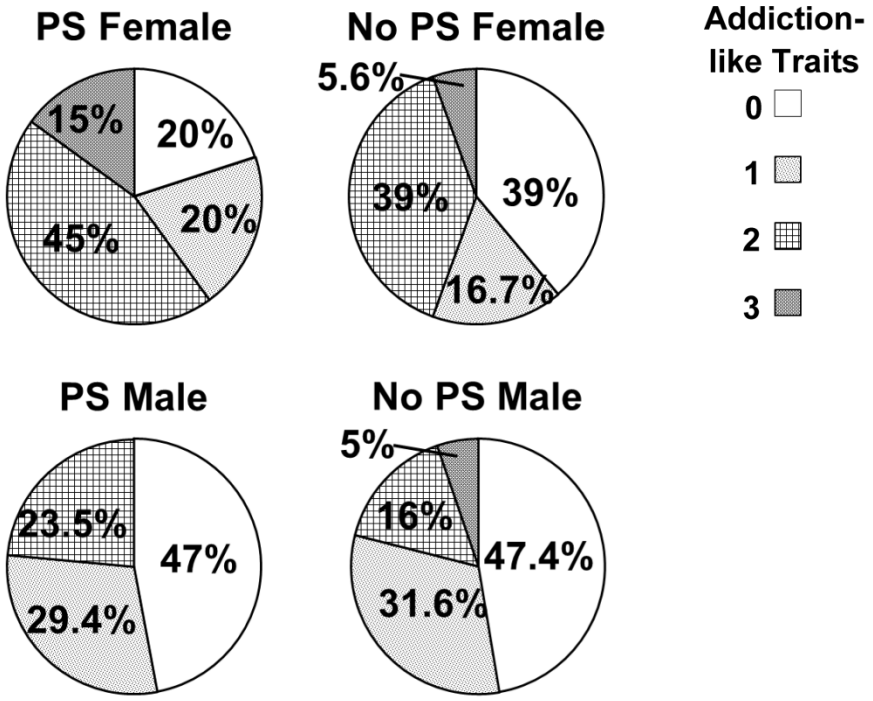

Figure 4.7 The percentage of each group that was positive for 0, 1, 2, and 3 addiction-like traits. As described in the Methods, individuals were considered positive for an addiction-like trait if they scored in the upper 35% of the distribution of the entire population. Females were positive for more traits than males, and rats with a history of prenatal stress (PS) met more addiction-like criteria than No PS controls. The effect of PS was stronger in females than it was in males, seemingly because PS decreased and increased the percentage of females satisfying 0 and 3 addiction-like traits, respectively.

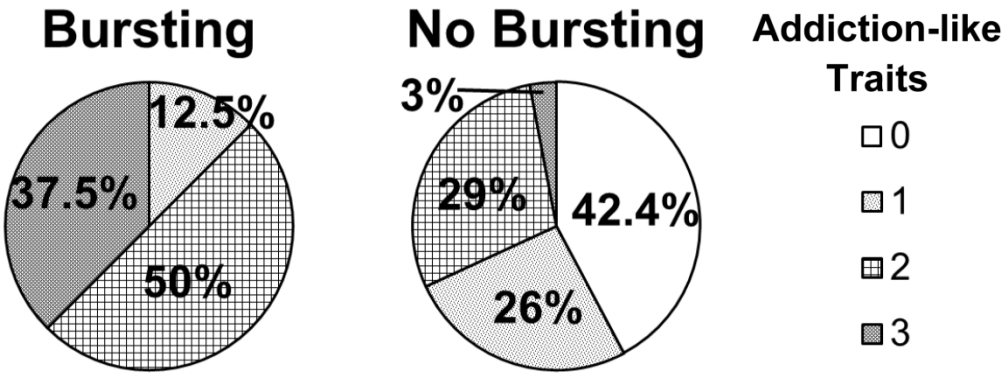

Figure 4.8 The number of addiction-like traits satisfied by rats that did (n = 8) and did not (n = 66) develop a "bursting" pattern of cocaine intake. A "bursting" episode consisted of 6 or more infusions self-administered within a 5-minute time span, as originally reported by Belin et al (2009). Rats showing evidence of "bursting" displayed their rapid pattern of drug intake at least once per session across sessions 31-34. These individuals were positive for significantly more addiction-like traits than those that never showed this pattern.

	Stress	No Stress	P value
Litters	13	12	
% with any naturally-occurring deaths	23.1	25	p = 0.91
Naturally-occurring deaths/litter	0.42 ± 0.27	0.31 ± 0.26	p = 0.77
Natural male/female ratio	1.08 ± 0.16	1.11 ± 0.16	p = 0.89
Culled male/female ratio	0.88 ± 0.07	0.94 ± 0.08	p = 0.61
Natural litter size	13.85 ± 0.59	13 ± 0.85	p = 0.42
Culled litter size	9.85 ± 0.11	9.83 ± 0.11	p = 0.93

Table 4.1 A comparison of litters derived from repeatedly-stressed (n = 13) and non-stressed (n = 12) mothers. The mean ± SEM is shown for each litter characteristic, except the percentage of litters with at least one naturally-occurring death in the perinatal period. A difference in the latter was assessed with a chi-square test. All other measures were independently evaluated with ANOVAs. As indicated by the p values listed in the far right column, repeated maternal stress had no significant effect on any characteristic measured.

CHAPTER V

CONCLUSIONS

1. Summary of findings and working hypotheses

A history of prenatal stress (PS) has been shown to heighten male rats' responsiveness to drugs. Little was known, however, about how PS affects the already-enhanced drug vulnerability of females (No PS female > No PS male) or whether it affects males and females in different ways. The results presented here indicate that PS affects the development of drug abuse and addiction-related behaviors, and a key component of their underlying neural circuitry, in a sex-dependent fashion.

The experiment described in Chapter II was designed to test whether PS alters a sex-dependent steady-state dopaminergic signaling pattern in striatal regions that are implicated in drug reward (Di Chiara, 1999, Koob and Le Moal, 2001). Based on work suggesting that PS males develop along a sex-atypical trajectory, it was predicted that PS would reduce the sex difference in dopamine (DA) concentrations that has been observed in previous reports (Castner et al., 1993, Xiao and Becker, 1994). The hypothesis was supported by the finding that PS reduced extracellular DA concentrations in the NAc and dorsal striatum of males, but not females. By reducing DA in males, PS negated the sex difference that was observed in No PS controls, and provides suggestive evidence that PS-enhanced responsiveness to drugs may stem from incomplete masculinization of the striatum.

Whether sex-specific effects of PS could also be observed at the behavioral level was the focus of Chapters III and IV. In Chapter III, it was predicted that PS would render males more so than females hypersensitive to cocaine in 2 paradigms of drug abuse/addiction potential: the acquisition of self-administration and the development of behavioral sensitization. The prediction

was partially confirmed by evidence that PS promoted acquisition and overall drug intake in self-administering males, but not females. However, the opposite pattern was found when a separate cohort was tested for behavioral sensitization: PS selectively augmented sensitization in *females.*

Several possibilities were considered plausible explanations for sex-dependent effects of PS being paradigm specific. First, PS may selectively enhance subjective (i.e. hedonic) and non-subjective (e.g. locomotor activating) drug effects in males and females, respectively. Yet, PS augmented sensitization to amphetamine in males (Henry et al., 1995) and promoted oral ethanol self-administration in females (Darnaudery et al., 2007, Van Waes et al., 2011a), suggesting that subjective and non-subjective effects can be increased by PS in both sexes. Alternatively, the direction of sex-dependent PS effects may be determined by the extent of one's drug history. PS may promote initial drug responsiveness in males, but enhance the long-term changes associated with drug use predominantly in females. Interestingly, there is some evidence to support this interpretation. When tested for oral ethanol self-administration, PS increased drug intake and preference in females given at least 1 month of exposure (Darnaudery et al., 2007, Van Waes et al., 2011a), but failed to have an effect in males provided many weeks of daily intake (Van Waes et al., 2011b). In contrast, sensitivity to drugs is enhanced by PS when males are tested within 1 week of initial exposure (Deminiere et al., 1992, Henry et al., 1995, Koehl et al., 2000, Yang et al., 2006, Thomas et al., 2009).

The experiment described in Chapter IV utilized a prolonged cocaine self-administration regimen to determine if differences between PS and No PS females emerge after more extensive drug exposure than was provided in Chapter III (i.e., outside of the acquisition period). Total cocaine intake was controlled for by limiting the number of available infusions for virtually all test sessions. Based on findings presented in Chapter III, PS was predicted to increase the rate of drug intake over the course of the first 9 sessions (an index of acquisition) exclusively in males. By the conclusion of testing, however, effects of PS were predicted to be seen primarily in females.

Contrary to the first prediction, no difference between PS and No PS males was observed during the acquisition period. The experimental design precluded a replication of the methodology used in Chapter III, providing a potential explanation for the failure of PS to influence drug use during this time. As testing progressed, however, PS sex-dependently increased the *compulsive-like* pursuit of cocaine. In support of the second prediction, PS females met more addiction-like criteria than No PS controls. Together with results presented in Chapter III, these data suggest that PS in females (1) facilitates the neuroplastic changes that accompany prolonged drug use and (2) augments the development of addictive-like behaviors.

Overall, the findings presented here indicate that PS has long-term and sex-dependent consequences for reward-related processes. In rats with little or no drug history, PS shifted the neural and behavioral phenotype of males in a feminine direction, making them less like No PS males and more like No PS females. This is consistent with a literature that has been accumulating since the 1970's. In rats with a substantial history of drug exposure, however, an influence of PS in males was not evident. Instead, effects of PS emerged in females, such that PS females were more susceptible to cocaine than sex-matched controls. Thus, a history of early-life stress may facilitate the transition from controlled to compulsive patterns of drug use, and to a disproportionate degree in women vs. men.

2. Possible mechanisms responsible for sex-dependent effects of PS

The presumptive mechanism mediating many effects of PS, at least in males, has been maternal stress-induced interference with the testosterone surge that normally occurs late in gestation. Because testosterone initiates the process of sexual differentiation during this time, it is perhaps no surprise that PS is associated with incomplete masculinization of males' brain and behavior. Unlike No PS rats, however, PS individuals are also raised by mothers with a history of repeated stress. Thus, effects of PS need not necessarily be attributed to *prenatal* programming. Rather, some may reflect changes in maternal care that are independent of and/or interact with prenatal experience. This may be

especially germane for females, who acquire feminine characteristics based on the relative *absence* of fetal testosterone.

As reviewed in Chapter I, maternal care plays a critical role in establishing a rat's life-long health prospects. Repeated maternal stress diminishes the quality of care provided to the litter, and cross-fostering PS pups to non-stressed mothers abolishes some of the negative consequences associated with PS. Overall, the literature suggests that impoverished maternal care may contribute to some of the effects of PS reported in this dissertation. To better delineate the prenatal vs. postnatal contributions of PS effects described here, a correlate of maternal licking/grooming (LG) behaviors was tested for its ability to predict pups' DA signaling patterns (Chapter II) and compulsive-like cocaine pursuit (Chapter IV) many weeks later.

In a recent report, the ratio of males to females observed on postnatal day 1 ("prenatal sex ratio") predicted the amount of LG behaviors shown by the dam, *independent of subsequent alterations to litter composition made by the experimenter* (de Medeiros et al., 2010). Thus, the prenatal (but not postnatal) sex ratio may provide an indirect measure of LG frequency. Interestingly, when used as a covariate in analyses that are described in Chapters II and IV, it was found to be a significant predictor of (1) extracellular DA in drug-naïve rats and (2) the number of addiction-like traits expressed in a cohort trained to self-administer cocaine. In addition, its predictive value was different for PS and No PS individuals, even though the litters provided by repeatedly-stressed and non-stressed mothers were no different in any aspect of overall composition (Tables 2.1 and 4.1). As shown in Appendix A and B, follow-up analyses were performed to determine whether these relationships were the same for males and females, with the hope that doing so would offer insight into the mediating mechanisms responsible for PS effects that were sex dependent.

After sex-based litter averages were created from data originally presented in Chapters II and IV, a consistent pattern emerged: (1) the prenatal ratio of males to females was negatively correlated with traits observed in adult No PS males (but not females) and (2) PS altered these correlations. In males

from No PS litters, the prenatal sex ratio was a modest (i.e., near significant) predictor of 2 parameters of DAergic signaling in the dorsal striatum: steady-state extracellular concentrations (Figure A.1) and diffusion of DA away from the probe (Figure A.3). In contrast, no significant correlations were observed in rats with a history of PS. In a separate cohort that was trained to self-administer cocaine, the sex ratio predicted males' (but not females') propensity to seek out the drug. In males, however, PS shifted the direction of the correlation from negative to positive (Figure B.1).

Together, these analyses indicate that PS disrupts the relationship between the prenatal sex ratio and some of the traits studied in adult males. Based on the work of de Medeiros et al. (2010), this may be a reflection of changes in early-life maternal care, which would be consistent with reports on maternal stress and pup-directed behaviors (Champagne and Meaney, 2006, Baker et al., 2008, Del Cerro et al., 2010). Because the prenatal sex ratio has no discernible relationship with traits observed in adult *females*, however, LG's role in reward-related behaviors may be largely or entirely restricted to males.

Interestingly, there are direct and indirect links between LG and sensitivity to reward (see Matthews and Robbins, 2003, Francis and Kuhar, 2008 for reviews). Indirectly, the relationship has been best documented with the maternal separation (MS) paradigm, in which neonates are removed from the mother on a daily basis during the first 2 weeks of postnatal life. Brief (15 minutes/day) separation increases maternal displays of LG once the pups are returned to the nest, relative to litters being completely untouched, or handled but not removed from the home environment (Francis and Kuhar, 2008). As adults, MS males are more resistant to cocaine and ethanol self-administration than non-separated controls (Ploj et al., 2003, Roman et al., 2003, Jaworski et al., 2005, Moffett et al., 2006); in addition, their drug use is negatively correlated with variation in LG (Francis and Kuhar, 2008). In contrast, MS has no effect on ethanol self-administration in females (Roman et al., 2004, Gustafsson et al., 2005). Because both sexes have not been included in the same MS project (although see Matthews et al., 1999 for sex differences with an MS paradigm that

has a less clear effect on LG bouts), it remains unknown whether effects of MS are indeed sex specific. What is evident is that MS makes males more resistant to drug reward, and these protective effects seem to be mediated by early-life LG episodes.

To isolate effects that are *uniquely* attributable to maternal care, recent work has supplemented or replaced maternally-provided LG bouts with experimenter-administered tactile stimulation ("artificial TS"). TS in the form of gentle brush strokes mimics the stimulation that pups ordinarily receive from LG and produces effects that are generally consistent with those of MS. TS blunted the locomotor-activating effects of amphetamine in males (Lovic et al., 2006) and reduced behavioral sensitization when the drug was given repeatedly to males and females (Muhammad et al., 2011). Based on the latter example, in which the reduction was independent of sex, LG may have protective effects in females that are comparable to those in males. Whether this extends to the drug self-administration paradigm, however, remains unknown.

The discussion above clearly indicates that maternal care plays a role in establishing susceptibility to drugs. At least when tested for self-administration, the protective effects of LG bouts appear to be stronger in males than in females. In data presented here, a predictor of LG frequency was, likewise, more strongly correlated with drug seeking and DA signaling in males than in females. In males, PS altered correlations that were observed in No PS controls, perhaps because maternal stress preferentially diminishes the quality of care provided by *high* LG dams (i.e., mothers that would ordinarily provide the highest frequency of LG bouts; Champagne and Meaney, 2006). As depicted in Figure 5.1, the milder effects on postnatal care provided by other dams may explain why no *overall* effect of PS in males materialized when drug seeking was analyzed in Chapter IV. Indeed, only after the prenatal sex ratio was added to the model did an effect of PS become evident.

It is proposed here that the prenatal sex ratio adjusts for variation in the severity of the decrease in LG frequency induced by maternal stress. Because maternal stress diminishes LG frequency in only a subpopulation of mothers,

many PS litters likely received early-life care that was comparable to that of No PS litters. If so, any influence of PS in these litters would presumably be driven by events occurring prior to birth. Based on evidence obtained from males without a history of PS (Figure B.1), however, drug seeking seems to be powerfully influenced by *postnatal* events (as argued here, variation in maternal care). Thus, it is speculated that PS affected only a subpopulation of PS males that were tested for drug seeking, and that this subpopulation received early-life care that was disproportionately impaired by maternal stress. A comparable process is likely to have occurred in rats tested with microdialysis; because PS *did* have an overall, sex-dependent effect on DAergic signaling (Chapter II), however, the contributions of LG were likely supplemented by programming effects in utero.

To sum, some evidence suggests that variation in maternal care contributes to differences in DAergic signaling and drug-seeking proclivity of PS vs. No PS males. Because an influence of PS on drug seeking emerged only after accounting for a predictor of LG frequency, maternal care may be a predisposing factor for compulsive-like pursuit developing in a *subgroup* of PS males. Given the weaker predictive value of LG in the opposite sex (at least in this set of studies), LG bouts likely play a smaller role in shaping individual differences in females than in males. Thus, while it is entirely possible that PS and No PS females received different levels of maternal care, when considered along with the findings of de Medeiros et al. (2010), the analyses presented here suggest that variation in LG is unlikely to account for the sex-dependent increase in addiction-like traits reported in Chapter IV.

Interestingly, PS did alter the relationship between females' motivation for cocaine ("breaking point" [BP] on a progressive ratio [PR] schedule) and a different litter characteristic: the ratio of males to females *after* culling ("postnatal sex ratio"). As shown in Figure B.2, BP scores were negatively correlated with the postnatal sex ratio of females raised by mothers that were not subjected to stress, even after controlling for the sex ratio before culling. No significant relationship was observed in females that had a history of PS, with or without

controlling for the prenatal sex ratio. Likewise, correlations failed to reach significance in either of the male groups. Taken together, these analyses indicate that (1) pre-weaning litter composition influences the proclivity of females (but not males) to work for drug access as adults and (2) this relationship is abolished by PS.

Previously, it was the *postnatal* sex ratio that was thought to be linked with maternal behavior (Hard and Larsson, 1968, Moore and Morelli, 1979, Richmond and Sachs, 1984). In the only study (as far as I can tell) that separated the effects of the postnatal and prenatal sex ratio, however, maternal behavior was only influenced by the latter (de Medeiros et al., 2010). Effects of the former were only studied in males, and indicated that being raised in a female-biased environment (FB males) made them less sexually attractive to stimulus females. Consequently, when compared to males raised in male-biased or sex-unbiased litters, FB males mounted females less often, but intromitted and ejaculated at the same frequency. Overall, the work of de Medeiros et al. (2010) suggests that the development of sex-typical reproductive behaviors is subtly altered by a preponderance of interactions with littermates of the opposite sex.

Obviously, using these findings to better interpret results from animals of the opposite sex is not a straightforward process. First, because males and females display very different reproductive behaviors in adulthood, it seems unlikely that their development will be influenced by litter composition in the same way. Second, it is unclear if and/or how variation in mating behavior relates to differences in BP scores for cocaine, especially since the former and latter were measured in males and females, respectively. Nevertheless, one possibility is that BP is sensitive to variation in the pre-weaning social environment. If, for example, play patterns contribute to differences in drug vulnerability, PS may abolish the postnatal sex ratio – BP correlation via demasculinization of opposite-sex littermate play behaviors. PS reduces rough-and-tumble play selectively in males (Ward and Stehm, 1991), such that their activity becomes comparable to that of PS and No PS females (Poole and Fish, 1976, Olioff and Stewart, 1978). Consequently, the relationship between postnatal sex ratio and BP may appear

“flattened” for PS females, relative to No PS controls (Figure B.2), because of less inter-litter variation in male-typical patterns of play.

To summarize, it is argued here that the group-dependent ability of litter composition to predict traits measured in adulthood provides some insight into the mechanisms mediating sex-dependent effects of PS. It is speculated that early-life LG exposure is an important mediator of PS effects, but only in males. Conversely, PS-heightened motivation for cocaine in females may be mediated, at least in part, by a change in how they interact with littermates. Other early-life changes associated with PS, such as fetal glucocorticoid and/or testosterone-mediated programming, may have supplemented these effects and be uniquely responsible for traits not predicted by litter composition (e.g., extracellular DA in the NAc of males).

3. Conclusions and overall hypothesis

Clearly, the early-life period is an incredibly important time for the development of reward-related processes. Maternal stress late in gestation (PS) creates a life-long susceptibility to drugs of abuse in the developing young. As shown here, however, the consequences are not the same for males and females. Some evidence suggests that these sex differences are mediated by fundamentally different mechanisms.

Figure 5.2 uses the results reported here and elsewhere to summarize what is currently known about the relationship between PS, sex differences, and drug effects on brain and behavior. Briefly, PS alters a well-documented sex difference in striatal DAergic signaling. Presumably, the female-like steady-state extracellular DA profile of PS males reflects a disruption of sex-typical brain development. Incomplete striatal masculinization then heightens their responsiveness to the rewarding, locomotor activating, and neurochemical-stimulating properties of drugs. For reasons that are not yet clear, however, these effects of PS seem to dissipate when drugs are administered chronically. As shown in B.1, only a small subgroup of PS males continues to seek out drugs at a rate above what is seen in No PS controls, and only after adjusting for – presumably – differences in early-life maternal care. Otherwise, the difference

between the sexes resembles what is observed in No PS controls, with females displaying greater susceptibility than males. As illustrated in Figure 5.2, however, PS actually *widens* the sex difference. Here it is hypothesized that this is mediated via a sex-dependent facilitation of drug-associated neuroadaptations in circuitry that mediates both behavioral sensitization and reward processes (Robinson and Berridge, 1993, 2003). Augmented neuroplasticity is the proposed mechanism by which PS increased females' (but not males') sensitization to cocaine (Chapter III) and likelihood of developing compulsive-like traits of drug pursuit (Chapter IV). Unfortunately, it is difficult to speculate about the processes mediating these changes in further detail, in part because so little is known about the brains of these animals. While it is likely that there are many contributing factors, given the vast neurobiological changes that accompany chronic drug use (see Chapter I for a sampling), more work is undoubtedly needed to narrow the list of potential candidates.

Additional mediators that may work independent of or in conjunction with neural sensitization are stress-responsive peptides, such as cocaine and amphetamine-regulated transcript (CART). As shown in Figure C.1, PS blunted CART mRNA levels in the NAc of females that were administered a series of cocaine injections. CART, which is upregulated in response to acute drug exposure, is thought to offer some protection against drug properties (see Rogge et al., 2008 for review). When microinjected into the NAc of rats previously sensitized to amphetamine, for example, CART dampened the locomotor response induced by a challenge injection (Kim et al., 2007). It also reduced the self-administration of cocaine on a PR schedule, suggesting that CART's regulatory role extends to drug reward (Jaworski et al., 2008). By inhibiting CART activity, PS may disinhibit the reward circuits activated by drugs of abuse. Why this did not translate to faster acquisition of drug taking in Chapter III is unclear, and will require more testing to better understand. At least for now, it suggests that CART may work in tandem with other factors, such as drug-associated sensitization processes, to mediate effects of PS.

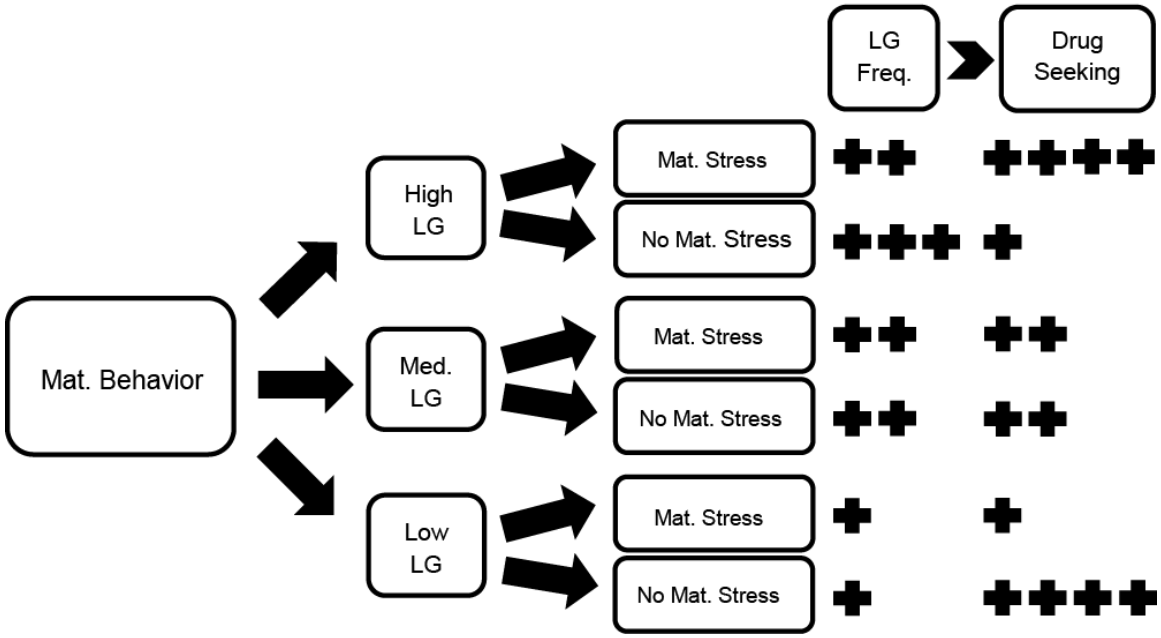

Figure 5.1 The hypothesized relationship between maternal stress (Mat. Stress), pup-directed licking/grooming (LG) bouts, and males' drug seeking in the absence of reinforcement ("no drug" active pokes). The number of plus symbols on the right indicates (1) the relative frequency of LG bouts experienced in the early postnatal period and (2) the amount of drug seeking during signaled drug-inaccessible periods of the self-administration regimen. A predictor of LG frequency, the prenatal ratio of males to females in the litter, was strongly correlated with the drug-seeking behavior of male pups once they became adults. The direction of the correlation, however, was completely different for rats that did and did not have a history of prenatal stress (PS). The relationship was positive and negative for PS and No PS males, respectively, suggesting that LG has protective effects against drug seeking in No PS controls. The positive relationship observed in PS rats may be mediated by the disproportionately severe decrease in LG bouts of certain dams, as a consequence of maternal stress. While it is well documented that maternal stress decreases LG frequency, the effect is primarily driven by its action on mothers that would ordinarily show the highest levels of LG (High LG), based on their behaviors with previous litters. Less affected are dams that show intermediate (Med. LG) and, in particular, low (Low LG) levels of maternal care. It is suggested here that PS creates a subgroup of males that develop a compulsive-like pattern of drug seeking, and this is mediated via the preferential diminishment of LG bouts initiated by *high* LG mothers.

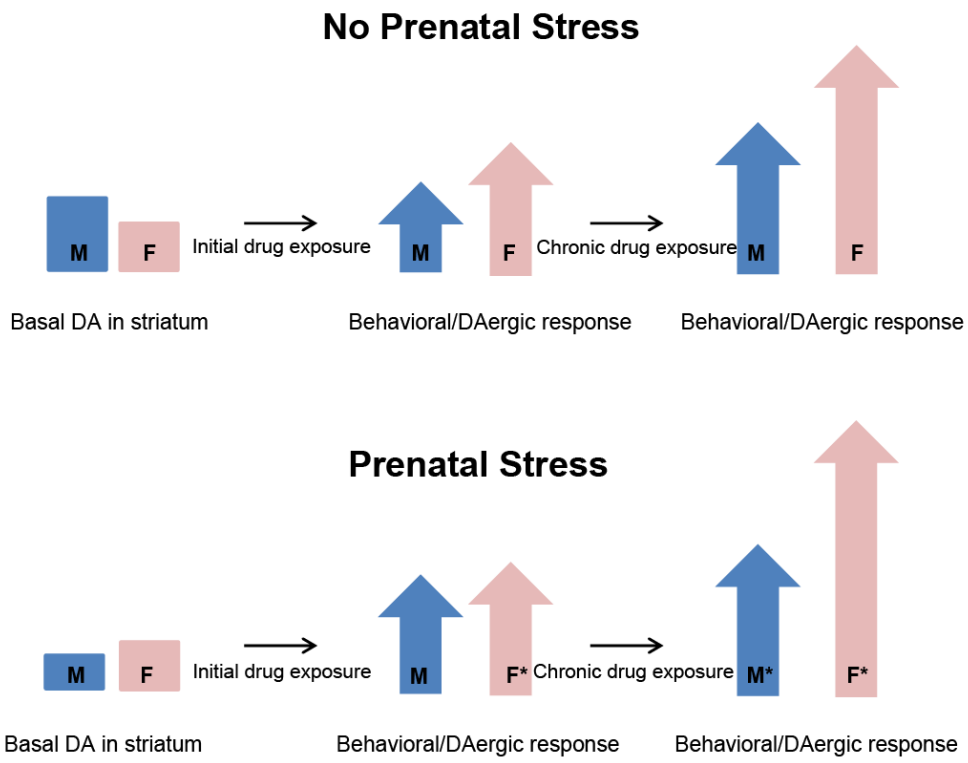

Figure 5.2. An overview of changes in sexual dimorphisms as a consequence of prenatal stress (PS). Males (M) and Females (F) are represented by blue and pink shapes, respectively. Well-established sex differences in rats without a history of PS are displayed along the top. In drug-naïve No PS rats, males have higher basal dopamine (DA) levels in the striatum than ovariectomized (OVXed) females. When first administered a drug of abuse, females show a greater behavioral and striatal DAergic response than males. This sex difference becomes even more pronounced after *chronic* drug treatment. Importantly, PS changes the sexual dimorphism at each of these stages. In drug-naïve subjects, PS diminishes basal DA levels exclusively in males, making them appear "feminized." Consequently, PS reverses the direction of the sex difference seen in No PS controls. A comparable effect is evident when provided their first experience with drugs – PS abolishes the sex difference seen in No PS controls by promoting the neurochemical and behavioral response of males. After chronic drug exposure, however, PS selectively augments the drug responsiveness of *females*. In doing so, PS widens the sex difference observed in rats with a chronic drug history. As demonstrated in Chapter IV, this translates into a greater risk of drug pursuit becoming compulsive-like in appearance. * denotes a DAergic response that has not been shown empirically, but is presumed based on corresponding behavioral changes.

APPENDICES

APPENDIX A

SUPPLEMENTARY FIGURES FOR CHAPTER II

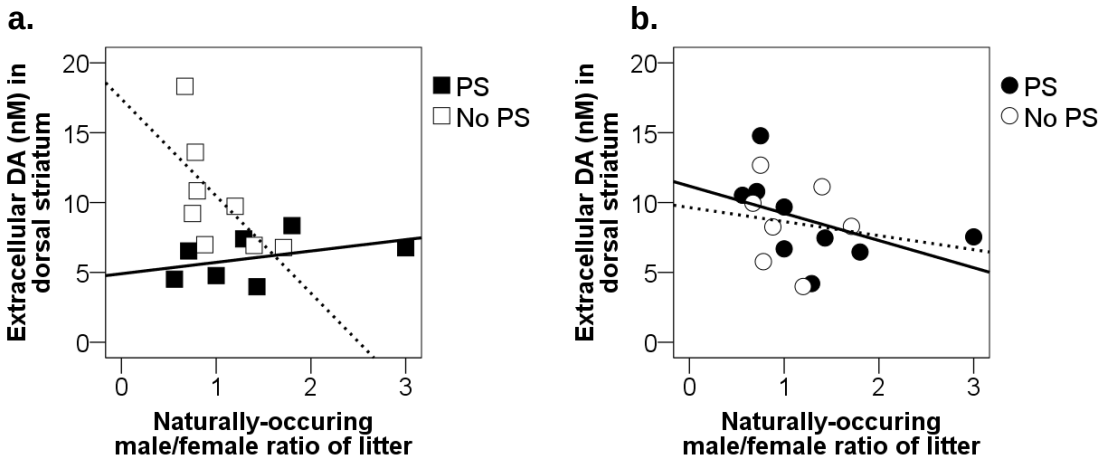

Figure A.1 Relationship between the prenatal sex ratio (the naturally-occurring ratio of males to females in the litter) and steady-state extracellular dopamine (DA) concentrations in the dorsal striatum of drug-naïve males (a) and females (b) tested with in vivo microdialysis. After obtaining sex-based litter averages of sampled DA, ANOVA indicated that the sex ratio was a modest overall predictor of future DA concentrations (F[1, 23] = 3.83; p = 0.063). In addition, its predictive value was sex and PS dependent (Sex X PS X Sex ratio; F[1, 23] = 3.51; p = 0.074). (a) Follow-up analyses revealed a very different relationship between these measures in PS vs. No PS males (F[1, 11] = 6.68; p = 0.025). A negative and near-significant correlation was observed in No PS rats (Pearson r = - 0.65; p = 0.08), even after controlling for the post-culling sex ratio (partial r = - 0.68; p = 0.095). In contrast, no correlation was found in males with a history of PS, with (partial r = 0.32; p = 0.54) or without (Pearson r = 0.41; p = 0.36) controlling for litter composition after culling. (b) No overall or PS-dependent relationship was evident in females (estradiol and oil-treated rats pooled together) after performing similar analyses. The correlation between DA and the litter sex ratio failed to reach or approach significance in either group. Solid and dotted lines represent the lines of best fit for sex-matched PS and No PS groups, respectively.

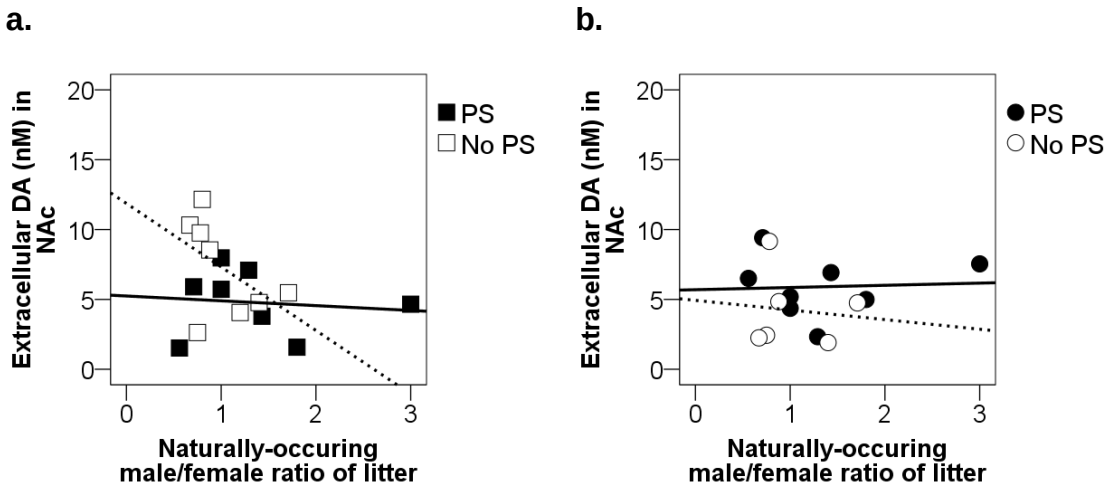

Figure A.2 Relationship between the prenatal sex ratio (the naturally-occurring ratio of males to females in the litter) and steady-state extracellular dopamine (DA) concentrations in the nucleus accumbens (NAc) of drug-naïve males (a) and females (b) tested with in vivo microdialysis. Unlike samples obtained from the dorsal striatum, DA concentrations in the NAc were not significantly related to prenatal litter composition for any group or treatment condition. Solid and dotted lines represent the lines of best fit for sex-matched PS and No PS groups, respectively.

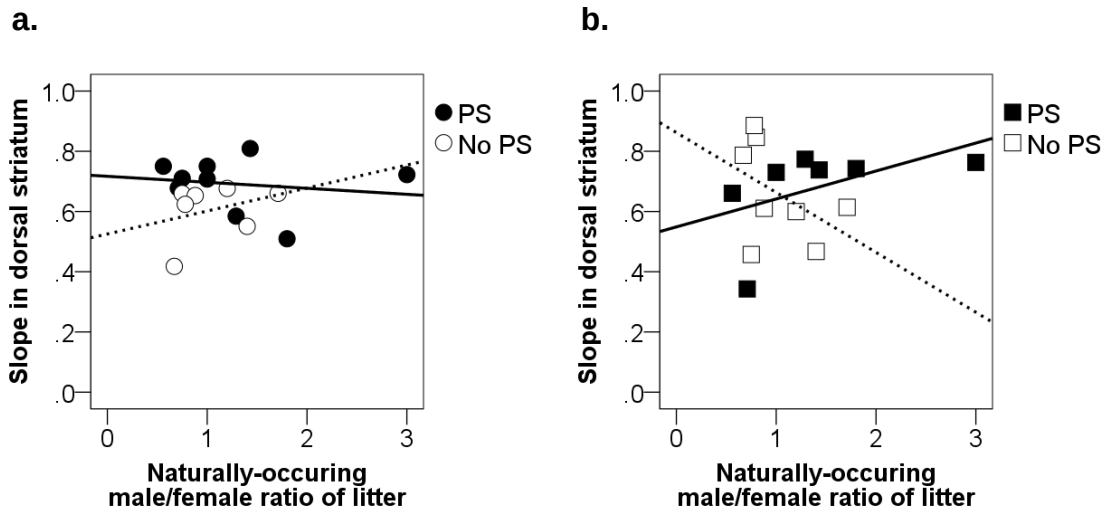

Figure A.3 Relationship between the prenatal sex ratio (the naturally-occurring ratio of males to females in the litter) and the slope of the curve used to calculate extracellular dopamine (DA) concentrations in the dorsal striatum of drug-naïve males (a) and females (b). The ability of the litter sex ratio to predict the slope was sex and PS dependent (Sex X PS X Sex ratio; F[1, 23] = 3 .67; p = 0.068). Follow-up ANOVAs did not indicate that the sex ratio had a differential relationship with the slope for male or female groups, or PS and No PS rats. Solid and dotted lines represent the lines of best fit for sex-matched PS and No PS groups, respectively.

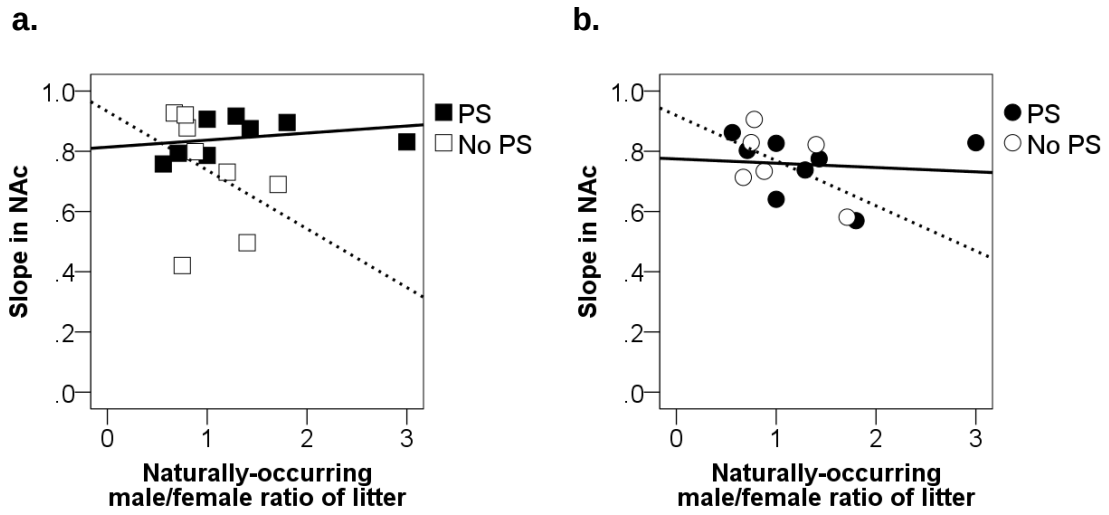

Figure A.4 The relationship between the prenatal sex ratio (the naturally-occurring ratio of males to females in the litter) and the slope of the curve used to calculate extracellular dopamine (DA) concentrations in the nucleus accumbens (NAc) of drug-naïve males (a) and females (b). No significant overall or treatment-specific effect of prenatal sex ratio was observed. Solid and dotted lines represent the lines of best fit for sex-matched PS and No PS groups, respectively.

APPENDIX B

SUPPLEMENTARY FIGURES FOR CHAPTER IV

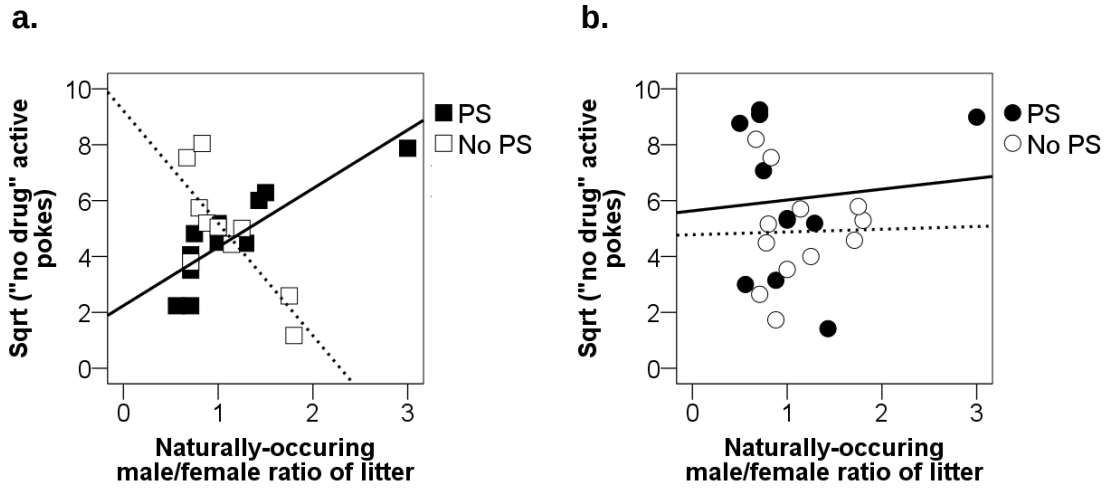

Figure B.1 Relationship between the prenatal sex ratio (naturally-occurring ratio of males to females in the litter) and drug seeking in the absence of reinforcement (averaged "no drug" active pokes for both sexes in each litter, following a square root [sqrt] transformation) for males (a) and females (b) tested for cocaine self-administration. A PS and sex-specific relationship was observed (Sex X PS X Sex ratio; $F[1, 36] = 5.65$ $p = 0.023$). (a) The prenatal sex ratio differentially predicted the drug-seeking behavior of PS vs. No PS males (PS X Sex ratio; $F[1, 17] = 33.4$; $p < 0.001$). The correlation was positive for PS males (Pearson $r = 0.86$; $p = 0.001$) and negative for No PS controls (Pearson $r = -0.8$; $p = 0.006$). (b) In contrast, no overall or stress-specific relationship was observed in females. Correlations for litters with (Pearson $r = 0.1$; $p = 0.78$) and without (Pearson $r = 0.02$; $p = 0.95$) a history of PS failed to reach significance. Solid and dotted lines represent the lines of best fit for sex-matched PS and No PS groups, respectively.

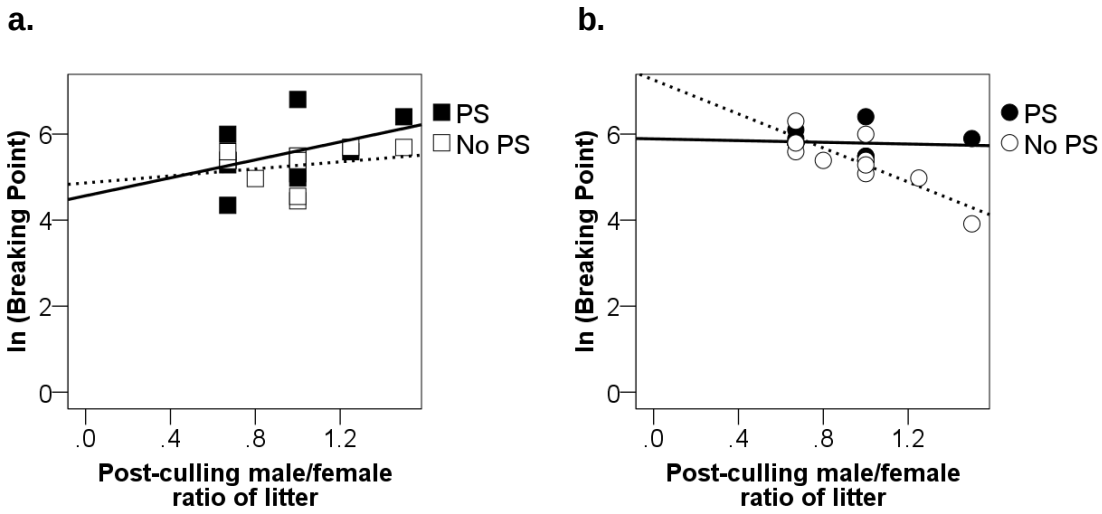

Figure B.2 Relationship between the ratio of males to females in the litter *after* culling and motivation for cocaine (transformed and averaged "breaking point" values for both sexes in each litter) for males (a) and females (b) tested with a progressive ratio schedule of cocaine self-administration. The relationship was different for males vs. females (Sex X Surviving sex ratio; $F[1, 37] = 10.8$; $p < 0.01$) and PS vs. No PS individuals (PS X Surviving sex ratio; $F[1, 37] = 5.48$; $p = 0.025$). (a) In males, no overall or PS-dependent effect of the sex ratio was observed. (b) In females, the post-culling sex ratio was a significant predictor of motivation for cocaine ($F[1, 19] = 15.11$; $p = 0.001$), especially in No PS controls (PS X Postnatal sex ratio; $F[1, 19] = 12.35$; $p < 0.01$). A negative and highly-significant correlation was found in No PS rats (Pearson $r = - 0.85$; $p < 0.001$), even after controlling for the sex ratio before culling (partial $r = - 0.8$; $p = 0.003$). In contrast, no significant correlation was seen in females with a history of PS (Pearson $r = - 0.09$; $p = 0.79$) after performing identical analyses (partial $r = - 0.1$; $p = 0.79$). Solid and dotted lines represent the lines of best fit for sex-matched PS and No PS groups, respectively.

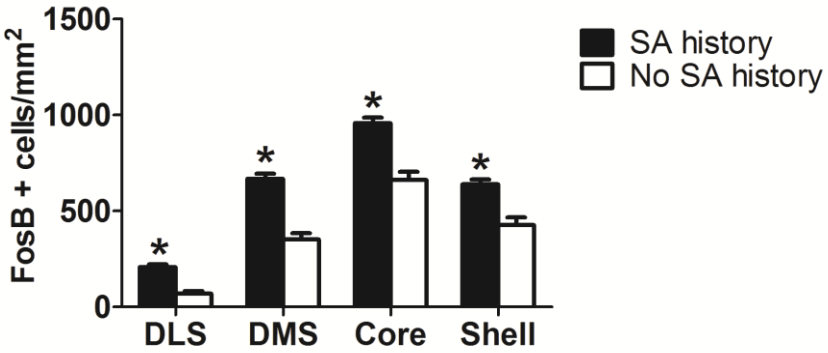

Figure B.3 FosB immunoreactivity in rats that did (SA history; n = 73) and did not (No SA history; n = 26) participate in cocaine self-administration (SA) testing. Brains were collected 48 hours after the final test session, with littermates never tested for SA serving as drug-naïve controls. Higher overall FosB levels were observed in the SA rats ($F[1, 45.47] = 41.2$; $p < 0.001$), which reflects staining intensity in the dorsolateral (DLS) and dorsomedial (DMS) compartments of the striatum, as well as the core and shell regions of the nucleus accumbens. * denotes a significant difference between rats with and without a history of SA at $p < 0.05$.

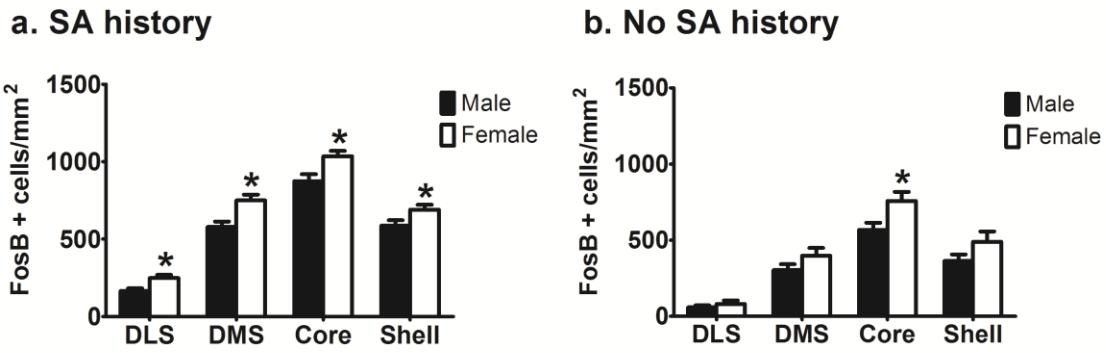

Figure B.4 FosB immunoreactivity in males and females with (a; SA history) and without (b; No SA history) prior cocaine self-administration (SA) experience. Females expressed more FosB than males ($F[1, 45.47] = 9.71$; $p < 0.01$), independent of prenatal stress, brain region, and SA experience. Nevertheless, pairwise comparisons revealed a significant sex difference in each region of rats tested with SA, while the same was true for only the nucleus accumbens core in drug-naïve controls. Brain regions sampled were the dorsolateral (DLS) and dorsomedial (DMS) compartments of the striatum, as well as the core and shell regions of the nucleus accumbens. * indicates a significant difference between males and females at $p < 0.05$.

APPENDIX C

SEX-DEPENDENT EFFECTS OF PRENATAL STRESS ON STRESS PEPTIDE LEVELS FOLLOWING COCAINE EXPOSURE

Methods

Subjects

For breeding purposes, male (275–300 g) and virgin female (200–225 g) Sprague–Dawley rats (Harlan Sprague–Dawley Indianapolis, IN) were housed under a 14/10 hour light/dark cycle (lights on at ZT0000 and off at ZT1400) in an environment maintained at 20–21 °C. Animals had *ad libitum* access to water and phytoestrogen-free rodent chow (2014 Teklad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI). All procedures were performed according to the protocol approved by the University of Michigan Committee for Use and Care of Animals.

One week after arrival, males and sexually-receptive females were paired overnight for mating. Vaginal lavage was to determine sexual receptivity, as well as to check for sperm following paired mating. When sperm was detected (designated as gestational day 0 [GD 0]), the female was removed and individually housed. Each male was allowed to sire no more than 1 litter.

Beginning on GD 15, a random selection of impregnated females (n = 14) were subjected to repeated restraint stress, which consisted of placement in a Plexiglass restrainer for 45 minutes 3 times per day (beginning at ZT0300, ZT0600, and ZT1000) through GD 21. Females not subjected to restraint (n = 13) were left undisturbed, except for weekly cage changes, throughout pregnancy. On the day of birth (postnatal day 1 [PD 1]), pups were briefly removed from the cage and weighed. Litter size was then culled to 10 per dam whenever possible), with an equal or near-equal ratio of males to females.

Surviving pups were placed back with the mother and left undisturbed until PD 21. On PD 21, pups were separated from their mother and housed in isosexual groups of 4-6 siblings/cage. All rats were handled at least once daily from approximately PND 33 to 50 to check for the timing of vaginal opening and preputial separation of females and males, respectively.

Ovariectomy

At approximately 55 days of age, female subjects were anesthetized by inhalation of 2.5% isoflurane and subjected to bilateral ovariectomy (OVX) via a dorsal approach (Hu and Becker, 2003). Males were anesthetized for approximately 10 minutes in duration to control for isoflurane exposure, but were not castrated. After 7 days of recovery, all females underwent vaginal lavage testing to confirm the cessation of cycling. Males were handled to control for possible handling effects.

Habituation

Following recovery from surgery, rats were habituated to locomotor testing conditions. Habituation entailed removal from the animal colony, administration of a 0.1 ml injection of peanut oil (subcutaneous [SC]), and individual placement in a circular plastic tub (60 cm high, 50 cm in diameter) for 30 minutes. Following the 30-minute period, rats were given a 1 ml/kg intraperitoneal (IP) injection of saline and returned to the tub for a 60 minute duration. At the conclusion of the 60-minute period, rats were removed from the tubs and returned to their home cages. Each subject received a total of 2-3 habituation sessions, with no more than 1 occurring in a 24 hour period. Habituation sessions were performed during the light cycle, with “white noise” playing in the background.

Locomotor testing

At least 24 hours after completing their final habituation session, rats were again transferred from the animal colony to the locomotor testing environment. All females were subsequently administered 5 µg of 17ß-Estradiol benzoate (EB) dissolved in 0.1 ml peanut oil (SC). Conversely, males were injected with 0.1 ml peanut oil alone. Immediately after, rats were individually placed in plastic tubs for a 30-minute period. After 30 minutes, rats received the first of a series of 3 IP

injections of either cocaine (each 15 mg/kg) or saline (each 1 ml/kg), with each injection being separated by 60 minutes in time. Immediately following each injection, animals were returned to the plastic tub. As with habituation sessions, all testing was performed during the light cycle. White noise was played in the background.

In situ hybridization

Sixty minutes after the final injection of cocaine or saline, rats were deeply anesthetized with an IP injection of Fatal-Plus solution. Brains were then rapidly removed, flash frozen with dry ice, and stored at -80 ºC until sectioning.

Coronal brain sections (14 µm) were cut on a cryostat and mounted onto slides (FisherBiotech ProbeOn Plus; Fisher Scientific, Pittsburgh, PA) in preparation for in situ hybridization. A riboprobe was used to localize corticotropin-releasing-hormone (CRH) mRNA within the central amygdala (CeA; -1.88 to -2.12 from Bregma), while an oligoprobe was used to do the same for cocaine- and amphetamine-regulated transcript (CART) in the core and shell regions of the nucleus accumbens (NAc; +1.6 from Bregma). All sections were fixed in 4% paraformaldehyde at room temperature for 60 minutes. Next, tissue was acetylated with 0.25% acetic anhydride and dehydrated in ascending ethanol concentrations. Finally, sections were hybridized with ^{35}S-labeled antisense overnight at 58 ºC.

Statistical Analysis

After hybridization, slides were exposed to Kodak Biomax film for 96 hours (CRH) or 14 days (CART). Films were then scanned and analyzed with Image J software. Background density was subtracted from the integrated optical density (OD) of each region of interest.

CART OD values from the core and shell regions were independently analyzed with analyses of variance (ANOVAs). Sex (2 levels), prenatal stress (2 levels), and drug (2 levels) were the between-subject factors. Significant effects ($p < 0.05$) were followed up with Bonferroni post hoc tests to compensate for multiple comparisons.

Due to low group sizes, CRH OD in the CeA was analyzed separately for

males and females. Prenatal stress (2 levels) and drug (2 levels) were the between-subject factors. Again, significant effects ($p < 0.05$) were followed up with Bonferroni post hoc tests to compensate for multiple comparisons.

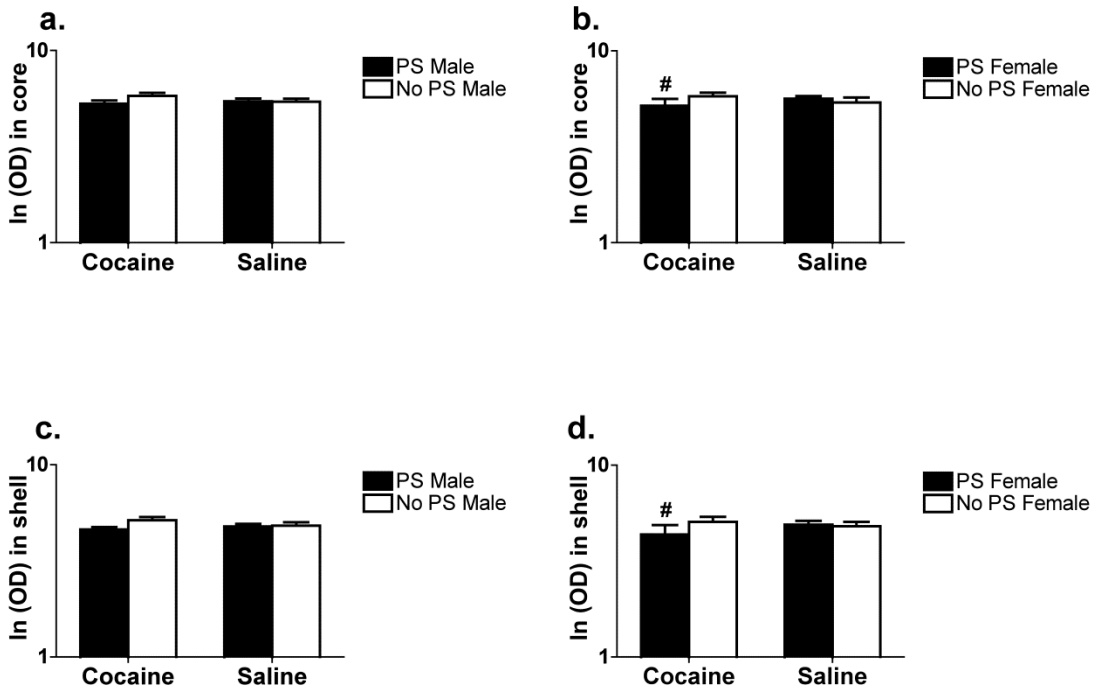

Figure C.1 Optical density (OD) of CART mRNA in the nucleus accumbens core (a-b) and shell (c-d) following a natural log (ln) transformation. Prenatal stress (PS) tended to reduce CART levels in rats administered cocaine, but not saline (PS X Drug; $F[1, 34] = 3.75$; $p = 0.06$ in the core; PS X Drug; $F[1, 33] = 2.9$; $p = 0.01$ in the shell). Pairwise comparisons suggested that this reduction was stronger in females than in males. In females, the difference between cocaine-treated PS and No PS groups approached significance in both areas (denoted by #). No such effect of PS was observed in cocaine-treated males.

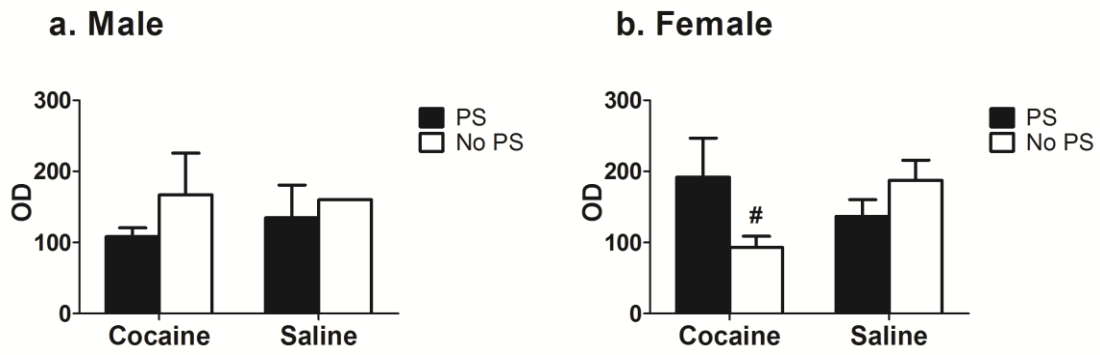

Figure C.2 Optical density (OD) of CRH mRNA in the central amygdala of males (a) and females (b). (b) In females, prenatal stress (PS) and drug had a synergistic effect (PS X Drug; F[1, 11] = 4.43; p = 0.06) that resulted in PS rats exhibiting great CRH expression after cocaine than identically-treated No PS controls (denoted by #; p = 0.065).

APPENDIX D

CHAPTER CITATIONS

CHAPTER II:

Thomas MB, Yang H, Becker JB (2012) Sex-specific effects of prenatal stress on steady-state extracellular dopamine in the nucleus accumbens and dorsal striatum. Submitted.

CHAPTER III:

Thomas MB, Hu M, Lee TM, Bhatnagar S, Becker JB (2009) Sex-specific susceptibility to cocaine in rats with a history of prenatal stress. *Physiology & Behavior* 97:270-277.

CHAPTER IV:

Thomas MB & Becker JB (2012) Sex differences in prenatal stress effects on cocaine pursuit. In Prep.

REFERENCES

Adrover E, Berger MA, Perez AA, Tarazi FI, Antonelli MC (2007) Effects of prenatal stress on dopamine D2 receptor asymmetry in rat brain. Synapse 61:459-462.

Andersen SL, Rutstein M, Benzo JM, Hostetter JC, Teicher MH (1997) Sex differences in dopamine receptor overproduction and elimination. Neuroreport 8:1495-1498.

Andersen SL, Teicher MH (2000) Sex differences in dopamine receptors and their relevance to ADHD. Neurosci Biobehav Rev 24:137-141.

Andersen SL, Thompson AP, Krenzel E, Teicher MH (2002) Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. Psychoneuroendocrinology 27:683-691.

Anderson DK, Rhees RW, Fleming DE (1985) Effects of prenatal stress on differentation of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the rat brain. Brain Res 332:113-118.

Anker JJ, Carroll ME (2010) Sex differences in the effects of allopregnanolone on yohimbine-induced reinstatement of cocaine seeking in rats. Drug Alcohol Depend 107:264-267.

Anker JJ, Larson EB, Gliddon LA, Carroll ME (2007) Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. Experimental and Clinical Psychopharmacology 15:472-480.

Anker JJ, Perry JL, Gliddon LA, Carroll ME (2009) Impulsivity predicts the escalation of cocaine self-administration in rats. Pharmacol Biochem Behav 93:343-348.

Anthony JC, Warner LA, Kessler RC (1994) Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: basic findings from the national comorbidity survey. Experimental and Clinical Psychopharmacology 2:244-268.

American Psychiatric Association (2003) Diagnostic and statistical manual of mental disorders: DSM-IV-TR. Washington D.C: Amercian Psychiatric Association.

Austin KB, Beiswanger C, Bronzino JD, Austinlafrance RJ, Galler JR, Morgane PJ (1992) Prenatal protein malnutrition alters behavioral state modulation of inhibition and facilitation in the dentate gyrus. Brain Res Bull 28:245-255.

Austin KB, Bronzino J, Morgane PJ (1986) Prenatal protein malnutrition affects synaptic potentiation in the dentate gyrus of rats in adulthood. Dev Brain Res 29:267-273.

Back SE, Hartwell K, DeSantis SM, Saladin M, McRae-Clark AL, Price KL, Maria M, Baker NL, Spratt E, Kreek MJ, Braly KT (2010) Reactivity to laboratory stress provocation predicts relapse to cocaine. Drug Alcohol Depend 106:21-27.

Baker S, Chebli M, Rees S, LeMarec N, Godbout R, Bielajew C (2008) Effects of gestational stress. 1. evaluation of maternal and juvenile offspring behavior. Brain Res 1213:98-110.

Barbazanges A, Piazza PV, Le Moal M, Maccari S (1996) Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. J Neurosci 16:3943-3949.

Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 153:31-43.

Barker DJP (1990) The fetal and infant origins of adult disease. Br Med J 301:1111-1111.

Barker DJP (1995) Fetal origins of coronary heart disease. Br Med J 311:171-174.

Barker DJP (1998) In utero programming of chronic disease. Clin Sci 95:115-128.

Barker DJP, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. Br Med J 301:259-262.

Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension, and hyperlipemia (syndrome x): relation to reduced fetal growth. Diabetologia 36:62-67.

Barros VG, Rodriguez P, Martijena ID, Perez A, Molina VA, Antonelli MC (2006) Prenatal stress and early adoption effects on benzodiazepine receptors and anxiogenic behavior in the adult rat brain. Synapse 60:609-618.

Bayer SA (1981) A correlated study of neurogenesis, morphogenesis and cytodifferentiation in the rat nucleus accumbens. In: The neurobiology of the Nucleus Accumbens (Chronister, R. B. and De France, J. F., eds), pp 173-197 Brunswick, Maine: Haer Institute for Electrophysiological Research.

Bayer SA, Altman J, Russo RJ, Zhang X (1993) Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14:83-144.

Becker JB (1990a) Direct effect of 17 beta-estradiol on striatum: sex differences in dopamine release. Synapse 5:157-164.

Becker JB (1990b) Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. Neurosci Lett 118:169-171.

Becker JB, Berkley KJ, Geary N, Hampson E, Herman JP, Young E (2008) Sex differences in the brain: from genes to behavior. New York: Oxford University Press.

Becker JB, Cha JH (1989) Estrus cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. Behav Brain Res 35:117-125.

Becker JB, Hu M (2008) Sex differences in drug abuse. Front Neuroendocrinol 29:36-47.

Becker JB, Ramirez VD (1980) Dynamics of endogenous catecholamine release from brain fragments of male and female rats. Neuroendocrinology 31:18-25.

Becker JB, Ramirez VD (1981a) Experimental studies on the development of sex differences in the release of dopamine from striatal tissue fragments in vitro. Neuroendocrinology 32:168-173.

Becker JB, Ramirez VD (1981b) Sex differences in the amphetamine-stimulated release of catecholamines from rat striatal tissue in vitro. Brain Res 204:361-372.

Becker JB, Rudick CN (1999) Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: a microdialysis study. Pharmacol Biochem Behav 64:53-57.

Beckhardt S, Ward IL (1983) Reproductive functioning in the prenatally stressed

female rat. Dev Psychobiol 16:111-118.

Belin D, Balado E, Piazza PV, Deroche-Gamonet V (2009) Pattern of intake and drug craving predict the development of cocaine addiction-like behavior in rats. Biol Psychiatry 65:863-868.

Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V (2011) High-novelty-preference rats are predisposed to compulsive cocaine self-administration. Neuropsychopharmacology 36:569-579.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320:1352-1355.

Berger MA, Barros VG, Sarchi MI, Tarazi FI, Antonelli MC (2002) Long-term effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. Neurochem Res 27:1525-1533.

Beveridge TJR, Smith HR, Daunais JB, Nader MA, Porrino LJ (2006) Chronic cocaine self-administration is associated with altered functional activity in the temporal lobes of non human primates. Eur J Neurosci 23:3109-3118.

Beydoun H, Saftlas AF (2008) Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. Paediatr Perinat Epidemiol 22:438-466.

Bhatnagar S, Lee TM, Vining C (2005) Prenatal stress differentially affects habituation of corticosterone responses to repeated stress in adult male and female rats. Horm Behav 47:430-438.

Blatt GJ, Chen JC, Rosene DL, Volicer L, Galler JR (1994) Prenatal protein malnutrition effects on the serotonergic system in the hippocampal formation: an immunocytochemical, ligand binding, and neurochemical study. Brain Res Bull 34:507-518.

Bloom SL, Sheffield JS, McIntire DD, Leveno KJ (2001) Antenatal dexamethasone and decreased birth weight. Obstet Gynecol 97:485-490.

Bohn MC, Dean D, Hussain S, Giuliano R (1994) Development of messenger RNAs for glucocorticoid and mineralocorticoid receptors in rat hippocampus. Dev Brain Res 77:157-162.

Bowman RE, Zrull MC, Luine VN (2001) Chronic restraint stress enhances radial arm maze performance in female rats. Brain Res 904:279-289.

Brady KT, Randall CL (1999) Gender differences in substance use disorders. Psychiatr Clin North Amer 22:241-252.

Broaddus WC, Bennett JP (1990) Postnatal development of striatal dopamine function. 1. an examination of D1 and D2 receptors, adenylate cyclase regulation and presynaptic dopamine markers. Dev Brain Res 52:265-271.

Brown RM, Lawrence AJ (2009) Neurochemistry underlying relapse to opiate seeking behaviour. Neurochem Res 34:1876-1887.

Bruinink A, Lichtensteiger W, Schlumpf M (1983) Prenatal and postnatal ontogeny and characterization of dopaminergic D2, serotonergic S2, and spirodecanone binding sites in rat forebrain. J Neurochem 40:1227-1236.

Caine SB, Bowen CA, Yu G, Zuzga D, Negus SS, Mello NK (2004) Effect of gonadectomy and gonadal hormone replacement on cocaine self-administration in female and male rats. Neuropsychopharmacology 29:929-942.

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A 95:5335-5340.

Cameron NM, Champagne FA, Fish C, Ozaki-Kuroda K, Meaney MJ (2005) The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care. Neurosci Biobehav Rev 29:843-865.

Camp DM, Robinson TE (1988) Susceptibility to sensitization. 2. The influence of gonadal hormones on enduring changes in brain monoamines and behavior produced by the repeated administration of d-amphetamine or restraint stress. Behav Brain Res 30:69-88.

Carboni E, Barros VG, Ibba M, Silvagni A, Mura C, Antonelli MC (2010) Prenatal restraint stress: an in vivo microdialysis study on catecholamine release in the rat prefrontal cortex. Neuroscience 168:156-166.

Carboni E, Vacca C (2003) Conditioned place preference: a simple method for investigating reinforcing properties in laboratory animals. Drugs of Abuse: Neurological Reviews and Protocols 79:481-498.

Carlezon WA (2003) Place conditioning to study drug reward and aversion. Opioid Research: Methods and Protocols 84:243-249.

Carroll ME, Anker JJ (2010) Sex differences and ovarian hormones in animal models of drug dependence. Horm Behav 58:44-56.

Carroll ME, Lac ST (1997) Acquisition of IV amphetamine and cocaine self-

administration in rats as a function of dose. Psychopharmacology 129:206-214.

Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP (2004) Sex and estrogen influence drug abuse. Trends in Pharmacological Sciences 25:273-279.

Carroll ME, Morgan AD, Lynch WJ, Campbell UC, Dess NK (2002) Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: phenotype and sex differences. Psychopharmacology 161:304-313.

Castner SA, Xiao L, Becker JB (1993) Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. Brain Res 610:127-134.

Champagne FA, Meaney MJ (2006) Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. Biol Psychiatry 59:1227-1235.

Chen DY, Liu F, Shang QG, Song XQ, Miao XP, Wang ZZ (2011) Association between polymorphisms of DRD2 and DRD4 and opioid dependence: evidence from the current studies. Am J Med Genet B 156B:661-670.

Chen R, Osterhaus G, McKerchar T, Fowler SC (2003) The role of exogenous testosterone in cocaine-induced behavioral sensitization and plasmalemmal or vesicular dopamine uptake in castrated rats. Neurosci Lett 351:161-164.

Chin J, Sternin O, Wu HBK, Burrell S, Lu D, Jenab S, Perrotti LI, Quinones-Jenab V (2002) Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration. Brain Res 945:123-130.

Cicero TJ, Aylward SC, Meyer ER (2003) Gender differences in the intravenous self-administration of mu opiate agonists. Pharmacol Biochem Behav 74:541-549.

Clarke AS, Wittwer DJ, Abbott DH, Schneider ML (1994) Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. Dev Psychobiol 27:257-269.

Coirini H, Magarinos AM, Denicola AF, Rainbow TC, McEwen BS (1985) Further studies of brain aldosterone binding sites employing new mineralocorticoid and glucocorticoid receptor markers in vitro. Brain Res 361:212-216.

Crabbe JC, Phillips TJ, Belknap JK (2010) The complexity of alcohol drinking:

studies in rodent genetic models. Behav Genet 40:737-750.

Crombag HS, Shaham Y (2002) Renewal of drug seeking by contextual cues after prolonged extinction in rats. Behav Neurosci 116:169-173.

Dalley JW, Fryer TD, Brichard L, Robinson ESJ, Theobald DEH, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW (2007) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science 315:1267-1270.

Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L, Levin N (1987) Regulation of ACTH secretion: variations on a theme of B. Recent Prog Horm Res 43:113-173.

Darnaudery M, Louvart H, Defrance L, Leonhardt M, Morley-Fletcher S, Gruber SH, Galietta G, Mathe AA, Maccari S (2007) Impact of an intense stress on ethanol consumption in female rats characterized by their pre-stress preference: Modulation by prenatal stress. Brain Res 1131:181-186.

Davis BA, Clinton SM, Akil H, Becker JB (2008) The effects of novelty-seeking phenotypes and sex differences on acquisition of cocaine self-administration in selectively bred High-Responder and Low-Responder rats. Pharmacol Biochem Behav 90:331-338.

De Kloet ER (1991) Brain corticosteroid receptor balance and homeostatic control. Front Neuroendocrinol 12:95-164.

De Kloet ER, Wallach G, McEwen BS (1975) Differences in corticosterone and dexamethasone binding to rat brain and pituitary. Endocrinology 96:598-609.

de Medeiros CB, Rees SL, Llinas M, Fleming AS, Crews D (2010) Deconstructing early life experiences: distinguishing the contributions of prenatal and postnatal factors to adult male sexual behavior in the rat. Psychological Science 21:1494-1501.

De Vries TJ, Homberg JR, Binnekade R, Raaso H, Schoffelmeer ANM (2003) Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology 168:164-169.

De Vries TJ, Schoffelmeer ANM, Binnekade R, Mulder AH, Vanderschuren L (1998) MK-801 reinstates drug-seeking behaviour in cocaine-trained rats. Neuroreport 9:637-640.

De Vries TJ, Schoffelmeer ANM, Binnekade R, Raaso H, Vanderschuren L (2002) Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. Neuropsychopharmacology 26:18-26.

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 75:134-143.

de Wit H, Stewart J (1983) Drug reinstatement of heroin-reinforced responding in the rat. Psychopharmacology 79:29-31.

Dejong W (1994) Relatpse prevention: an emerging technology for promoting long-term drug abstinence. Int J Addict 29:681-705.

Del Cerro MCR, Perez-Laso C, Ortega E, Martin JLR, Gomez F, Perez-Izquierdo MA, Segovia S (2010) Maternal care counteracts behavioral effects of prenatal environmental stress in female rats. Behav Brain Res 208:593-602.

Deminiere JM, Piazza PV, Guegan G, Abrous N, Maccari S, Le Moal M, Simon H (1992) Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. Brain Res 586:135-139.

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for addiction-like behavior in the rat. Science 305:1014-1017.

Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning disorder. Eur J Pharmacol 375:13-30.

Di Paolo T, Falardeau P, Morissette M (1988) Striatal D2 dopamine agonst binding sites fluctuate during the rat estrous cycle. Life Sciences 43:665-672.

Diergaarde L, Pattij T, Poortvliet I, Hogenboom F, de Vries W, Schoffelmeer ANM, De Vries TJ (2008) Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. Biol Psychiatry 63:301-308.

Dluzen DE, McDermott JL (2008) Sex differences in dopamine- and vesicular monoamine-transporter functions: implications for methamphetamine use and neurotoxicity. In: Drug addiction: research frontiers and treatment advances, vol. 1139 (Kuhar, M. J., ed), pp 140-150 Oxford: Blackwell Publishing.

Dluzen DE, Ramirez VD (1987) Intermittent infusion of progesterone potentiates

whereas continuous infusion reduces amphetamine-stimulated dopamine release from ovariectomized estrogen-primed rat striatal fragments superfused in vitro. Brain Res 406:1-9.

Dluzen DE, Ramirez VD (1990) In vitro progesterone modulation of amphetamine-stimulated dopamine release from the corpus striatum o ovariectomized estrogen-treated female rats: response characteristics. Brain Res 517:117-122.

Dluzen DE, Ramirez VD (1991) Modulatory effects of progesterone upon dopamine release from the corpus striatum of ovariectomized estrogen-treated rats are stereo-specific. Brain Res 538:176-179.

Dodic M, Hantzis V, Duncan J, Rees S, Koukoulas I, Johnson K, Wintour EM, Moritz K (2002) Programming effects of short prenatal exposure to cortisol. Faseb J 16:1017-1026.

Dodic M, May CN, Wintour EM, Coghlan JP (1998) An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. Clin Sci 94:149-155.

Dodic M, Samuel C, Moritz K, Wintour EM, Morgan J, Grigg L, Wong J (2001) Impaired cardiac functional reserve and left ventricular hypertrophy in adult sheep after prenatal dexamethasone exposure. CircRes 89:623-629.

Donny EC, Caggiula AR, Rowell PP, Gharib MA, Maldovan V, Booth S, Mielke MM, Hoffman A, McCallum S (2000) Nicotine self-administration in rats: estrous cycle effects, sex differences and nicotinic receptor binding. Psychopharmacology 151:392-405.

Early RJ, McBride BW, Vatnick I, Bell AW (1991) Chronic heat stress and prenatal development in sheep. 2. placental cellularity and metabolism. J Anim Sci 69:3610-3616.

Economidou D, Pelloux Y, Robbins TW, Dalley JW, Everitt BJ (2009) High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence. Biol Psychiatry 65:851-856.

Epstein DH, Preston KL, Stewart J, Shaham Y (2006) Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology 189:1-16.

Evanson NK, Herman JP, Sakai RR, Krause EG (2010) Nongenomic Actions of Adrenal Steroids in the Central Nervous System. J Neuroendocrinol 22:846-861.

Everitt BJ, Dickinson A, Robbins TW (2001) The neuropsychological basis of addictive behaviour. Brain Res Rev 36:129-138.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481-1489.

Farkas LM, Huttner WB (2008) The cell biology of neural stem and progenitor cells and its significance for their proliferation versus differentiation during mammalian brain development. Current Opinion in Cell Biology 20:707-715.

Fatseas M, Denis C, Massida Z, Verger M, Franques-Reneric P, Auriacombe M (2011) Cue-induced reactivity, cortisol response and substance use outcome in treated heroin dependent individuals. Biol Psychiatry 70:720-727.

Fattore L, Spano MS, Altea S, Angius F, Fadda P (2007) Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. Br J Pharmacol 152:795-804.

Fattore L, Spano MS, Altea S, Fadda P, Fratta W (2010) Drug- and cue-induced reinstatement of cannabinoid-seeking behaviour in male and female rats: influence of ovarian hormones. Br J Pharmacol 160:724-735.

Feltenstein MW, Byrd EA, Henderson AR, See RE (2009) Attenuation of cocaine-seeking by progesterone treatment in female rats. Psychoneuroendocrinology 34:343-352.

Feltenstein MW, Henderson AR, See RE (2011) Enhancement of cue-induced reinstatement of cocaine-seeking in rats by yohimbine: sex differences and the role of the estrous cycle. Psychopharmacology 216:53-62.

Feltenstein MW, See RE (2007) Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. Drug Alcohol Depend 89:183-189.

Fishell G, Van der Kooy D (1987) Pattern formation in the striatum: developmental changes in the distribution of striatonigral neurons. J Neurosci 7:1969-1978.

Flagel SB, Robinson TE (2007) Quantifying the psychomotor activating effects of cocaine in the rat. Behav Pharmacol 18:297-302.

Fleming DE, Kinghorn EW, Rhees RW, Anderson RH, Smythe E (1992) Biological predictors of masculine sexual behavior in prentally stressed

and nonstressed rats. Bulletin of the Psychonomic Society 30:513-514.

Forgie ML, Stewart J (1994) Sex differences in the locomotor-activating effects of amphetamine: role of circulating testosterone in adulthood. Physiol Behav 55:639-644.

Fowden AL (1995) Endocrine regulation of fetal growth. Reprod Fertil Dev 7:351-363.

Francis DD, Kuhar MJ (2008) Frequency of maternal licking and grooming correlates negatively with vulnerability to cocaine and alcohol use in rats. Pharmacol Biochem Behav 90:497-500.

Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD (1996) Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet 348:1478-1480.

French NP, Hagan R, Evans SF, Godfrey M, Newnham JP (1999) Repeated antenatal corticosteroids: size at birth and subsequent development. Am J Obstet Gynecol 180:114-121.

Frye CA, Orecki ZA (2002a) Prenatal stress alters reproductive responses of rats in behavioral estrus and paced mating of hormone-primed rats. Horm Behav 42:472-483.

Frye CA, Orecki ZA (2002b) Prenatal stress produces deficits in socio-sexual behavior of cycling, but not hormone-primed, Long-Evans rats. Pharmacol Biochem Behav 73:53-60.

Fuchs RA, Evans KA, Mehta RH, Case JM, See RE (2005) Influence of sex and estrous cyclicity on conditioned cue-induced reinstatement of cocaine-seeking behavior in rats. Psychopharmacology 179:662-672.

Galeeva A, Ordyan N, Pivina S, Pelto-Huikko M (2006) Expression of glucocorticoid receptors in the hippocampal region of the rat brain during postnatal development. J Chem Neuroanat 31:216-225.

Galeeva A, Pelto-Huikko M, Pivina S, Ordyan N (2010) Postnatal ontogeny of the glucocorticoid receptor in the hippocampus. In: Hormones of the Limbic System, vol. 82 (Litwack, G., ed), pp 367-389 San Diego: Elsevier Academic Press Inc.

Galler JR, Tonkiss J (1991) Prenatal protein malnutrition and maternal behavior in Sprague-Dawley rats. J Nutr 121:762-769.

Gao SB, Suenaga T, Oki Y, Yukie M, Nakahara D (2011) Cocaine enhances

resistance to extinction of responding for brain-stimulation reward in adult prenatally stressed rats. Behav Brain Res 223:411-416.

Gelbard HA, Teicher MH, Faedda G, Baldessarini RJ (1989) Postnatal development of dopamine-D1 and dopamine-D2 receptor sites in rat striatum. Dev Brain Res 49:123-130.

Gerber GJ, Stretch R (1975) Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol Biochem Behav 3:1055-1061.

Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311:461-464.

Gerfen CR (1985) The neostriatal mosaic. 1. compartmental organization of projections from the striatal to the substantia nigra in the rat. J Comp Neurol 236:454-476.

Giacomuzzi SM, Riemer Y, Ertl M, Kemmler G, Rossler H, Hinterhuber H, Kurz M (2005) Gender differences in health-related quality of life on admission to a maintenance treatment program. Eur Addict Res 11:69-75.

Giorgi O, Demontis G, Porceddu ML, Mele S, Calderini G, Toffano G, Biggio G (1987) Developmental and age-related changes in D1 dopamine receptors and dopamine content in the rat striatum. Dev Brain Res 35:283-290.

Goeders NE (2002a) The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 27:13-33.

Goeders NE (2002b) Stress and cocaine addiction. J Pharmacol Exp Ther 301:785-789.

Goeders NE, Guerin GF (1996) Role of corticosterone in intravenous cocaine self-administration in rats. Neuroendocrinology 64:337-348.

Gotz M, Huttner WB (2005) The cell biology of neurogenesis. Nature Reviews Molecular Cell Biology 6:777-788.

Gracy KN, Dankiewicz LA, Weiss F, Koob GF (2000) Heroin-specific stimuli reinstate operant heroin-seeking behavior in rats after prolonged extinction. Pharmacol Biochem Behav 65:489-494.

Grissom N, Bhatnagar S (2009) Habituation to repeated stress: get used to it. Neurobiol Learn Mem 92:215-224.

Groenewegen HJ, Wright CI, Beijer AVJ, Voorn P (1999) Convergence and

segregation of ventral striatal inputs and outputs. Annals of the New York Academy of Sciences 877:49-63.

Gustafsson L, Ploj K, Nylander I (2005) Effects of maternal separation on voluntary ethanol intake and brain peptide systems in female Wistar rats. Pharmacol Biochem Behav 81:506-516.

Gutteling BM, de Weerth C, Willemsen-Swinkels SHN, Huizink AC, Mulder EJH, Visser GHA, Buitelaar JK (2005) The effects of prenatal stress on temperament and problem behavior of 27-month-old toddlers. Eur Child Adolesc Psych 14:41-51.

Guzmán C, Cabrera R, Cárdenas M, Larrea F, Nathanielsz PW, Zambrano E (2006) Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. The Journal of Physiology 572:97-108.

Hales CN, Barker DJP, Clark PMS, Cox LJ, Fall C, Osmond C, Winter PD (1991) Fetal and infant growth and impaired glucose-tolerance at age 64. Br Med J 303:1019-1022.

Han F, Ozawa H, Matsuda K, Nishi M, Kawata M (2005) Colocalization of mineralocorticoid receptor and glucocorticoid receptor in the hippocampus and hypothalamus. Neurosci Res 51:371-381.

Haney M, Castanon N, Cador M, Lemoal M, Mormede P (1994) Cocaine sensitivity in Roman High and Low-Avoidance rats is modulated by sex and gonadal hormone status. Brain Res 645:179-185.

Hard E, Larsson K (1968) Dependence of adult mating behavior in male rats on the presence of littermates in infancy. Brain Behav Evol 1:405-419.

Harrod SB, Booze RM, Welch M, Browning CE, Mactutus CF (2005a) Acute and repeated intravenous cocaine-induced locomotor activity is altered as a function of sex and gonadectomy. Pharmacol Biochem Behav 82:170-181.

Harrod SB, Mactutus CF, Browning CE, Welch M, Booze RM (2005b) Home cage observations following acute and repeated IV cocaine in intact and gonadectomized rats. Neurotoxicology and Teratology 27:891-896.

Henry C, Guegant G, Cador M, Arnauld E, Arsaut J, Le Moal M, Demotesmainard J (1995) Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens. Brain Res 685:179-186.

Henry C, Kabbaj M, Simon H, Lemoal M, Maccari S (1994) Prenatal stress

increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. J Neuroendocrinol 6:341-345.

Hernandez-Avila CA, Rounsaville BJ, Kranzler HR (2004) Opioid-, cannabis- and alcohol-dependent women show more rapid progression to substance abuse treatment. Drug Alcohol Depend 74:265-272.

Herrenkohl LR (1979) Prenatal stress reduces fertility and fecundity in female offspring. Science 206:1097-1099.

Herrenkohl LR, Scott S (1984) Prenatal stress and postnatal androgen effects on reproduction in female rats. Experientia 40:101-103.

Hopf FW, Chang SJ, Sparta DR, Bowers MS, Bonci A (2010) Motivation for alcohol becomes resistant to quinine adulteration after 3 to 4 months of intermittent alcohol self-administration. Alcoholism 34:1565-1573.

Hu M, Becker JB (2003) Effects of sex and estrogen on behavioral sensitization to cocaine in rats. J Neurosci 23:693-699.

Hu M, Becker JB (2008) Acquisition of cocaine self-administration in ovariectomized female rats: Effect of estradiol dose or chronic estradiol administration. Drug Alcohol Depend 94:56-62.

Hu M, Crombag HS, Robinson TE, Becker JB (2004) Biological basis of sex differences in the propensity to self-administer cocaine. Neuropsychopharmacology 29:81-85.

Huizink AC, de Medina PGR, Mulder EJH, Visser GHA, Buitelaar JK (2003) Stress during pregnancy is associated with developmental outcome in infancy. J Child Psychol Psychiatry Allied Discip 44:810-818.

Huizink AC, Mulder EJH, Buitelaar JK (2004) Prenatal stress and risk for psychopathology: Specific effects or induction of general susceptibility? Psychol Bull 130:115-142.

Hunt WA, Barnett LW, Branch LG (1971) Relapse rates in addiction programs. J Clin Psychol 27:455-456.

Ikegami M, Jobe AH, Newnham J, Polk DH, Willet KE, Sly P (1997) Repetitive prenatal glucocorticoids improve lung function and decrease growth in preterm lambs. Am J Respir Crit Care Med 156:178-184.

Jackson LR, Robinson TE, Becker JB (2006) Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. Neuropsychopharmacology 31:129-138.

Jaferi A, Bhatnagar S (2006) Corticosterone can act at the posterior paraventricular thalamus to inhibit hypothalamic-pituitary-adrenal activity in animals that habituate to repeated stress. Endocrinology 147:4917-4930.

Jaferi A, Nowak N, Bhatnagar S (2003) Negative feedback functions in chronically stressed rats: role of the posterior paraventricular thalamus. Physiol Behav 78:365-373.

Jaworski J, Francis D, Brommer C, Morgan E, Kuhar M (2005) Effects of early maternal separation on ethanol intake, GABA receptors and metabolizing enzymes in adult rats. Psychopharmacology 181:8-15.

Jaworski JN, Hansen ST, Kuhar MJ, Mark GP (2008) Injection of CART (cocaine- and amphetamine-regulated transcript) peptide into the nucleus accumbens reduces cocaine self-administration in rats. Behav Brain Res 191:266-271.

Jentsch JD, Redmond DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH (1997a) Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 277:953-955.

Jentsch JD, Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology 146:373-390.

Jentsch JD, Taylor JR, Elsworth JD, Redmond DE, Roth RH (1999) Altered frontal cortical dopaminergic transmission in monkeys after subchronic phencyclidine exposure: involvement in frontostriatal cognitive deficits. Neuroscience 90:823-832.

Jentsch JD, Tran A, Le D, Youngren KD, Roth RH (1997b) Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. Neuropsychopharmacology 17:92-99.

Joels M, De Kloet ER (1994) Mineralocorticoid and glucocorticoid receptors in the brain: implications for ion permeability and transmitter systems. Progress in Neurobiology 43:1-36.

Justice AJH, de Wit H (1999) Acute effects of d-amphetamine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology 145:67-75.

Justice AJH, de Wit H (2000) Acute effects of d-amphetamine during the early

and late follicular phases of the menstrual cycle in women. Pharmacol Biochem Behav 66:509-515.

Kalivas PW, Sorg BA, Hooks MS (1993) The pharmacology and neural circuitry of sensitization to psychostimulants. Behav Pharmacol 4:315-334.

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug-induced and stress-induced sensitization of motor activity. Brain Res Rev 16:223-244.

Kapoor A, Matthews SG (2005) Short periods of prenatal stress affect growth, behaviour and hypothalamo-pituitary-adrenal axis activity in male guinea pig offspring. J Physiol-London 566:967-977.

Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O, Piazza PV (2010) Transition to addiction is associated with a persistent impairment in synaptic plasticity. Science 328:1709-1712.

Kerchner M, Ward IL (1992) SDN-MPOA volume in male rats is decreased by prenatal stress, but is not related to ejaculatory behavior. Brain Res 581:244-251.

Kerstetter KA, Aguilar VR, Parrish AB, Kippin TE (2008) Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. Psychopharmacology 198:63-75.

Kim S, Yoon HS, Kim JH (2007) CART peptide 55-102 microinjected into the nucleus accumbens inhibits the expression of behavioral sensitization by amphetamine. Regul Pept 144:6-9.

Kim WR, Sun W (2011) Programmed cell death during postnatal development of the rodent nervous system. Dev Growth Diff 53:225-235.

Kippin TE, Fuchs RA, Mehta RH, Case JM, Parker MP, Bimonte-Nelson HA, See RE (2005) Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. Psychopharmacology 182:245-252.

Kippin TE, Szumlinski KK, Kapasova Z, Rezner B, See RE (2008) Prenatal stress enhances responsiveness to cocaine. Neuropsychopharmacology 33:769-782.

Knackstedt LA, Kalivas PW (2009) Glutamate and reinstatement. Current Opinion in Pharmacology 9:59-64.

Koehl M, Bjijou Y, Le Moal M, Cador M (2000) Nicotine-induced locomotor activity is increased by preexposure of rats to prenatal stress. Brain Res

882:196-200.

Koehl M, Darnaudery M, Dulluc J, Van Reeth O, Le Moal M, Maccari S (1999) Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. J Neurobiol 40:302-315.

Koenig JI, Elmer GI, Shepard PD, Lee PR, Mayo C, Joy B, Hercher E, Brady DL (2005) Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. Behav Brain Res 156:251-261.

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52-58.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97-129.

Krozowski Z, Funder JW (1981) Mineralocorticoid receptors in rat anterior pituitary: toward a re-definition of mineralocorticoid hormone. Endocrinology 109:1221-1224.

Kucerova J, Vrskova D, Sulcova A (2009) Impact of repeated methamphetamine pretreatment on intravenous self-administration of the drug in males and estrogenized or non-estrogenized ovariectomized female rats. Neuroendocrinol Lett 30:663-670.

Landon J, Davison M, Krageloh CU, Thompson NM, Miles JL, Vickers NH, Fraser M, Breier BH (2007) Global undernutrition during gestation influences learning during adult fife. Learn Behav 35:79-86.

Langley-Evans SC, Phillips GJ, Jackson AA (1994) In utero exposure to maternal low-protein diets induces hypertension in weanling rats, independently of maternal blood pressure changes. Clin Nutr 13:319-324.

Laplante DP, Barr RG, Brunet A, Du Fort GG, Meaney ML, Saucier JF, Zelazo PR, King S (2004) Stress during pregnancy affects general intellectual and language functioning in human toddlers. Pediatr Res 56:400-410.

Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME (2007) Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. Experimental and Clinical Psychopharmacology 15:461-471.

Larson EB, Carroll ME (2007) Estrogen receptor beta, but not alpha, mediates estrogen's effect on cocaine-induced reinstatement of extinguished

cocaine-seeking behavior in ovariectomized female rats. Neuropsychopharmacology 32:1334-1345.

Larson EB, Roth ME, Anker JJ, Carroll ME (2005) Effect of short- vs. long-term estrogen on reinstatement of cocaine-seeking behavior in female rats. Pharmacol Biochem Behav 82:98-108.

Lavalaye J, Booij J, Reneman L, Habraken JBA, van Royen EA (2000) Effect of age and gender on dopamine transporter imaging with I-123 FP-CIT SPET in healthy volunteers. Eur J Nucl Med 27:867-869.

Lawson A, Ahima R, Krozowski Z, Harlan R (1991) Postnatal development of corticosteroid receptor immunoreactivity in the rat hippocampus. Dev Brain Res 62:69-79.

Lemaire V, Lamarque S, Le Moal M, Piazza PV, Abrous DN (2006) Postnatal stimulation of the pups counteracts prenatal stress-induced deficits in hippocampal neurogenesis. Biol Psychiatry 59:786-792.

Lesage J, Blondeau B, Grino M, Breant B, Dupouy JP (2001) Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. Endocrinology 142:1692-1702.

Letchworth SR, Nader MA, Smith HR, Friedman DP, Porrino LJ (2001) Progression of changes in dopamine transporter binding site density as a result of cocaine self-administration in rhesus monkeys. J Neurosci 21:2799-2807.

Levesque D, Gagnon S, Dipaolo T (1989) Striatal D1 dopamine receptor density fluctuates during the rat estrous cycle. Neurosci Lett 98:345-350.

Li YF, Shao CH, Zhang DD, Zhao M, Lin L, Yan PR, Xie YY, Jiang KD, Jin L (2006) The effect of dopamine D2, D5 receptor and transporter (SLC6A3) polymorphisms on the cue-elicited heroin craving in Chinese. Am J Med Genet B 141B:269-273.

Li YL, Kolb B, Robinson TE (2003) The location of persistent amphetamine-induced changes in the density of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate-putamen. Neuropsychopharmacology 28:1082-1085.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to

stress. Science 277:1659-1662.

Longshore D, Hsieh S, Anglin MD (1993) Ethnic and gender differences in drug users perceived need for treatment. Int J Addict 28:539-558.

Lovic V, Fleming AS, Fletcher PJ (2006) Early life tactile stimulation changes adult rat responsiveness to amphetamine. Pharmacol Biochem Behav 84:497-503.

Lu L, Grimm JW, Dempsey J, Shaham Y (2004) Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. Psychopharmacology 176:101-108.

Lynch WJ (2008) Acquisition and maintenance of cocaine self-administration in adolescent rats: effects of sex and gonadal hormones. Psychopharmacology 197:237-246.

Lynch WJ (2009) Sex and ovarian hormones influence vulnerability and motivation for nicotine during adolescence in rats. Pharmacol Biochem Behav 94:43-50.

Lynch WJ, Arizzi MN, Carroll ME (2000) Effects of sex and the estrous cycle on regulation of intravenously self-administered cocaine in rats. Psychopharmacology 152:132-139.

Lynch WJ, Carroll ME (1999) Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology 144:77-82.

Lynch WJ, Carroll ME (2000) Reinstatement of cocaine self-administration in rats: sex differences. Psychopharmacology 148:196-200.

Lynch WJ, Roth ME, Carroll ME (2002) Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology 164:121-137.

Lynch WJ, Taylor JR (2005) Decreased motivation following cocaine self-administration under extended access conditions: Effects of sex and ovarian hormones. Neuropsychopharmacology 30:927-935.

Maccari S, Darnaudery M, Morley-Fletcher S, Zuena AR, Cinque C, Van Reeth O (2003) Prenatal stress and long-term consequences: implications of glucocorticoid hormones. Neurosci Biobehav Rev 27:119-127.

Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le Moal M (1995) Adoption reverses the long-term impairment in glucocorticoid feedback

induced by prental stress. J Neurosci 15:110-116.

Marchlewska-Koj A, Kruczek M, Kapusta J, Pochron E (2003) Prenatal stress affects the rate of sexual maturation and attractiveness in bank voles. Physiol Behav 79:305-310.

Masterpasqua F, Chapman RH, Lore RK (1976) Effects of prenatal psychological stress on sexual behavior and reactivity of male rats. Dev Psychobiol 9:403-411.

Matthews K, Robbins TW (2003) Early experience as a determinant of adult behavioural responses to reward: the effects of repeated maternal separation in the rat. Neurosci Biobehav Rev 27:45-55.

Matthews K, Robbins TW, Everitt BJ, Caine SB (1999) Repeated neonatal maternal separation alters intravenous cocaine self-administration in adult rats. Psychopharmacology 141:123-134.

McCance-Katz EF, Carroll KM, Rounsaville BJ (1999) Gender differences in treatment-seeking cocaine abusers: implications for treatment and prognosis. Am J Addict 8:300-311.

McCarthy MM, Arnold AP (2011) Reframing sexual differentiation of the brain. Nat Neurosci 14:677-683.

McCormick CM, Smythe JW, Sharma S, Meaney MJ (1995) Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. Brain research Developmental brain research 84:55-61.

McEwen BS, Weiss JM, Schwartz LS (1968) Selective retention of corticosterone by limbic structures in rat brain. Nature 220:911-912.

McFarland K, Ettenberg A (1997) Reinstatement of drug-seeking behavior produced by heroin-predictive environmental stimuli. Psychopharmacology 131:86-92.

McGivern RF, Poland RE, Taylor AN, Branch BJ, Raum WJ (1986) Prenatal stress feminizes adult male saccharin preference and maze learning: antagonism by propranolol. Monographs in neural sciences 12:172-178.

Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 24:1161-1192.

Meaney MJ, Aitken DH, Vanberkel C, Bhatnagar S, Sapolsky RM (1988) Effect of

neonatal handling on age-related impairments associated with the hippocampus. Science 239:766-768.

Meaney MJ, Sapolsky RM, Aitken DH, McEwen BS (1985) H-3 dexamethasone binding in the limbic brain of the fetal rat. Dev Brain Res 23:297-300.

Meaney MJ, Szyf M, Seckl JR (2007) Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends Mol Med 13:269-277.

Menendez-Delmestre R, Segarra AC (2011) Testosterone is essential for cocaine sensitization in male rats. Physiol Behav 102:96-104.

Miles JL, Landon J, Davison M, Krageloh CU, Thompson NM, Triggs CM, Breier BH (2009) Prenatally undernourished rats show increased preference for wheel running v. lever pressing for food in a choice task. Br J Nutr 101:902-908.

Moffett MC, Harley J, Francis D, Sanghani SP, Davis WI, Kuhar MJ (2006) Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. J Pharmacol Exp Ther 317:1210-1218.

Moore CL, Morelli GA (1979) Mother rats interact differently with male and female offspring. Journal of Comparative and Physiological Psychology 93:677-684.

Morgane PJ, Austinlafrance R, Bronzino J, Tonkiss J, Diazcintra S, Cintra L, Kemper T, Galler JR (1993) Prenatal malnutrition and development of the brain. Neurosci Biobehav Rev 17:91-128.

Morgane PJ, Miller M, Kemper T, Stern W, Forbes W, Hall R, Bronzino J, Kissane J, Hawrylewicz E, Resnick O (1978) Effects of protein malnutrition on developing central nervous system in rat. Neurosci Biobehav Rev 2:137-230.

Morgane PJ, Mokler DJ, Galler JR (2002) Effects of prenatal protein malnutrition on the hippocampal formation. Neurosci Biobehav Rev 26:471-483.

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

Morley-Fletcher S, Puopolo M, Gentili S, Gerra G, Macchia T, Laviola G (2004) Prenatal stress affects 3,4-methylenedioxymethamphetamine pharmacokinetics and drug-induced motor alterations in adolescent female rats. Eur J Pharmacol 489:89-92.

Morley-Fletcher S, Rea M, Maccari S, Laviola G (2003) Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. Eur J Neurosci 18:3367-3374.

Mozley LH, Gur RC, Mozley PD, Gur RE (2001) Striatal dopamine transporters and cognitive functioning in healthy men and women. Am J Psychiat 158:1492-1499.

Mueller BR, Bale TL (2008) Sex-specific programming of offspring emotionality after stress early in pregnancy. J Neurosci 28:9055-9065.

Muhammad A, Hossain S, Pellis SM, Kolb B (2011) Tactile stimulation during development attenuates amphetamine sensitization and structurally reorganizes prefrontal cortex and striatum in a sex-dependent manner. Behav Neurosci 125:161-174.

Murrin LC, Ferrer JR (1984) Ontogeny of the rat striatum: correspondence of dopamine terminals, opiate receptors and acetylcholinesterase. Neurosci Lett 47:155-160.

Murrin LC, Gibbens DL, Ferrer JR (1985) Ontogeny of dopamine, serotonin, and spirodecanone receptors in rat forebrain: an autoradiographic study. Dev Brain Res 23:91-109.

Murrin LC, Zeng W (1989) Dopamine D1 receptor development in the rat striatum: early localization in striosomes. Brain Res 480:170-177.

Murrin LC, Zeng WY (1986) Postnatal ontogeny of dopamine D2 receptors in rat striatum. Biochem Pharmacol 35:1159-1162.

Murrin LC, Zeng WY (1990) Ontogeny of dopamine D1 receptors in rat forebrain: a quantitative autoradiographic study. Dev Brain Res 57:7-13.

Nader MA, Daunais JB, Moore T, Nader SH, Moore RJ, Smith HR, Friedman DP, Porrino LJ (2002) Effects of cocaine self-administration on striatal dopamine systems in rhesus monkeys: initial and chronic exposure. Neuropsychopharmacology 27:35-46.

Newnham JP, Evans SF, Godfrey M, Huang W, Ikegami M, Jobe A (1999) Maternal, but not fetal, administration of corticosteroids restricts fetal growth. The Journal of maternal-fetal medicine 8:81-87.

Nishio H, Kasuga S, Ushijima M, Harada Y (2001) Prenatal stress and postnatal development of neonatal rats – sex-dependent effects on emotional behavior and learning ability of neonatal rats. International Journal of

Developmental Neuroscience 19:37-45.

Normand M, Lalonde J, Lavoie M, Barden N (1980) Adrenocortical responses to adrenocorticotropin in the rat. Can J Physiol Pharmacol 58:1279-1285.

Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR (1998) Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. The Journal of Clinical Investigation 101:2174-2181.

O'Connor EC, Chapman K, Butler P, Mead AN (2011) The predictive validity of the rat self-administration model for abuse liability. Neurosci Biobehav Rev 35:912-938.

O'Connor TG, Heron J, Golding J, Beveridge M, Glover V (2002) Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years – report from the Avon Longitudinal Study of Parents and Children. Br J Psychiatry 180:502-508.

O'Connor TG, Heron J, Golding J, Glover V, Team AS (2003) Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis. J Child Psychol Psychiatry Allied Discip 44:1025-1036.

Olioff M, Stewart J (1978) Sex differences in play behavior of prepubescent rats. Physiol Behav 20:113-115.

Otten W, Kanitz E, Tuchscherer M, Nurnberg G (2001) Effects of prenatal restraint stress on hypothalamic-pituitary-adrenocortical and sympatho-adrenomedullary axis in neonatal pigs. Anim Sci 73:279-287.

Ozawa H, Ito T, Ochiai I, Kawata M (1999) Cellular localization and distribution of glucocorticoid receptor immunoreactivity and the expression of glucocorticoid receptor messenger RNA in rat pituitary gland – A combined double immunohistochemistry study and in situ hybridization histochemical analysis. Cell and Tissue Research 295:207-214.

Pampfer S, Donnay I (1999) Apoptosis at the time of embryo implantation in mouse and rat. Cell Death Differ 6:533-545.

Parsons LH, Smith AD, Justice JB, Jr. (1991) The in vivo microdialysis recovery of dopamine is altered independently of basal level by 6-hydroxydopamine lesions to the nucleus accumbens. J Neurosci Methods 40:139-147.

Parsons LH, Justice JB (1992) Extracellular concentration and in vivo recovery

of dopamine in the nucleus accumbens using microdialysis. J Neurochem 58:212-218.

Paulson PE, Robinson TE (1995) Amphetamine-induced time-dependent sensitization of dopamine transmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. Synapse 19:56-65.

Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates. San Diego, California: Academic Press.

Peris J, Decambre N, Coleman-Hardee ML, Simpkins JW (1991a) Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal H-3 dopamine release. Brain Res 566:255-264.

Peris J, Decambre N, Colemanhardee ML, Simpkins JW (1991b) Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal H-3 dopamine release. Brain Res 566:255-264.

Perrotti LI, Russo SJ, Fletcher H, Chin J, Webb T, Jenab S, Quinones-Jenab V (2001) Ovarian hormones modulate cocaine-induced locomotor and stereotypic activity. In: Biological Basis of Cocaine Addiction, vol. 937 (QuinonesJenab, V., ed), pp 202-216 New York: New York Acad Sciences.

Piazza NJ, Vrbka JL, Yeager RD (1989a) Telescoping of alcoholism in women alcoholics. Int J Addict 24:19-28.

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989b) Factors that predict individual vulnerability to amphetamine self-administration. Science 245:1511-1513.

Piazza PV, Le Moal M (1997) Glucocorticoids as a biological substrate of reward: physiological pathophysiological implications. Brain Res Rev 25:359-372.

Pierce RC, Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Rev 25:192-216.

Ploj K, Roman E, Nylander I (2003) Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. Neuroscience 121:787-799.

Poole TB, Fish J (1976) Investigation of individual, age and sexual differences in play of rattus novegicus (mammalia rodentia). J Zool 179:249-260.

Porrino LJ, Lyons D, Smith HR, Daunais JB, Nader MA (2004) Cocaine self-

administration produces a progressive involvement of limbic, association, and sensorimotor striatal domains. J Neurosci 24:3554-3562.

Post RM, Rose H (1976) Increasing effects of repetitive cocaine administration in rat. Nature 260:731-732.

Quinones-Jenab V, Jenab S (2010) Progesterone attenuates cocaine-induced responses. Horm Behav 58:22-32.

Randall CL, Roberts JS, Del Boca FK, Carroll KM, Connors GJ, Mattson ME (1999) Telescoping of landmark events associated with drinking: a gender comparison. J Stud Alcohol 60:252-260.

Rao PA, Molinoff PB, Joyce JN (1991) Ontogeny of dopamine D1 and D2 receptor subtypes in rat basal ganglia: a quantitative autoradiographic study. Dev Brain Res 60:161-177.

Reinisch JM, Simon NG, Karow WG, Gandelman R (1978) Prenatal exposure to prednisone in humans and animals retards intrauterine growth. Science 202:436-438.

Reul J, De Kloet ER (1985) 2 Receptor systems for corticosterone in rat brain: micordistribution and differential occupation. Endocrinology 117:2505-2511.

Reul J, De Kloet ER (1986) Anatomical resolution of 2 types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis. J Steroid Biochem Mol Biol 24:269-272.

Reyes-Castro LA, Rodriguez JS, Rodriguez-Gonzalez GL, Wimmer RD, McDonald TJ, Larrea F, Nathanielsz PW, Zambrano E (2011) Pre- and/or postnatal protein restriction in rats impairs learning and motivation in male offspring. International Journal of Developmental Neuroscience 29:177-182.

Rezvani AH, Eddins D, Slade S, Hampton DS, Christopher NC, Petro A, Horton K, Johnson M, Levin ED (2008) Neonatal 6-hydroxydopamine lesions of the frontal cortex in rats: Persisting effects on locomotor activity, learning and nicotine self-administration. Neuroscience 154:885-897.

Rhees RW, Al-Saleh HN, Kinghorn EW, Fleming DE, Lephart ED (1999) Relationship between sexual behavior and sexually dimorphic structures in the anterior hypothalamus in control and prenatally stressed male rats. Brain Res Bull 50:193-199.

Rice D, Barone S (2000) Critical periods of vulnerability for the developing

nervous system: evidence from humans and animal models. Environ Health Perspect 108:511-533.

Rich-Edwards JW, Stampfer MJ, Manson JE, Rosner B, Hankinson SE, Colditz GA, Willett WC, Hennekens CH (1997) Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. Br Med J 315:396-400.

Richardson HN, Zorrilla EP, Mandyam CD, Rivier CL (2006) Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. Endocrinology 147:2506-2517.

Richmond G, Sachs BD (1984) Maternal discrimination of pup sex in rats. Dev Psychobiol 17:87-89.

Rivest R, Falardeau P, Di Paolo T (1995) Brain dopamine transporter: gender differences and effect of chronic haloperidol. Brain Res 692:269-272.

Rivier CL, Plotsky PM (1986) Mediation by corticotropin releasing factor (CRF) of adenohypophyseal hormone secretion. Annu Rev Physiol 48:475-494.

Roberts DCS, Bennett SAL, Vickers GJ (1989) The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. Psychopharmacology 98:408-411.

Robinson TE (1984) Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology 84:466-475.

Robinson TE, Becker JB (1982) Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur J Pharmacol 85:253-254.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res Rev 11:157-198.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18:247-291.

Robinson TE, Berridge KC (2003) Addiction. Annu Rev Psychol 54:25-53.

Robinson TE, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse 39:257-266.

Robinson TE, Gorny G, Savage VR, Kolb B (2002) Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats. Synapse 46:271-279.

Robinson TE, Jurson PA, Bennett JA, Bentgen KM (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats. Brain Res 462:211-222.

Robinson TE, Kolb B (1997) Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 17:8491-8497.

Robinson TE, Kolb B (1999a) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598-1604.

Robinson TE, Kolb B (1999b) Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. Synapse 33:160-162.

Rodriguez A, Bohlin G (2005) Are maternal smoking and stress during pregnancy related to ADHD symptoms in children? J Child Psychol Psychiatry 46:246-254.

Rogge G, Jones D, Hubert GW, Lin Y, Kuhar MJ (2008) CART peptides: regulators of body weight, reward and other functions. Nat Rev Neurosci 9:747-758.

Roman E, Hyytiä P, Nylander I (2003) Maternal separation alters acquisition of ethanol intake in male ethanol-preferring AA rats. Alcoholism: Clinical and Experimental Research 27:31-37.

Roman E, Ploj K, Nylander I (2004) Maternal separation has no effect on voluntary ethanol intake in female Wistar rats. Alcohol 33:31-39.

Rosenfeld P, Sutanto W, Levine S, Dekloet ER (1988a) Ontogeny of type I and type II corticosteroid receptors in the rat hippocampus. Dev Brain Res 42:113-118.

Rosenfeld P, Sutanto W, Levine S, De Kloet ER (1990) Ontogeny of mineralocorticoid (type 1) receptors in brain and pituitary: an in vivo autoradiographical study. Dev Brain Res 52:57-62.

Rosenfeld P, Van Eekelen JAM, Levine S, De Kloet ER (1988b) Ontogeny of the

type 2 glucocorticoid receptor in discrete rat brain regions: an immunocytochemical study. Dev Brain Res 42:119-127.

Rosenfeld P, Vaneekelen JAM, Levine S, De Kloet ER (1988c) Ontogeny of the type 2 glucocorticoid receptor in discrete rat brain regions: an immunocytochemical study. Dev Brain Res 42:119-127.

Rossi NA, Reid LD (1976) Affective states associated with morphine injections. Physiological Psychology 4:269-274.

Roth ME, Carroll ME (2004) Sex differences in the acquisition of IV methamphetamine self-administration and subsequent maintenance under a progressive ratio schedule in rats. Psychopharmacology 172:443-449.

Roth ME, Casimir AG, Carroll ME (2002) Influence of estrogen in the acquisition of intravenously self-administered heroin in female rats. Pharmacol Biochem Behav 72:313-318.

Roth ME, Cosgrove KP, Carroll ME (2004) Sex differences in the vulnerability to drug abuse: a review of preclinical studies. Neurosci Biobehav Rev 28:533-546.

Roussel S, Boissy A, Montigny D, Hemsworth PH, Duvaux-Ponter C (2005) Gender-specific effects of prenatal stress on emotional reactivity and stress physiology of goat kids. Horm Behav 47:256-266.

Russo SJ, Festa ED, Fabian SJ, Gazi FM, Kraish M, Jenab S, Quinones-Jenab V (2003) Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. Neuroscience 120:523-533.

Russo SJ, Sun WL, Minerly ACE, Weierstall K, Nazarian A, Festa ED, Niyomchai T, Akhavan A, Luine V, Jenab S, Jenab VQ (2008) Progesterone attenuates cocaine-induced conditioned place preference in female rats. Brain Res 1189:229-235.

Sales N, Martres MP, Bouthenet ML, Schwartz JC (1989) Ontogeny of dopaminergic D-2 receptors in the rat nervous system: Characterization and detailed autoradiographic mapping with [125I]iodosulpride. Neuroscience 28:673-700.

Salomon S, Bejar C, Schorer-Apelbaum D, Weinstock M (2011) Corticosterone mediates some but not other behavioural changes induced by prenatal stress in rats. J Neuroendocrinol 23:118-128.

Sanchis-Segura C, Spanagel R (2006) Behavioural assessment of drug

reinforcement and addictive features in rodents: an overview. Addict Biol 11:2-38.

Sarrieau A, Sharma S, Meaney MJ (1988) Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors. Dev Brain Res 43:158-162.

Saunders BT, Robinson TE (2010) A cocaine cue acts as an incentive stimulus in some but not others: implications for addiction. Biol Psychiatry 67:730-736.

Saunders BT, Robinson TE (2011) Individual variation in the motivational properties of cocaine. Neuropsychopharmacology 36:1668-1676.

Schetter CD (2009) Stress processes in pregnancy and preterm birth. Curr Dir Psychol 18:205-209.

Schetter CD (2011) Psychological science on pregnancy: stress processes, biopsychosocial models, and emerging research issues. In: Annual Review of Psychology, Vol 62, vol. 62 (Fiske, S. T. et al., eds), pp 531-558 Palo Alto: Annual Reviews.

Shaham Y, Erb S, Stewart J (2000) Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Rev 33:13-33.

Shaham Y, Shalev U, Lu L, de Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology 168:3-20.

Shaham Y, Stewart J (1995) Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology 119:334-341.

Shahbazi M, Moffett A, Williams B, Frantz K (2008) Age- and sex-dependent amphetamine self-administration in rats. Psychopharmacology 196:71-81.

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacological Reviews 54:1-42.

Silvagni A, Barros VG, Mura C, Antonelli MC, Carboni E (2008) Prenatal restraint stress differentially modifies basal and stimulated dopamine and noradrenaline release in the nucleus accumbens shell: an 'in vivo' microdialysis study in adolescent and young adult rats. Eur J Neurosci 28:744-758.

Silverman JL, Koenig JI (2007) Evidence for the involvement of ER beta and

RGS9-2 in 17-beta estradiol enhancement of amphetamine-induced place preference. Horm Behav 52:146-155.

Simmons D, Self DW (2009) Role of mu- and delta-opioid receptors in the nucleus accumbens in cocaine-seeking behavior. Neuropsychopharmacology 34:1946-1957.

Singh RR, Cullen-McEwen LA, Kett MM, Boon WM, Dowling J, Bertram JF, Moritz KM (2007) Prenatal corticosterone exposure results in altered AT(1)/AT(2), nephron deficit and hypertension in the rat offspring. J Physiol-London 579:503-513.

Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ (2006) Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. Arch Gen Psychiatry 63:324-331.

Sircar R, Kim D (1999) Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in Fischer, Lewis, and Sprague-Dawley rats. J Pharmacol Exp Ther 289:54-65.

Smith AD, Justice JB (1994) The effect of inhibition of synthesis, release, metabolism and uptake on the microdialysis extraction of dopamine. J Neurosci Meth 54:75-82.

Spanagel R, Hölter SM (1999) Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? Alcohol and Alcoholism 34:231-243.

Stafford D, LeSage MG, Glowa JR (1998) Progressive-ratio schedules of drug delivery in the analysis of drug self-administration: a review. Psychopharmacology 139:169-184.

Staley JK, Krishnan-Sarin S, Zoghbi S, Tamagnan G, Fujita M, Seibyl JP, Maciejewski PK, O'Malley S, Innis RB (2001) Sex differences in I-123 beta-CIT SPECT measures of dopamine and serotonin transporter availability in healthy smokers and nonsmokers. Synapse 41:275-284.

Stewart J (1984) Reinstatement of heroin and cocaine self-administration behavior in the rat by intracerebral application of morphine in the ventral tegmental area. Pharmacol Biochem Behav 20:917-923.

Stewart J, Vezina P (1988) A comparison of the effects of intra-accumbens injections of amphetamine and morphine on reinstatement of heroin intravenous self-administration behavior. Brain Res 457:287-294.

Stretch R, Gerber GJ (1973) Drug-induced reinstatement of amphetamine self-

administration behavior in monkeys. Canadian Journal of Psychology-Revue Canadienne De Psychologie 27:168-177.

Stretch R, Gerber GJ, Wood SM (1971) Factors affecting behavior maintained by response-contingent intravenous infusions of amphetamine in squirrel monkeys. Can J Physiol Pharmacol 49:581-589.

Szuran TF, Pliska V, Pokorny J, Welzl H (2000) Prenatal stress in rats: effects on plasma corticosterone, hippocampal glucocorticoid receptors, and maze performance. Physiol Behav 71:353-362.

Szyf M, Weaver ICG, Champagne FA, Diorio J, Meaney MJ (2005) Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. Front Neuroendocrinol 26:139-162.

Szymanska M, Budziszewska B, Jaworska-Feil L, Basta-Kaim A, Kubera M, Leskiewicz M, Regulska M, Lason W (2009) The effect of antidepressant drugs on the HPA axis activity, glucocorticoid receptor level and FKBP51 concentration in prenatally stressed rats. Psychoneuroendocrinology 34:822-832.

Tamura M, Sajo M, Kakita A, Matsuki N, Koyama R (2011) Prenatal stress inhibits neuronal maturation through downregulation of mineralocorticoid receptors. J Neurosci 31:11505-11514.

Teicher MH, Andersen SL, Hostetter JC (1995) Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Dev Brain Res 89:167-172.

Thomas MB, Hu M, Lee TM, Bhatnagar S, Becker JB (2009) Sex-specific susceptibility to cocaine in rats with a history of prenatal stress. Physiol Behav 97:270-277.

Thompson T, Schuster CR (1964) Morphine self-administration, food-reinforced, and avoidance behaviors in rhesus monkeys. Psychopharmacology 5:87-94.

Thompson WR (1957) Influence of prenatal maternal anxiety on emotionality in young rats. Science 125:698-699.

Tonkiss J, Galler JR, Formica RN, Shukitthale B, Timm RR (1990) Fetal protein malnutrition impairs acquisition of a DRL task in adult rats. Physiol Behav 48:73-77.

Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12:227-462.

Vale W, Vaughan J, Smith M, Yamamoto G, Rivier J, Rivier C (1983) Effects of synthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neurohypophyseal peptides, and other substances on cultured corticotropic cells. Endocrinology 113:1121-1131.

Vallee M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S (1997) Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. J Neurosci 17:2626-2636.

Vallee M, Mayo W, Maccari S, Le Moal M, Simon H (1996) Long-term effects of prenatal stress and handling on metabolic parameters: Relationship to corticosterone secretion response. Brain Res 712:287-292.

Van den Bergh BRH, Marcoen A (2004) High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8-and 9-year-olds. Child Dev 75:1085-1097.

Van den Bergh BRH, Mennes M, Oosterlaan J, Stevens V, Stiers P, Marcoen A, Lagae L (2005) High antenatal maternal anxiety is related to impulsivity during performance on cognitive tasks in 14-and 15-year-olds. Neurosci Biobehav Rev 29:259-269.

Van den Bergh BRH, Mennes M, Stevens V, Van Der Meere J, Borger N, Stiers P, Marcoen A, Lagae L (2006) ADHD deficit as measured in adolescent boys with a continuous performance task is related to antenatal maternal anxiety. Pediatr Res 59:78-82.

Van der Kooy D (1984) Developmental relationships between opiate receptors and dopamine in the formation of caudate putamen patches. Dev Brain Res 14:300-303.

Van der Kooy D, Fishell G (1987) Neuronal birthdate underlies the development of striatal compartments. Brain Res 401:155-161.

Van Eekelen JAM, Bohn MC, De Kloet ER (1991) Postnatal ontogeny of minealocorticoid and glucocorticoid receptor gene expression in regions of the rat telencephalon and diencephalon. Dev Brain Res 61:33-43.

Van Etten ML, Anthony JC (2001) Male-female differences in transitions from first drug opportunity to first use: searching for subgroup variation by age, race, region, and urban status. Journal of Women's Health and Gender-Based Medicine 10:797-804.

Van Haaren F, Meyer ME (1991) Sex differences in locomotor activity after acute

and chronic cocaine administration. Pharmacol Biochem Behav 39:923-927.

van Olphen J, Eliason MJ, Freudenberg N, Barnes M (2009) Nowhere to go: how stigma limits the options of female drug users after release from jail. Substance Abuse Treatment Prevention and Policy 4.

Van Waes V, Darnaudery M, Marrocco J, Gruber SH, Talavera E, Mairesse J, Van Cama G, Casolla B, Nicoletti F, Mathe AA, Maccari S, Morley-Fletcher S (2011a) Impact of early life stress on alcohol consumption and on the short- and long-term responses to alcohol in adolescent female rats. Behav Brain Res 221:43-49.

Van Waes V, Enache M, Berton O, Vinner E, Lhermitte M, Maccari S, Darnaudery M (2011b) Effect of prenatal stress on alcohol preference and sensitivity to chronic alcohol exposure in male rats. Psychopharmacology 214:197-208.

Vanderschuren L, Everitt BJ (2004) Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 305:1017-1019.

Vanderschuren L, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 151:99-120.

Vengeliene V, Celerier E, Chaskiel L, Penzo F, Spanagel R (2009) Compulsive alcohol drinking in rodents. Addict Biol 14:384-396.

Wadhwa PD, Entringer S, Buss C, Lu MC (2011) The contribution of maternal stress to preterm birth: issues and considerations. Clin Perinatol 38:351-384.

Waites CL, Craig AM, Garner CC (2005) Mechanisms of vertebrate synaptogenesis. In: Annu Rev Neurosci, vol. 28, pp 251-274 Palo Alto: Annual Reviews.

Walker QD, Ray R, Kuhn CM (2006) Sex differences in neurochemical effects of dopaminergic drugs in rat striatum. Neuropsychopharmacology 31:1193-1202.

Walker QD, Rooney MB, Wightman RM, Kuhn CM (2000) Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. Neuroscience 95:1061-1070.

Ward IL (1972) Prenatal stress feminizes and demasculinizes behavior of males.

Science 175:82-84.

Ward IL (1974) Sexual behavior differentiation: prenatal hormonal and environmental control. In: Sex differences in behavior (Friedman, R. C. et al., eds), pp 3-17 New York: Wiley.

Ward IL, Stehm KE (1991) Prenatal stress feminizes juvenile play patterns in male rats. Physiol Behav 50:601-605.

Ward IL, Weisz J (1980) Maternal stress alters plasma testosterone in fetal males. Science 207:328-329.

Ward IL, Weisz J (1984) Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. Endocrinology 114:1635-1644.

Weeks JR (1962) Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. Science 138:143-144.

Weinstock M (2007) Gender differences in the effects of prenatal stress on brain development and behaviour. Neurochem Res 32:1730-1740.

Weinstock M (2008) The long-term behavioural consequences of prenatal stress. Neurosci Biobehav Rev 32:1073-1086.

Weinstock M (2011) Sex-dependent changes induced by prenatal stress in cortical and hippocampal morphology and behaviour in rats: an update. Stress 14:604-613.

Weinstock M, Matlina E, Maor GI, Rosen H, McEwen BS (1992) Prenatal stress selectively alters the reactivity of the hypothalamic pituitary adrenal system in the female rat. Brain Res 595:195-200.

Weinstock M, Poltyrev T, Schorer-Apelbaum D, Men D, McCarty R (1998) Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats. Physiol Behav 64:439-444.

Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smith DL, Ben-Shahar O (2000) Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. Proc Natl Acad Sci U S A 97:4321-4326.

Weissenborn R, Robbins TW, Everitt BJ (1997) Effects of medial prefrontal or anterior cingulate cortex lesions on responding for cocaine under fixed-ratio and second-order schedules of reinforcement in rats.

Psychopharmacology 134:242-257.

Weisz J, Ward IL (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106:306-316.

Welberg LAM, Seckl JR (2001) Prenatal stress, glucocorticoids and the programming of the brain. J Neuroendocrinol 13:113-128.

Whitnall MH, Mezey E, Gainer H (1985) Co-localization of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. Nature 317:248-250.

Wiesel TN, Hubel DH (1963) Single cell responses in striate cortex of kittens deprived of vision in one eye. Journal of Neurophysiology 26:1003-1017.

Wise R, Murray A, Bozarth M (1990) Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. Psychopharmacology 100:355-360.

Wolf ME (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Progress in Neurobiology 54:679-720.

Xiao L, Becker JB (1994) Quantitative microdialysis determination of extracellular striatal dopamine concentration in male and female rats: effects of estrous cycle and gonadectomy. Neurosci Lett 180:155-158.

Yang HY, Zhao W, Hu M, Becker JB (2007) Interactions among ovarian hormones and time of testing on behavioral sensitization and cocaine self-administration. Behav Brain Res 184:174-184.

Yang JL, Li WH, Liu XH, Li ZX, Li HY, Yang GF, Xu L, Li LJ (2006) Enriched environment treatment counteracts enhanced addictive and depressive-like behavior induced by prenatal chronic stress. Brain Res 1125:132-137.

Zahm DS, Brog JS (1992) On the significance of subterritories in the accumbens part of the rat ventral striatum. Neuroscience 50:751-767.

Zambrano E, Rodriguez-Gonzalez GL, Guzman C, Garcia-Becerra R, Boeck L, Diaz L, Menjivar M, Larrea F, Nathanielsz PW (2005) A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. J Physiol-London 563:275-284.

Zeng WY, Hyttel J, Murrin LC (1988) Ontogeny of dopamine D1 receptors in the rat striatum. J Neurochem 50:862-867.

Zhao W, Becker JB (2010) Sensitization enhances acquisition of cocaine self-administration in female rats: estradiol further enhances cocaine intake after acquisition. Horm Behav 58:8-12.

Zhen XC, Goswami S, Abdali SA, Frankfurt M, Friedman E (2007) Estrogen-modulated frontal cortical CaMKII activity and behavioral supersensitization induced by prolonged cocaine treatment in female rats. Psychopharmacology 191:323-331.

Zilberman M, Tavares H, el-Guebaly N (2003) Gender similarities and differences: the prevalence and course of alcohol- and other substance-related disorders. J Addict Dis 22:61-74.