Sex differences in behavioral and neural cross-sensitization and escalated cocaine taking as a result of intermittent social defeat stress in rats

A thesis

submitted by

Elizabeth Nicole Holly[1]

In partial fulfillment of the requirements

for the degree of

Master of Science

in

Psychology

TUFTS UNIVERSITY

May 2012

Committee Members:

Klaus A. Miczek[1,2], Joseph F. DeBold[1], and Emmanuel Pothos[2]

[1]Department of Psychology, [2]Department of Neuroscience, Tufts University,

Medford, MA

ABSTRACT

Brief episodes of social stress can result in cross-sensitization to cocaine in rodents, characterized by augmented locomotor activation, dopamine (DA) levels in the nucleus accumbens (NAc), and cocaine taking during a 24 hour "binge" in male rats. However, females are more vulnerable than males at each phase of cocaine addiction, and while these sex differences have been replicated in rats, the role of social stress in females has remained largely neglected.

Long-Evans rats were subjected to four episodes, 72 hours apart, of social defeat by an aggressive resident of the same sex. Ten days later, rats were either assessed for (1) behavioral sensitization as determined by locomotor activity in response to acute cocaine (10 mg/kg, ip) (2) DA sensitization to acute cocaine as measured by *in vivo* microdialysis of the NAc, or (3) intravenous self-administration of cocaine (0.3 mg/kg/infusion, fixed ratio 1) in an unlimited access "binge".

Both stressed males and females showed elevated locomotor activity 5-10 minutes after cocaine injection, but in females the effect was both larger and more prolonged, regardless of estrous cycle phase. While stressed males showed a significant increase in extracellular DA in the NAc compared to non-stressed males, there was no difference in the percent baseline DA levels between stressed males and both groups of females. However, the augmentation in extracellular DA persisted in stressed females, whereas it returned to baseline within 30 min for all other groups. Finally, while stressed males and all females had similar cocaine intake during the first 24 hrs of the cocaine binge, stressed females had a significantly longer "binge".

These data suggest that socially stressed females exhibit a more robust and longer lasting behavioral cross-sensitization, as well as more disregulated cocaine taking, possibly due to alterations in the dopaminergic response in the nucleus accumbens. Furthermore, estradiol appears to play a facilitatory role in both behavioral and dopaminergic sensitization, although future studies need to assess these effects more directly.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my committee. I could not have performed these experiments without your guidance in experimental design and methodology. Secondly, I would also like to thank Dr. Akiko Shimamoto for providing me excellent advising, training, and technical knowledge. Additionally, I would like to recognize Chris Boyson and Tom Sopko for their tremendous help with self-administration, as well as my skilled undergraduate research assistants, Rachel Doyle, Andrew Terrano, and Melanie Monroe. Finally, I would like to acknowledge the funding from the National Institute on Drug Abuse, Grant DA-031734 awarded to KAM.

TABLE OF CONTENTS

I. INTRODUCTION

The World Health Organization reports an estimated 14.3 to 20.5 million people worldwide used cocaine in 2009, with cocaine use concentrated in developed or cocaine producing countries and the United States responsible for nearly 25% of the world's cocaine consumption (UNODC, 2011). Cocaine is the second most prevalent illicit drug in the United States, behind only marijuana (SAMHSA, 2010). According to the most recent National Survey on Drug Use and Health (SAMHSA, 2010), 37.2 million Americans report using cocaine in their lifetime, with 3.9 million reporting taking cocaine within the last year, and 1.5 million within the last month. Cocaine is responsible for more emergency room visits than any other illicit drug, with 482,188 incidences reported in 2008 alone. Moreover, cocaine is the second most common illicit drug for which treatment was sought in the last year, with over 787,000 entering substance abuse treatment for cocaine in 2010 (SAMHSA, 2010).

Interestingly, of the 37.2 million Americans (roughly 11.9% of the US population) who used cocaine at least once, only just over 1 million reported cocaine addiction or dependence in 2010 (SAMHSA, 2010). Addiction is a chronic disorder, behaviorally and neurobiologically distinct from occasional use. Compulsive drug taking is characterized by three stages: (1) binge/intoxication, (2) withdrawal/negative affect, and (3) preoccupation/anticipation, or "craving" (Koob & Le Moal, 1997). As only a small proportion of cocaine users develop dependence, over recent decades research has been directed at determining possible risk factors leading to an increased risk or vulnerability to cocaine addiction. Both hormonal

and neurophysiological sex differences have been shown to increase the subjective cocaine reward in regards to positive feelings and euphoria, as well as potentiate cocaine addiction in females (see (Anker & Carroll, 2011) for review). Additionally, stress, particularly psychosocial stress, has been demonstrated to augment cocaine taking and the neurochemical response to cocaine (see (Sinha, 2008) for review). Clinical data suggest a correlational interaction between sex and stress (Fox & Sinha, 2009), such that drug taking in females may be facilitated by an interaction between estrogens and reward and stress-related systems. This set of experiments sought to evaluate sex differences engendered by social stress in the behavioral and neurochemical response to cocaine, as well as cocaine taking behavior in rodents.

1. THEORIES AND MODELS OF COCAINE ADDICTION

1.1 Cocaine mechanism of action

The central effects of cocaine at reinforcing doses are a result of its blockade of the reuptake mechanisms for monoamine neurotransmitters, particularly the dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporters (Reith, Kim, & Lajtha, 1986). While cocaine has an equal affinity for the three monoamine transporters (Reith et al., 1986; Ritz, Lamb, Goldberg, & Kuhar, 1987), it is hypothesized that the actions at the dopamine transporter (DAT) in particular are responsible for the reinforcing effects of cocaine. Roberts and et al. (1977) first assessed the role of the ascending catecholaminergic systems in intravenous (IV) cocaine self-administration. They found that lesions of the DAergic terminals in the nucleus accumbens (NAc) caused animals to cease responding for cocaine, whereas lesions of ascending NEergic innervation had no effect. Loh and Roberts (1990)

extended these findings to show that ascending 5-HTergic lesions also had no effect on cocaine self-administration. However, Ritz and her colleagues (1987) were the first to directly show specificity towards the DAT in cocaine's mechanism of action. They established that while cocaine does indeed bind to other sites, the potencies of cocaine and cocaine analogs in IV self-administration are directly correlated with the affinity to striatal DA, but not 5-HT or NE, transporters.

Furthermore, drug discrimination studies have shown that administration of other DAT antagonists results in responding at the cocaine appropriate lever, which can be blocked by DA antagonism (Kleven, Anthony, & Woolverton, 1990), although these discriminative effects of cocaine have been observed with 5-HT and NE transporter antagonism as well (Cunningham & Callahan, 1991; Kleven & Koek, 1998). Interestingly, the cocaine induced "high" has been shown to be positively correlated with DAT occupancy in humans (Volkow et al., 1997).

Studies on genetically modified mice have provided a slightly less clear picture of DAT involvement in the rewarding effects of cocaine. Knockout mice lacking the DAT continue to self-administer cocaine (Rocha et al., 1998) as well as develop conditioned place preference for cocaine (Sora et al., 1998). However, completely knocking out the DAT may lead to extensive neuroadaptations and compensatory changes in the monoamine system, thus making results with complete DAT depletion difficult to interpret (S. R. Jones et al., 1999; S. R. Jones et al., 1998; Thomsen, Han, Gu, & Caine, 2009). Giros and colleagues (1996) had shown just two years earlier that both cocaine and amphetamine had no effect on locomotor activity or DA release or uptake in DAT knockout mice. Recently,

Thomsen et al. (2009) demonstrated that a separately derived line of DAT knockout mice would not self-administer cocaine, in direct opposition to the previous Rocha et al. (1998) findings. Additionally, in this experiment Thomsen and colleagues (2009) showed that other operant behaviors were not diminished by DAT knockout, and that self-administration of direct DA agonists in these genetically modified mice was still intact, indicating motor function and motivation are not depleted in their knockout mice. Furthermore, they found that serotonin transporter (SERT) knockout mice reliably self-administered cocaine, furthering the hypothesis that DAT, but not SERT, is critical in mediating the rewarding effects of cocaine. In further support of this hypothesis, animals with intact, functional DAT that does not bind to cocaine do not develop a conditioned place preference for (R. Chen et al., 2006) or self-administer cocaine (Thomsen, Han, et al., 2009).

The DAT controls the termination of DA signaling via mediation of reuptake from the synaptic cleft (Gether, Andersen, Larsson, & Schousboe, 2006; Iversen, 2006; Torres & Amara, 2007). Along with the transporters for 5-HT and NE, DAT is a member of the neurotransmitter/sodium symporter solute carrier 6 (NSS-SLC6) family (Broer, 2006; N. H. Chen, Reith, & Quick, 2004; Hoglund, Adzic, Scicluna, Lindblom, & Fredriksson, 2005).

The DAT, like all SLC6 transporters, is characterized by 12 transmembrane (TM) helices formed through the association of an unexpected repeat of a 5TM helical bundle, such that TM1-TM5 are superimposed to TM6-TM10 in apparent symmetry across an axis of TM3 and TM8 through the center of the membrane (see (Abramson & Wright, 2009) and (Gether et al., 2006) for review). Recently, the

isolation and crystallization of the LeuT transporter, a bacterial homologue of the DAT, from the bacteria *Aquifax aeolicus* has provided more insight into the tertiary and quaternary structure, and thus function, of the SLC6 family of transporters (Yamashita, Singh, Kawate, Jin, & Gouaux, 2005). The exact molecular mechanisms by which cocaine binds to the DAT, as well as its relationship with the DA binding site, are still poorly understood. Work by Beuming and colleagues (2008) using molecular modeling with LeuT has indicated that cocaine and its analogs bind to a site deeply buried in the tertiary structure of the DAT, and that this site overlaps with the binding site for DA. Huang et al. (2009), however, computationally determined a different DAT-ligand binding mode, in which the cocaine- and dopamine- binding sites are close, but not overlapping.

Several neuronal pathways are involved in the progress of the addiction cycle (for review, see (Koob & Volkow, 2010)). The rewarding effects of cocaine are thought to be mediated by monoamines, particularly DA, in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) (Roberts & Koob, 1982). Dopamine in the NAc provides incentive salience to environmental stimuli (Berridge & Valenstein, 1991) leading to promotion of goal directed behavior (Chang, Sawyer, Lee, & Woodward, 1994). Indeed, all drugs of abuse, including cocaine, appear to increase DA levels in the NAc by direct actions in the VTA and/or NAc (Di Chiara, 1992; Koob, Sanna, & Bloom, 1998; Nestler, 2001; Wise, 1996).

Acutely, due to the DAT and SERT blockade, cocaine elevates extracellular levels of both DA (Bradberry & Roth, 1989; Parsons & Justice, 1993) and 5-HT (Parsons & Justice, 1993). DA in the NAc can then enhance inward K^+ flow when

interacting with D1 receptors, or conversely reduce inward K^+ flow by acting on D2 receptors (Uchimura & North, 1990), altering excitability in the NAc. Not surprisingly, activation of DA D2 receptors in the striatum has been shown to increase drug-seeking behavior, in contrast with the attenuated drug seeking behavior caused by D1 receptor activation (Becker & Hu, 2008; Self, Barnhart, Lehman, & Nestler, 1996). Moreover, knocking out the D1 receptor (Caine et al., 2007) or directly antagonizing the D1 receptor in the central amygdala (Caine, Heinrichs, Coffin, & Koob, 1995; McGregor & Roberts, 1993) blocks cocaine self-administration in rodents.

Currently, a leading hypothesis is that cocaine addiction is driven by neuroadaptations in this mesocorticolimbic dopamine system, as well as the glutamatergic corticolimbic circuitry in which dopamine projections are imbedded (Everitt & Robbins, 2005; Kalivas & O'Brien, 2008; Nestler, 2002; Thomas, Kalivas, & Shaham, 2008). Behavioral sensitization is paired with a neuronal sensitization, in which the DAergic increase in the NAc as a result of acute cocaine is potentiated in animals that have received repeated cocaine or amphetamine administration (Kalivas, 1995; Kalivas & Duffy, 1993; Paulson & Robinson, 1995; Robinson, Jurson, Bennett, & Bentgen, 1988).

1.2 Brief review of animal models relevant to drug abuse

1.2.1 Sensitization (Behavioral and Dopaminergic) Repeated intermittent administration of psychostimulants leads to a progressive increase in the subsequent response to the same or lower doses of the same compound, a phenomenon known as behavioral sensitization (Robinson & Berridge, 1993; Segal

& Mandell, 1974). Behavioral sensitization is generally quantified by an increase in locomotor activity, such as walking, rearing, and stereotyped behaviors (such as climbing, sniffing, and circling) (Kalivas, 1995; Kalivas & Duffy, 1993; Robinson & Berridge, 1993). Behavioral sensitization has been associated with an enhancement of extracellular dopamine in the striatum, a phenomenon known as dopaminergic sensitization (Akimoto, Hamamura, & Otsuki, 1989; Kalivas, 1995; Kalivas & Duffy, 1990, 1993; Kalivas & Stewart, 1991; Parsons & Justice, 1993; Paulson & Robinson, 1995; Pierce, Duffy, & Kalivas, 1995; Vanderschuren & Kalivas, 2000). Thus, behavioral sensitization is likely mediated by adaptations in the mesolimbic DAergic pathway, particularly in the cell bodies in the VTA and terminals in the NAc. Incidentally, cross-sensitization to psychostimulants can be induced by repeated administration of other psychostimulants (Bonate, Swann, & Silverman, 1997; Liu, Morgan, & Roberts, 2007; Wuo-Silva et al., 2011) or various types of stress (Covington et al., 2005; Cruz, Quadros, Hogenelst, Planeta, & Miczek, 2011; de Jong, Wasilewski, van der Vegt, Buwalda, & Koolhaas, 2005; Kalivas, Richardson-Carlson, & Van Orden, 1986; Nikulina, Covington, Ganschow, Hammer, & Miczek, 2004; Prasad, Ulibarri, & Sorg, 1998; Quadros & Miczek, 2009; Sorg, 1992; Sorg & Kalivas, 1991)

1.2.2 Conditioned Place Preference Rather than study an animal's direct response to a drug that is reinforced, an alternative approach is to study the conditioned place preference (CPP) for a drug. This procedure relies on an animal's learned preference for a location where it received a drug injection and experienced the effects of the drug. The effects of a drug or control treatment serve as the

unconditioned stimulus (UCS), which is repeatedly paired with a neutral environmental stimulus, which ultimately becomes the conditioned stimulus (CS). When the animal is subsequently exposed to these conditioned stimuli in the absence of drug, the preference of the drug CS can serve as a measure of reward. Intact mesocorticolimbic DA system is required to express drug-induced CPP (for recent review, see (Prus, James, & Rosecrans, 2009)).

1.2.3 IV Self-Administration Rather than the aforementioned studies of passive drug administration, IV self-administration assesses the active, controlled process of drug administration, and as such is a much more direct measure of drug reinforcement. First reported in rodents by Weeks (1962), self-administration is an experimental technique which allows subjects to voluntarily receive a drug, generally in an operant conditioning paradigm. A variety of first and second order schedules of reinforcement have been used in this model to assess acquisition and escalation of drug taking, as well as drug seeking in reinstatement tests following extinction or withdrawal (for review, see (Roberts, 2010)). Furthermore, much like sensitization and conditioned place preference, mesocorticolimbic DA is necessary for cocaine self-administration (Ettenberg, Pettit, Bloom, & Koob, 1982; Roberts & Koob, 1982).

In conclusion, each of these three paradigms measures different aspects of cocaine or other psychomotor stimulant reinforcement (locomotor or dopaminergic response, preference for drug associated context, or active reinforcement though self-administration), each of these paradigms are mediated by mesocorticolimbic

DA pathway, which is directly associated with the reinforcing properties of cocaine and other drugs.

2. GENDER DIFFERENCES IN COCAINE ADDICTION

2.1 Gender differences

Depending on cultural factors, which sex has been predominant in abusing drugs has varied over time (Anker & Carroll, 2011). As of 2010, 40% of cocaine users in the United States were women (SAMHSA, 2010). Yet, over past decades, cocaine use in women has been on the rise, while remaining relatively steady for men (SAMHSA, 2010). While women represented 38.5% of individuals initiating cocaine use within the last year across all age groups, women were responsible for 50.2% of first year initiation in the 12-18 year old age group (SAMHSA, 2010).

Expanding upon prevalence and incidence data, clinical research indicates that women demonstrate a higher vulnerability to cocaine addiction than men in every phase of the addiction cycle. Women initiate cocaine use at a younger age than men, with a mean age of first use for women of 19.2, compared with 20.7 for men (K. Chen & Kandel, 2002; SAMHSA, 2010). Also, in comparison with men, women escalate from first use to dependence at a much faster rate (McCance-Katz, Carroll, & Rounsaville, 1999; O'Brien & Anthony, 2005), are more likely to engage in binge-like patterns of drug use(O'Brien & Anthony, 2005), report stronger drug effects at lower doses, and use cocaine more often (K. Chen & Kandel, 2002). Moreover, women report greater difficulty in quitting (Becker & Hu, 2008), stronger cravings (Robbins, Ehrman, Childress, & O'Brien, 1999), and are more prone to relapse (Ignjatova & Raleva, 2009; Kosten, Gawin, Kosten, & Rounsaville, 1993), taking more

cocaine following a period of abstinence (Gallop et al., 2007). Women also experience less severe withdrawal symptoms than men (Perry, Nelson, & Carroll, 2008), indicating resilience to negative effects of cocaine in addition to a greater sensitivity of positive effects.

2.2 Role of gonadal hormones

Circulating ovarian hormones affect both the physiological and subjective effects of cocaine in women. Overall, clinical studies report that the mood altering effects of cocaine are enhanced during the follicular phase of the menstrual cycle, when plasma estrogen is high, compared to during the luteal phase of the menstrual cycle, when plasma estrogen is low and progesterone high (Evans & Foltin, 2006; Evans, Haney, & Foltin, 2002; Lukas et al., 1996; Sofuoglu, Dudish-Poulsen, Nelson, Pentel, & Hatsukami, 1999).

Interestingly, while Evans and colleagues (2002) reported increases in the cardiovascular response to cocaine correlated with estradiol levels in the menstrual cycle, most studies find no variation in physiological measures as a result of acute cocaine, indicating no difference in the pharmacokinetic profile between the two menstrual cycle phases (Evans & Foltin, 2006; Kaufman et al., 2001; Mendelson, Sholar, Siegel, & Mello, 2001). In comparison with men, women in the luteal phase, but not the follicular phase, report a longer latency to detect cocaine effects (Lukas et al., 1996), as well as report feeling less high in response to the same dose of cocaine (Sofuoglu et al., 1999). Importantly, the above-mentioned studies encompass both smoked and intranasally administered cocaine, indicating no effect of route of administration.

Altogether, these clinical studies suggest that either the presence of estrogen in the follicular phase enhances the positive drug effects, or, conversely, the presence of progesterone in the luteal phase reduces the positive drug effects. Indeed, exogenous progesterone administered during the follicular phase decreases reported positive cocaine effects in women (Evans & Foltin, 2006; Sofuoglu, Babb, & Hatsukami, 2002).

Kouri and colleagues (2002) found that combination oral contraceptives (containing both estrogen and progesterone), abolish differences in the physiological and subjective effects of cocaine across the menstrual cycle, and were not different from follicular phase women without oral contraceptive treatment. Potential explanations of the lack of effects may be that estrogen and progesterone could cancel each other out when administered in combination, or that the doses of estrogen in the oral contraceptives are too low to result in any effects. As of yet, no studies have been done with progesterone-only oral contraceptives, as the combination pill is the most commonly prescribed in the United States (Kouri et al., 2002).

In summary, females seem to be more sensitive to the rewarding and addictive, but not the aversive, effects of cocaine, and it appears that high plasma estrogen levels potentiate whereas high plasma progesterone levels attenuate the positive subjective responses.

3. SEX DIFFERENCES IN COCAINE REINFORCEMENT

3.1 Sex differences

In parallel to findings in humans, female rodents appear to be more sensitive to the rewarding effects of cocaine than males, in that equivalent doses have larger behavioral and neurochemical effects in females compared to males. Repeated cocaine administration produces behavioral sensitization, which is suggested to contribute to characteristic behaviors of drug addiction, particularly motivational aspects of drug craving and compulsive drug seeking (Robinson & Berridge, 1993; Vanderschuren & Kalivas, 2000). Interestingly, female rats exhibit greater behavioral sensitization in the form of larger and longer lasting locomotor activation than male rats after repeated cocaine administration (Carroll, Anderson, & Morgan, 2007; Dow-Edwards, 2010; Festa & Quinones-Jenab, 2004; Gulley, Hoover, Larson, & Zahniser, 2003; Hu & Becker, 2003). Additionally, females acquire conditioned place preference, a preclinical test of an animal's preference for a context associated with non-contingent drug reward, more quickly and at lower doses than males (Russo, Festa, et al., 2003; Russo, Jenab, et al., 2003).

Furthermore, females outperform males in every phase of IV cocaine self-administration. For example, female rats transition from initial sampling to regular use (referred to as acquisition) in cocaine self-administration at a much faster rate than males (Jackson, Robinson, & Becker, 2006; Lynch, 2008; Lynch & Carroll, 1999). In animal models of escalation, in which self-administration protocols are manipulated to result in an increase in drug taking, when compared to males female rats also showed higher rates of operant responding (Carroll, Morgan, Lynch, Campbell, & Dess, 2002; Fuchs, Evans, Mehta, Case, & See, 2005; Kippin et al., 2005), higher number of lever presses to obtain cocaine in a progressively increasing ratio

schedule of reinforcement (Cummings et al., 2011; Roberts, Bennett, & Vickers, 1989), and greater cocaine intake during extended access (Lynch & Taylor, 2004; Roth & Carroll, 2004). Furthermore, in comparison with males, female rats are also more resistant to extinction (Lynch & Carroll, 2000) and are more sensitive to both cocaine- (Anker, Perry, Gliddon, & Carroll, 2009; Kippin et al., 2005) and stress- (Anker & Carroll, 2010) induced reinstatement procedures.

3.2 Role of gonadal hormones

The impact of circulating gonadal hormones on cocaine reward has been extended to rodents as well. In male rats, testosterone does not alter locomotor activity following a cocaine injection (Minerly et al., 2010), and chronic administration following castration does not play a role in acquisition of conditioned place preference (Minerly et al., 2008). Although testosterone is necessary for the induction of behavioral sensitization following chronic cocaine administration (Chin et al., 2002; Menendez-Delmestre & Segarra, 2011), neither castration nor testosterone have an effect in cocaine self-administration in rats (Caine et al., 2004).

While the role of androgens on cocaine reinforcement in males is limited, estrogens seem to play a much greater role, particularly in female rats. Following ovariectomy (OVX), female rats exhibit a more robust behavioral sensitization, again in the form of larger and longer lasting locomotor activation, to repeated cocaine than intact male rats (Segarra et al., 2010). This effect is significantly potentiated in OVX rats receiving estradiol replacement (Hu & Becker, 2003; Segarra et al., 2010; Yang, Zhao, Hu, & Becker, 2007), as well as rats receiving both estradiol and

progesterone (Perrotti et al., 2001; Sircar & Kim, 1999). The effects of progesterone alone on behavioral sensitization are variable, depending on the experimental conditions. While progesterone administration decreases behavioral sensitization to cocaine when dosed in a time-release capsule (Sell, Scalzitti, Thomas, & Cunningham, 2000), it has no effect when administered systemically 4 hours prior to testing (Perrotti et al., 2001; Quinones-Jenab, Perrotti, Mc Monagle, Ho, & Kreek, 2000; Sircar & Kim, 1999). Conditioned place preference is also affected by gonadal hormones, with OVX rats administered estradiol showing a greater conditioned place preference than males (Segarra et al., 2010), while OVX rats receiving progesterone show reduced cocaine-induced conditioned place preference (Russo, Jenab, et al., 2003).

In IV cocaine self-administration, OVX rats treated with estradiol show faster acquisition of cocaine self-administration (Hu, Crombag, Robinson, & Becker, 2004; Jackson et al., 2006; Lynch, Arizzi, & Carroll, 2000; Lynch, Roth, Mickelberg, & Carroll, 2001), higher levels of cocaine self-administration during maintenance (Feltenstein & See, 2007; Lynch et al., 2000; Roberts et al., 1989), as well as potentiated cocaine intake in self-administration paradigms designed to escalate cocaine taking (Larson, Anker, Gliddon, Fons, & Carroll, 2007; Lynch & Taylor, 2005), greater resistance to extinction (Feltenstein & See, 2007; Kerstetter, Aguilar, Parrish, & Kippin, 2008), and heightened cocaine-primed reinstatement (Anker, Larson, Gliddon, & Carroll, 2007; Feltenstein & See, 2007; Kerstetter et al., 2008; Larson & Carroll, 2007; Larson, Roth, Anker, & Carroll, 2005) than those treated with vehicle, and all these effects are reversed by progesterone administration

(Anker et al., 2007; Feltenstein, Byrd, Henderson, & See, 2009; Jackson et al., 2006; Larson et al., 2007).

Overall, preclinical data parallel clinical findings in that the objective and subjective reinforcement for cocaine seems to be greater in females than in males and is heavily influenced by circulating gonadal hormones. As in humans, the rewarding effects of cocaine appear to be potentiated by estradiol and attenuated by progesterone, while largely unaffected by testosterone.

4. NEURONAL MECHANISMS UNDERLYING SEX DIFFERENCES IN COCAINE REWARD

Neuronal sex differences in the mesocorticolimbic dopaminergic system, particularly dopaminergic tone and receptor expression, are thought to mediate the observed behavioral differences in cocaine reward (see Figure 2, (Hu & Becker, 2003)).

4.1 Dopaminergic Tone

4.1.1 Sex differences While circulating estrogens play a role in the enhanced behavioral sensitization and self-administration, these effects are only slightly diminished by ovariectomy, indicating a partial neural cause in the observed sex differences (Hu & Becker, 2003). The putative mesolimbic DA reward pathway is sexually dimorphic, and these dimorphisms are hypothesized to underlie the reported differences in behavior. OVX females have significantly less extracellular striatal dopamine than castrated (CAST) males, as determined by *in vivo* microdialysis using the no net flux method (Castner, Xiao, & Becker, 1993; Xiao & Becker, 1994). Moreover, *in vivo* voltammetry experiments have shown that in

females cocaine substantially augments the extracellular DA release following electrical stimulation more so than in males, likely due to differential affinity for or autoreceptor control over the DAT (Walker, Ray, & Kuhn, 2006; Walker, Rooney, Wightman, & Kuhn, 2000).

The difference in basal dopaminergic tone between males and females may be responsible for less psychostimulant-induced behavioral activation in males, as greater DA increases would be necessary in males to overcome the higher basal DA activity (Becker & Hu, 2008). Accordingly, psychostimulant administration is thought to enhance existing sex differences in DA activation, as females show a higher relative percent increase in accumbal DA than males following amphetamine (Becker & Cha, 1989; Becker & Ramirez, 1981b).

4.1.2 Role of gonadal hormones Circulating estrogens can potentiate the striatal dopaminergic response to cocaine and other psychostimulants. For example, estradiol enhances striatal DA release *in vitro* (Becker, 1990a; Becker & Ramirez, 1981a) and *in vivo* (Becker, 1990b; Becker & Rudick, 1999; Dazzi et al., 2007; McEwen & Alves, 1999; Zhang, Yang, Yang, Jin, & Zhen, 2008), and intact, cycling females have greater levels of extracellular dopamine in the striatum than OVX females (Becker & Beer, 1986; Becker, Beer, & Robinson, 1984; Becker & Ramirez, 1981b). Amphetamine-stimulated DA release in the striatum is increased during estrus as shown by *in vitro* (Becker & Ramirez, 1981b)*, in vivo* microdialysis (Becker & Cha, 1989), and fast-cyclic voltammetry (Becker, 1990b) experiments. In addition, Zhang et al (2008) showed that OVX rats given estradiol exhibited a heightened neuronal sensitivity to the inhibitory effects of cocaine on VTA neuronal firing, while

OVX animals receiving vehicle did not. Indeed, OVX with estradiol leads to enhanced cocaine-induced DA release in NAc, or ventral striatum (Thompson, 1999; Thompson, Moore, & Smith, 2000; Thompson & Moss, 1995). Therefore, it is likely that the absence of estradiol may decrease striatal DA, which may explain why animals with lower estradiol, either due to natural, surgical, or pharmacological causes, demonstrate lower levels of cocaine seeking than those with higher estradiol levels (Larson et al., 2007; Larson & Carroll, 2007; Larson et al., 2005; Lynch et al., 2001).

4.2 DA receptor expression and activity

In addition to basal dopaminergic levels, sex differences exist in DA receptor expression and activity. Interestingly, male rats have approximately 10% more striatal D1 receptors than females (Andersen, Rutstein, Benzo, Hostetter, & Teicher, 1997), which may partially explain the elevated drug seeking observed in female rats. No sex differences in striatal D2 receptor densities have been reported in humans (Farde, Hall, Pauli, & Halldin, 1995; Munro et al., 2006) or rodents (Hruska, Ludmer, Pitman, De Ryck, & Silbergeld, 1982; D. Levesque & Di Paolo, 1988), although estradiol has been shown to down regulate D2 receptor expression (Bazzett & Becker, 1994) and increase DA turnover (Di Paolo, Rouillard, & Bedard, 1985) in the striatum. Ligand-bound estradiol receptors regulate transcript of proteins associated with the DA system (D. C. Jones & Miller, 2008) and, *in vitro*, estradiol interferes with the GTP-induced affinity shift of D2 receptors (D. Levesque & Di Paolo, 1993). Additionally, D2 receptor densities in the striatum have been reported to be greater following natural or synthetic estradiol elevations (Bazzett &

Becker, 1994; Czoty et al., 2009; Di Paolo, Falardeau, & Morissette, 1988; Pazos, Stoeckel, Hindelang, & Palacios, 1985; Zhou, Cunningham, & Thomas, 2002). In mouse cultures, estradiol modulates the G coupling process, resulting in alterations in adenylate cyclase activity stimulation by D1 and D2 agonists (Maus, Bertrand, et al., 1989; Maus, Cordier, Glowinski, & Premont, 1989).

Taken together, these sexual dimorphisms in both DAergic tone and DA receptor expression lead to exacerbation of sex differences in DA activity following cocaine administration, possibly underlying the enhanced sensitivity to cocaine observed in females.

5. STRESS AND COCAINE ADDICTION

5.1 Physiological stress response

Stress activates a homeostasis-driven adaptive response (McEwen, 2007). Physiologically, the stress response is mediated by two pathways. The autonomic nervous system response is mediated by the sympathoadrenal medullary (SAM) axis, while in the hypothalamic-pituitary-adrenal (HPA) axis, corticotropin releasing factor (CRF) is released from the paraventricular nucleus (PVN) of the hypothalamus, stimulating the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH then goes on to stimulate cortisol/corticosterone release from the adrenal medulla (Charmandari, Tsigos, & Chrousos, 2005; McEwen, 1999; Sinha, 2008).

In addition to stimulating the HPA axis, CRF is expressed in regions of the mesocorticolimbic system and acts as a central neurotransmitter (Rodaros, Caruana, Amir, & Stewart, 2007; Swanson, Sawchenko, Rivier, & Vale, 1983). CRF

administration dose-dependently increases the firing rate of VTA DA neurons (Wanat, Hopf, Stuber, Phillips, & Bonci, 2008), while acute and chronic CRF receptor antagonism decreases cocaine-induced DA release in the NAc (Lodge & Grace, 2005). Thus, it appears CRF may augment DA release in projection areas such as the NAc, enhancing drug reward and promoting drug-seeking behaviors. There is an overlapping neural circuitry between stress and reward circuits (Corominas, Roncero, & Casas, 2010; Sinha et al., 2007), such that stress system activation may serve to "prime" reward circuits, modulating reward related behavior via mesencephalic DA stimulation (Piazza & Le Moal, 1997; Sinha, 2001).

5.1.1 Sex differences in the stress response While in humans, some studies have shown no effect of gender on HPA reactivity to CRH (Hatzinger, Brand, Herzig, & Holsboer-Trachsler, 2011), those using behavioral tasks to induce stress have elicited sex differences. In healthy individuals, gender differences have been reported in stress reactivity from a temporal discounting task (Diller, Patros, & Prentice, 2011), mental arithmetic task (Tersman, Collins, & Eneroth, 1991; Veit, Brody, & Rau, 1997) and other behavioral tasks (Stoney, Davis, & Matthews, 1987; Tersman et al., 1991), such that males have a greater increase in blood pressure, while females demonstrate a greater increase in heart rate. Using interpersonal stress, Lévesque and colleagues (2010) found that women exerted both higher heart rate and blood pressure than males. Interestingly, a study by Schmaus, Laubmeier, Boquiren, Herzer, and Zakowski (2008) did not find gender differences in stress reactivity after initial stress exposure, but women exhibited significantly higher negative affect and heart rate upon repeated exposure, while men did not, indicating

sensitization. The slight variations in the nature of induced laboratory stress could explain why some studies report a gender difference in reactivity while others do not.

Female rats also have a greater behavioral sensitivity to stress, as measured by locomotor activity (van Haaren & Meyer, 1991). Additionally, intact female rats have higher corticosterone and adrenocorticotropic hormone (ACTH) levels than males at baseline (Atkinson & Waddell, 1997) and in response to stress (Handa, Burgess, Kerr, & O'Keefe, 1994; Rivier, 1999; Yoshimura et al., 2003), as well as longer lasting HPA activation (Heinsbroek, van Haaren, Feenstra, Boon, & van de Poll, 1991).

5.1.2 Role of gonadal hormones Ovarian hormones play a clear role in the heightened stress system activation. Women in the luteal phase react significantly more to cold pressor stress, as well as exhibit higher cortisol and aldosterone levels than women in the early follicular phase (Tersman et al., 1991), indicating a role of progesterone in stress reactivity in females. In a study of postmenopausal women, psychosocial stress in the form of the Trier Social Stress Test potentiated negative mood and anxiety in women receiving estradiol replacement for two months, but not those receiving placebo (Newhouse et al., 2008). In rodents, estradiol has an excitatory effect on the HPA axis (Dallman et al., 2004; McCormick & Mathews, 2007), increasing CRF release (Patchev, Hayashi, Orikasa, & Almeida, 1995; Swanson & Simmons, 1989) as well as increasing both ACTH and corticosterone (Burgess & Handa, 1992). Behaviorally, OVX+E rats exhibit heightened fear potentiated startle compared to OVX+V controls, and this facilitation is reversed by

progesterone treatment (Hiroi & Neumaier, 2006; Toufexis, Davis, Hammond, & Davis, 2004)

5.2 Stress and Drug Abuse in Humans

Given the interaction between CRF and DA in the mesocorticolimbic system, it is not surprising that stress and stress reactivity are major factors in vulnerability to drug abuse (Sinha, 2001, 2008). Both clinical and preclinical work show that stress plays a key role in initiation, escalation, and relapse to drug abuse (Shaham, Erb, & Stewart, 2000; Sinha, 2009; Sinha et al., 2007; Sinha & Li, 2007). Individuals suffering from high psychosocial stress initiate drug use earlier and take cocaine longer than low-stressed individuals (Karlsgodt, Lukas, & Elman, 2003), while adolescents experiencing stressful life events and survivors of childhood abuse show escalated levels of drug taking and addiction compared to relatively less stress controls (Barrett & Turner, 2006; Newcomb & Bentler, 1988; Newcomb & Harlow, 1986; Wills & Cleary, 1996; Wills, Vaccaro, & McNamara, 1992). Cocaine dependent and recently abstinent patients report higher levels of cocaine craving and anxiety following stress imagery than healthy controls (Fox, Hong, Siedlarz, & Sinha, 2008; Sinha, Catapano, & O'Malley, 1999; Sinha, Fuse, Aubin, & O'Malley, 2000; Sinha, Garcia, Paliwal, Kreek, & Rounsaville, 2006; Sinha et al., 2003).

Both stress and drug cue imagery have been shown to increase cocaine craving, subjective anxiety, pulse, systolic blood pressure, ACTH, cortisol, prolactin, and norepinephrine (Fox et al., 2008; Sinha et al., 2003), but only stress imagery has been shown to elevate diastolic blood pressure and plasma epinephrine levels (Sinha et al., 2003), indicating a significant but differential activation of the HPA and

SAM axes during drug cue and stress induced craving states. Indeed, in male cocaine abusers neuroendocrine reactivity to social stress in the laboratory predicts cocaine relapse (Back et al., 2010; Sinha et al., 2006). Interestingly, greater stress- but not drug-induced cocaine craving correlates with a shorter abstinence period before relapse, while stress-induced cortisol levels predict an increased amount of cocaine taken during relapse (Sinha et al., 2006).

5.2.1 Clinical evidence for gender differences Psychostimulants such as cocaine are known to stimulate the HPA axis in both humans and animals (Sarnyai, Mello, Mendelson, Eros-Sarnyai, & Mercer, 1996; Sorg & Steketee, 1992), and gender differences in HPA changes following acute cocaine administration have been reported, with females showing greater HPA activation following cocaine than males (reviewed by (Fox & Sinha, 2009)). Thus, it has been proposed that drug taking in females may be facilitated by an interaction between estrogens and reward-/stress-related systems, such that estrogens may enhance mesocorticolimbic DA pathway function (Anker & Carroll, 2011), and stress has been correlated with an augmented vulnerability to drug addiction in females (Fox & Sinha, 2009). Indeed, clinical work in humans indicates that women report greater feelings of craving for cocaine as well as negative emotion (i.e. anxiety, sadness) than men following stressful stimuli (Back, Brady, Jackson, Salstrom, & Zinzow, 2005; Fox et al., 2008; Fox & Sinha, 2009), and women are more likely than men to relapse during periods of high psychosocial stress (Back et al., 2008; Elman, Karlsgodt, & Gastfriend, 2001; Fox & Sinha, 2009; Hyman et al., 2008; McKay, Rutherford, Cacciola, Kabasakalian-McKay, & Alterman, 1996; Waldrop et al., 2010). Cocaine abusing men exhibit higher basal

ACTH and epinephrine tone than both cocaine abusing women and healthy controls, but women show a greater HPA and SAM response to stress and drug cues (Fox et al., 2009), and this heightened stress reactivity may lead to the increased vulnerability to cocaine relapse observed in females.

5.3 Stress and Drug Taking in Animals

Corticosterone administration increases cocaine self-administration in rats (Goeders & Guerin, 1996; Piazza & Le Moal, 1996), while pharmacological or surgical inactivation of the corticosterone prevents acquisition (Mantsch, Saphier, & Goeders, 1998; Piazza & Le Moal, 1996) and reinstatement (Goeders & Guerin, 1996; Mantsch & Goeders, 1999; Piazza & Le Moal, 1996). Stress manipulations such as rearing isolation, restraint, footshock, and intermittent social defeat have also been shown to increase cocaine self-administration (Goeders & Guerin, 1994; Miczek & Mutschler, 1996; Ramsey & Van Ree, 1993; Schenk, Lacelle, Gorman, & Amit, 1987), and brief footshock stress can reinstate cocaine seeking following extinction (Ahmed & Koob, 1997; Erb, Shaham, & Stewart, 1996; Mantsch & Goeders, 1998; Shaham et al., 2000).

5.3.1 Effects of intermittent social defeat stress in male rodents

Physiological effects Intermittent social defeat stress in particular has proven to be a particularly powerful animal model to induce an increased cocaine response and instigate escalated cocaine-seeking behavior. In male rats, intermittent social defeat stress results in long lasting physiological, behavioral, and neural changes (Covington et al., 2005). Social defeat in male rodents increases corticosterone (Covington et al., 2005; Koolhaas, De Boer, De Rutter, Meerlo, & Sgoifo, 1997; Pich et

al., 1993; Ribeiro Do Couto et al., 2006) and decreases the amplitude of circadian rhythms regulating heart rate and core body temperature for several weeks and months following the last defeat (Tornatzky & Miczek, 1995). These data indicate that in rodents there is no physiological habituation to intermittent social stress.

Behavioral effects Moreover, intermittent social defeat results in an array of behavioral effects. Acute and repeated social defeat induces behavioral cross-sensitization to psychomotor stimulants in the form of augmented locomotor activity and stereotypy in males (Boyson, Miguel, Quadros, Debold, & Miczek, 2011; Covington & Miczek, 2001, 2005; de Jong et al., 2005; Dietz, Dietz, Moore, Ouimet, & Kabbaj, 2008; Marrow, Overton, & Brain, 1999; Miczek, Mutschler, van Erp, Blank, & McInerney, 1999; Nikulina et al., 2004; Piazza, Deminiere, le Moal, & Simon, 1990; Quadros & Miczek, 2009; Yap, Covington, Gale, Datta, & Miczek, 2005; Yap & Miczek, 2007). Interestingly, this effect is only observed during adulthood, as adolescent male rats and hamsters do not exhibit cross-sensitization to psychostimulants due to social stress (Burke, Watt, & Forster, 2011; Kabbaj, Isgor, Watson, & Akil, 2002; Trzcinska, Bergh, DeLeon, Stellar, & Melloni, 2002).

Social defeat stress also enhances the conditioned place preference for amphetamine in both adolescent and adult male rodents (Burke et al., 2011; McLaughlin, Li, Valdez, Chavkin, & Chavkin, 2006), while footshock does not result in an augmented conditioned place preference (Burke et al., 2011). Furthermore, social defeat enhances cocaine-primed reinstatement of extinguished conditioned place preference for cocaine in male mice (Ribeiro Do Couto, Aguilar, Lluch, Rodriguez-Arias, & Minarro, 2009).

Finally, social defeat alters the reinforcing effects of cocaine. Socially defeated male rats acquire cocaine self-administration more quickly than non-defeated control males (Tidey & Miczek, 1996). Moreover, both male and female socially defeated rats show an increase in intake in late acquisition trials compared to non-defeated control (Haney, Maccari, Le Moal, Simon, & Piazza, 1995). Increases in cocaine intake in socially defeated male rats during the maintenance phase of self-administration have been observed (Boyson et al., 2011), although this effect seems to be greater at lower doses of cocaine (Miczek & Mutschler, 1996). While some studies have reported an increase in breakpoint on a progressive ratio schedule of reinforcement in cocaine self-administration in socially defeated male rats (Covington et al., 2005; Covington & Miczek, 2005; Covington, Tropea, Rajadhyaksha, Kosofsky, & Miczek, 2008; Quadros & Miczek, 2009), others have reported no effect at the same doses (Boyson et al., 2011; Covington & Miczek, 2001; Yap & Miczek, 2007). Finally, socially defeated male rats take significantly more cocaine in a 24 hour unlimited access "binge" protocol (Boyson et al., 2011; Covington et al., 2005; Covington & Miczek, 2001, 2005; Miczek, Nikulina, Shimamoto, & Covington, 2011; Quadros & Miczek, 2009). Thus, it appears that intermittent social defeat may enhance some aspects of cocaine reward in male rats.

Neurophysiological effects These alterations in behavior appear to be paralleled by long lasting neurophysiological changes (Covington et al., 2005; Nikulina et al., 2004). Cross-sensitization between psychomotor stimulant administration and intermittent social defeat stress observed in male rodents suggests shared neural mechanisms. Indeed, in male rats both psychomotor

stimulant administration and social defeat stress activate dopaminergic ventral tegmental area (VTA) neurons projecting to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) (Covington et al., 2008; Tidey & Miczek, 1996; Vanderschuren & Kalivas, 2000). In males, social defeat itself, or the mere exposure to a cage where social defeat has occurred (Tidey & Miczek, 1996, 1997), can increase DA in the NAc, as well as enhance extracellular DA in the NAc both at baseline and in response to acute d-amphetamine (Miczek et al., 1999) or cocaine (Miczek et al., 2011). These DAergic alterations may be related to the accelerated acquisition of cocaine self-administration in socially defeated male rats, as well as increased cocaine intake in the 24-hour binge (Miczek et al., 2011; Tidey & Miczek, 1997). Intermittent social defeat in adulthood also down regulates striatal DA D2 receptors in male rats (Dietz et al., 2008).

Brain-derived neurotrophic factor (BDNF) positively modulates DA release (Altar et al., 1992; Cordeira, Frank, Sena-Esteves, Pothos, & Rios, 2010). Interestingly, intermittent social defeat increases BDNF in pre-synaptic DA neurons of the VTA in male rats (Berton et al., 2006; Miczek et al., 2011), and this alteration in BDNF may be part of a stress-induced cascade leading to enhanced neural and behavioral sensitization, as well as cocaine taking and seeking behaviors (Graham et al., 2007; Grimm et al., 2003; Narita, Aoki, Takagi, Yajima, & Suzuki, 2003).

In addition to its effects on DA, intermittent social defeat in male rodents alters the interactions with the glutamatergic neurons in which the DA terminals are buried. Pretreatment with MPEP, a mGluR5 noncompetitive antagonist, during the induction of behavioral cross-sensitization blocks social stress-induced, but not

repeated amphetamine induced, sensitization to amphetamine (Yap et al., 2005). Additionally, amphetamine cross-sensitization in males induced by social defeat, as well as enhanced cocaine self-administration on a progressive ratio schedule or binge, can be blocked by the noncompetitive NMDA antagonist dizocilpine (Covington et al., 2008) and the AMPA antagonist AP5 (Yap et al., 2005).

5.3.2 Sex differences in the effects of stress in regards to cocaine In general, sex differences in stress reactivity as it pertains to preclinical models of drug taking and drug reincorcement related behavior has remained relatively understudied. Haney et al (1995) showed that females subjected to intermittent social defeat stress self-administered significantly more cocaine than intermittently stressed males in the late phases of acquisition. Moreover, they demonstrated while as a whole females had higher plasma corticosterone levels in response to a novel environment, history of social defeat stress did not augment the corticosterone response. Additionally, compared to males, females display an enhanced reinstatement due to yohimbine, an α-adrenergic agonist (Anker & Carroll, 2010; Feltenstein, Henderson, & See, 2011), and this effect can be blocked by concurrent administration of the progesterone metabolite allopregnanolone (Anker & Carroll, 2010). Moreover, while CRF induces reinstatement in both male and female rats, females exhibit a greater variability in responding, with a subset of high responding females demonstrating significantly greater cocaine seeking than their high responding male counterparts (Buffalari, Baldwin, Feltenstein, & See, 2012).

6. CURRENT STUDY

As previously discussed, reports on sex differences in the effects of stress on behavioral and dopaminergic sensitization to cocaine, as well as cocaine taking behavior, are extremely limited. As social stress is a particularly ethologically relevant stressor, and engenders a wide array of behavioral and neurophysiological changes in male rats, its effects on females was of particular interest. Again, only one study to date has examined the role of intermittent social defeat stress in female rats. Haney et al. (1995) demonstrated that intermittently stressed females acquire cocaine self-administration more rapidly than non-stressed females and take more cocaine during late acquisition trials than both stressed and non-stressed males, while novelty-induced corticosterone increases were not affected by stress history. However, other important behavioral, neurochemical, and endocrine measures were not examined. The present study sought to systematically examine sex and estrous cycle differences in behavioral and dopaminergic cross-sensitization to cocaine, as well as cocaine taking behavior in an unlimited access cocaine self-administration "binge", predicting a greater effect in intact females, with those in estrus or proestrus, when circulating ovarian hormones are higher, showing a larger effect than those in met/diestrus.

Male and female Long Evans rats were either handled daily or subjected to social defeat stress by an aggressive rat of the same sex on days 1, 4, 7, and 10. Rats were then assessed ten days later for (I) behavioral sensitization, as assessed by locomotor activity in response to acute cocaine (10 mg/kg, ip), (II) dopaminergic sensitization, as assessed by *in vivo* microdialysis of DA in the NAc in response to

acute cocaine (10 mg/kg, ip), or (III) cocaine taking, as assessed by an unlimited access cocaine self-administration "binge" (FR1, 0.3 mg/kg/infusion).

II. METHODS

General Methods

Subjects Male and female Long-Evans rats (Charles River, Wilmington, MA) weighing 200-225g upon arrival were individually housed in custom-built clear acrylic chambers (30x30.5x24.5cm) with cellulose pellet bedding (CelluDri™, Shepherd Specialty Papers, Kalamazoo, MI) and adapted to the facilities for at least one week prior to experimentation. Rats were randomly assigned to stressed or non-stressed control group. Stimulus males unrelated to the experiment were housed in close proximity to females to help ensure regular estrous cycles. Separate "resident" rats were housed in male-female pairs in large stainless steel cages (71x46x46 cm) as described previously (Miczek, 1979). Rats were housed on a reversed 12 hour light cycle (lights on at 20:00) and provided food and water *ad libitum*. All procedures were approved by the Tufts University Institutional Animal Care and Use Committee, following guidelines set forth in the Guide for Care and Use of Laboratory Animals (National Research Council, 1996).

Experimental Design Animals were exposed to either intermittent social defeat stress or daily handling for ten days, and subsequently evaluated for either (Experiment I) behavioral sensitization (n=57), assessed by locomotor activity in response to acute cocaine, (Experiment II) neural sensitization (n=54), assessed by *in vivo* microdialysis of the nucleus accumbens in response to acute cocaine, or

(Experiment III) cocaine taking (n=66), assessed by an unlimited cocaine self-administration "binge" (see Figure 3).

Estrous Cycle Examinations Vaginal smears were taken daily and examined using the Giemsa staining method (Staples & Geils, 1965). Estrous stage was categorized as either proestrus, estrus, or met/diestrus. Proestrus is characterized by round-nucleated epithelial cells, and estrus as cornified cells, while met/diestrus encompass everything else (namely polymorphonuclear leucocytes and occasional epithelial cells) (Brack, Jeffery, & Lovick, 2006). Smears were taken for at least two complete cycles to establish baseline cyclicity prior to stress manipulations. Initially, two females were excluded due to irregularity of cycles before experimental manipulations. After it was established that intermittent social defeat did not alter estrous cyclicity, rats were no longer excluded for irregular cycles.

Social Defeat Stress A modification of a previously described resident-intruder paradigm (Tornatzky and Miczek 1995; Shimamoto, Debold et al. 2011) was used. Briefly, rats were subjected to four social defeats, separated by approximately 72 hours. Experimental rats were defeated by a same sex resident, either a larger male screened for reliable aggressive behavior or a lactating dam. First, the opposite sex resident rat was removed, and the experimental rat placed in a small protective cage inside the larger resident cage for 10 minutes. This allowed for olfactory and visual instigation and threat, but prevented tactile contact. Next, the protective cage was removed and the experimental animal placed in the resident cage until defeated (defined as 10 bites and/or 6s held in supine position by resident) or until 5 minutes had elapsed from the first attack bite. Following the defeat, experimental

rats were again placed in the protective cage inside the resident cage for another 10 minutes, then finally returned to their home cage.

Experiment I: *Behavioral Sensitization*

Ten days after the last defeat (day 20) both stressed (n=16 male; n=12 female) and non-stressed (n=16 male, n=13 female) control rats were given a challenge injection of cocaine (10 mg/kg, ip) to evaluate locomotor sensitization (Covington & Miczek, 2001). Rats were injected with saline (ip) once per day for three days prior to testing to ensure habituation to handling and injection. On the day of testing, rats were moved to an adjacent experimental room and given an injection of saline. Five minutes following the injection, behavior was recorded for a period of five minutes. Then, rats were injected with cocaine, and behavior recorded for a period of 5 minutes both 5 and 25 minutes later (see Figure 4). For females, vaginal smears were taken immediately following the end of recording to evaluate the effects of estrous cycle on behavioral sensitization. Video recordings were later analyzed by a reliable scorer using a customized keyboard and computer software (The Observer Video-Pro© version 8.0, Noldus Information Technology, Wageningen, The Netherlands) for duration and frequency of walking.

Experiment II: *In vivo Microdialysis*

A separate cohort of stressed (n=15 male; n=12 female) and non-stressed (n=15 male; n=12 female) rats was assessed for dopamine levels in the nucleus accumbens (NAc) in response to acute cocaine (10 mg/kg, ip) 10 days after the last defeat (day 20). Stereotaxic surgery, sample collection, and monoamine analysis

have been described previously (Shimamoto, Debold, Holly, & Miczek, 2011). Briefly, rats were anesthetized with ketamine (100 mg/kg) and xylazine (6 mg/kg for males, 3 mg/kg for females) and implanted with a unilateral guide cannula (BASi, West Lafayette, IN) aimed at the NAc using the coordinates according to stereotaxic atlas (Paxinos & Watson, 1997): AP, +2.1mm from bregma; ML, +0.9mm (females) or +1.1mm (males) from midline; DV, -5.8mm from dura. Four female rats died from anesthesia during or immediately after surgery.

Rats were injected with saline (ip) once per day for three days prior to testing to ensure habituation to handling and injection. The day before sample collection, rats were anesthetized with isoflurane and the stylet in the cannula replaced with a 2-mm active membrane probe (BASi, West Lafayette, IN) connected to a syringe filled with artificial cerebrospinal fluid (aCSF, CMA Microdialysis Inc., North Chelmsford, MA). The infusion rate was set to 0.5 μL/min overnight, and increased to 1.5 μL/min 30 minutes prior to sample collection the next day.

Samples were collected every 10 minutes using a refrigerated fraction collector (CMA 142, CMA Microdialysis Inc., North Chelmsford, MA) in vials containing 5μL antioxidant (20 mM phosphate buffer including 25 mM EDTA-2NA and 0.5 mM ascorbic acid, pH 3.5). Tonic levels of DA were measured in five baseline samples, which was followed by saline (ip, at 55 minutes) and cocaine (10 mg/kg, ip, at 75 minutes) injections. Sample collection continued for 115 minutes following the cocaine injection in order to assess the time course of DA changes in response to cocaine.

Following sample collection, rats were deeply anesthetized with pentobarbitol

(100 mg/kg) and perfused with saline and 4% paraformaldehyde. Brains were removed for histological analysis of probe placement. Brains were sliced at 60 μm and mounted on gelatin coated slides, then stained with cresyl violet. Precise probe placement was determined using light microscopy. Six male and six female rats were excluded due to missed placements.

DA was analyzed by an HPLC instrument consisting of an LC10-AD pump (Shimadzu, Columbia, MD), a manual injector (model 7,125; Rheodyne, Cotati, CA) with a 0.1 mL sample loop, and an electrochemical detection (ECD) system (DECADE II, Antec Leyden BV, Zoeterwoude, Netherlands). A cation-exchange column (CAPCELL PAK, 1.5mmx250mm, 5μm ID, Shiseido, Tokyo, Japan) with temperature set at 30°Cwas used to separate monoamines. Mobile phase consisted of 150 mM ammonium acetate, 50 mM citric acid, 27μM EDTA, 10% methanol, and 1% acetonitrile, with pH adjusted to 4.6 and flow rate set at 0.2 mL/min. Concentrations of DA were calculated using a standard curve with known amounts of monoamines in a range of 1.875-18.75 pg. Thus, the limit of detection (LOD) for DA was 0.21 pg. Twelve male and two female rats with proper placements were excluded due to DA levels below the LOD or otherwise unable to be analyzed.

Experiment III: *IV Cocaine Self-Administration*

A third cohort of stressed (n=15 male; n=18 female) and non-stressed (n=15 male; n=18 female) rats was assigned to the self-administration experiment. One male developed an infection after the second social defeat and died. The remaining animals were implanted with a catheter (SILASTIC silicon tubing; inner diameter, 0.63 mm; outer diameter, 1.17 mm) in the right jugular vein under ketamine (100

mg/kg) and xylazine (6 mg/kg for males, 3 mg/kg for females) anesthesia. Seven animals died due to anesthesia. The catheter was passed subcutaneously to the rat's back, where exited through a small incision and was attached to a pedestal (Plastics One) mounted to a harness system (Instech Laboratories). Five days after surgery, rats were moved from their home cage to permanent housing in the IV self-administration chambers. To help ensure catheter patency, each morning catheters were flushed with 0.2 mL saline and 0.2 mL heparinized saline (20 IU/mL), and 0.17 mL pulses of saline were delivered every 30 minutes, except during the daily self-administration procedures. One rat was excluded due to loss of catheter patency.

Rats were allowed to self-administer cocaine (0.75 mg/kg/infusion) according to a fixed ratio 1 (FR1) schedule of reinforcement. Sessions were signaled by a stimulus light, and one wall of the home cage contained two retractable levers. Pressing the active lever resulted in an intravenous infusion, followed by a 30 second time out period, during which the stimulus light was turned off. Pressing the inactive lever did not result in any infusions, but lever presses were recorded. Rats performed two daily sessions, which were terminated after 15 infusions or 5 hours. Beginning on the first self-administration session, rats were shaped with additional reinforcers (Froot Loops, peanut butter, or female urine) to ensure rapid acquisition. Seventeen rats were excluded for failing to meet acquisition criteria by the time of the binge.

After five days of self-administration (day 10 after the last defeat, day 20 overall), rats were given continuous access to cocaine (0.3 mg/kg/infusion) in an unlimited binge protocol. The binge terminated after 2 hours without any infusions.

Two rats died from infection prior to binge.

Statistical Analysis

Unless otherwise noted, statistical analyses were carried out using Sigma Plot v11.0 (Systat Software, San Jose, CA). A Mann-Whitney Rank Sum Test was used to assess sex differences in quantitative aspects of the social defeat encounters. For behavioral sensitization, walking duration was analyzed using a split plot factor three-way repeated measures analysis of variance (ANOVA, SAS, SAS Institute, Cary, NC), followed by *a priori* driven two way ANOVAs with Holm-Sidak corrections for multiple comparisons. For neural sensitization, percent change from baseline DA concentrations were analyzed with three-way split plot, repeated measures ANOVAs (SAS, SAS Institue, Cary, NC), followed by *a priori* driven two-way repeated measures ANOVAs with Holm-Sidak corrections for multiple comparisons. Areas under the curve were calculated and analyzed using a Student's t-test. For cocaine "binge", time of last infusion and total cocaine intake were analyzed with two-way ANOVAs with Holm-Sidak corrections for multiple comparisons.

III. RESULTS

Social Defeat

No significant effect of sex in latency to the first bite (Mann-Whitney U=24747, n_1=211, n_2=238, n.s.), total number of bites (U=23064.5, n_1=211, n_2=238, n.s.), or total defeat encounter duration (U=23340, n_1=211, n_2=238, n.s) was observed, but there was a significant effect of sex in the proportion of defeats ending with the experimental animal in the submissive supine posture for over 6s, with a

significantly larger proportion of males exhibiting this behavior than females (U=22590.5, n_1=211, n_2=238, p<0.001).

Experiment I: Behavioral Sensitization

Significant effects of cocaine ($F_{2, 98}$=33.6, p<0.001), sex and estrous cycle ($F_{2,49}$=29.82, p<0.001), and intermittent social defeat stress ($F_{1,49}$=23.34, p<0.001) were observed for walk duration during the cocaine challenge.

In non-stressed rats there was no significant difference in walking duration prior to cocaine treatment, and only females in estrus spent significantly more time walking at 5-10 (p<0.05) and 25-30 (p<0.05) minutes following cocaine injection. Additionally, walking duration for estrous females during each time period was significantly higher than that of males (p<0.001), but not non-estrous females (Figure 5).

Within the stressed animals, at baseline females in estrus had a significantly longer walking duration than both males (p<0.05) and non-estrous females (p<0.01), but no difference was observed between males and non-estrous females. Walking duration 5-10 minutes after cocaine administration was significantly elevated for males (p<0.001, Figure 5a), non-estrous females (p<0.001, Figure 5b), and estrous females (p<0.001, Figure 5c). However, a significantly larger increase in comparison to males for walking duration was observed in both groups of females (p<0.001 for both), with estrous females spending significantly more time walking than non-estrous females (p<0.001).

Experiment II: *In vivo* Microdialysis

Accurate placements are shown in Figure 6.

No difference in average baseline DA concentration (pmol) was observed (non-stressed male M=1.698, SD=1.171; stressed male M=1.479, SD=0.276; non-stressed female M=1.306, SD=0.465; stressed female M=2.001, SD=0.778; $F_{1,23}$=0.0760, p=0.746). However, percent change from baseline was used to reduce intergroup and intersex variability. No statistical difference between all five baseline samples and the post-saline sample was observed across all groups ($F_{5,90}$=1.67, p=0.1503), so baseline samples were excluded from subsequent analysis and all post-cocaine samples were compared to the post-saline sample.

To analyze overall effects of sex, stress, and cocaine, a mixed three-way repeated measures analysis of variance (ANOVA) of percent change from baseline from saline though the end of sampling was performed. This analysis yielded no sex difference ($F_{1,19}$=3.84, p=0.0649), nor interactions of sex x stress ($F_{1,19}$=3.12, p=0.0932), sex x time ($F_{11,209}$=1.14, p=0.3283), or stress x time ($F_{11,209}$=1.08, p=0.3812). However, significant effects of stress ($F_{1,19}$=7.24, p=0.0145) and time ($F_{11,209}$=15.77, p<0.001), as well as a sex x stress x time interaction ($F_{11,209}$=2.49, p=0.0058) were observed.

As non-stressed females and both groups of males returned to baseline within 45 minutes of the cocaine injection, the last three samples (85-115 minutes post cocaine) were excluded in a second mixed three-way ANOVA. In this analysis, significant effects of sex ($F_{1,19}$=4.51, p=0.0470), stress ($F_{1,19}$=11.06, p=0.0036), and time ($F_{8,152}$=17.57, p<0.0001), with significant sex x stress x time ($F_{8,152}$=2.91,

p=0.0047) but not sex x stress ($F_{1,19}$=3.29, p=0.0857), sex x time ($F_{8,152}$=1.33, p=0.2326) or stress x time ($F_{8,152}$=1.47, p=0.1742) interactions were observed.

One-way repeated measures ANOVAs indicated both stressed and non-stressed rats in each sex group had a significant change from baseline DA in response to cocaine. In males (Figure 7a), a significant effect of cocaine was observed in non-stressed ($F_{11,44}$=5.571, p<0.001) and stressed ($F_{11,66}$=17.964, p<0.001) rats, with significant increases over baseline at 15-25 (p=0.006) and 25-35 (p<0.001) minutes post cocaine for non-stressed males and 15-25 (p<0.001), 25-35 (p<0.001), and 35-45 (p=0.006) for stressed males. Non-stressed females (Figure 7b) also showed a significant effect of cocaine ($F_{11,44}$=3.423, p=0.002), with significant elevation in extracellular DA relative to baseline at 15-25 (p=0.010) and 25-35 (p=0.048) minutes after cocaine, while stressed females showed an augmented DA percent increase from baseline ($F_{11,55}$=2.862, p=0.005) for every sample after cocaine injection (5-15 p<0.001; 15-25, p<0.001; 25-35 p=0.002; 35-45 p=0.007; 45-55 p=0.004; 55-65 p=0.015; 65-75 p=0.025; 75-85 p=0.025; 85-95 p=0.020; 95-105 p=0.030; 105-115 p=0.030).

As significant overall three way interactions were observed, hypothesis driven two-way repeated measures ANOVAs with Holm-Sidak adjustments were used for post hoc analysis of results. Within males (Figure 7a), a significant effect of stress was observed at 15-25 (p<0.001), 25-35 (p<0.001), and 35-45 (p=0.003) minutes following the cocaine injection, with stressed males significantly higher at each time point. Non-stressed and stressed females (Figure 7b), on the other hand, significantly differed 5-15 (p=0.001) following cocaine injection, and again from 45-

55 (p=0.031), 55-65 (p=0.012), 65-75 (p=0.037), 75-85 (p=0.037), 85-95 (p=0.008), and 95-105 (p=0.018) post cocaine, with no significant differences from 15-45 minutes following cocaine.

Two-way repeated measures ANOVAs with Holm-Sidak corrections were also performed as post hoc test to evaluate sex differences within each stress group. Comparing non-stressed male and females, there was no significant effect of sex ($F_{1,8}$=0.0742, p=0.792), although a significant difference between sexes was observed at 35-45 minutes following cocaine (p=.033), with females (M=207.85, SD=68.99) showing a higher elevation above baseline DA levels compared to males (M=128.733, SD=25.33).

Experiment III: IV Cocaine Self-Administration

In the unlimited access "binge", both sex and stress effects were observed in the overall time of binge. The effect of estrous cycle phase was first examined in all females. No effect of estrous cycle phase was found in total infusions ($F_{2,16}$=0.207, n.s., 8a), infusions within the first 24 hours ($F_{2,16}$=0.116, n.s., Figure 8b), or time of last infusion ($F_{2,16}$=0.512, n.s., Figure 8c). Therefore females were collapsed across estrous cycle for all subsequent analyses.

A two-way analysis of variance for sex and stress on total number of infusions across the binge yielded a significant effect of stress ($F_{1,34}$=13.792, p<0.001), but not sex ($F_{1,34}$=2.404, n.s.) or a sex x stress interaction ($F_{1,34}$=0.253, n.s., Figure 9a). Pairwise multiple comparisons with Holm-Sidak corrections showed significant stress effects within both males (non-stressed M=283, SEM=41.319; stressed M=528, SEM=67.837; p=0.042) and females (non-stressed M=362.912,

SEM=65.630; stressed M=684.7, SEM=100.312; p=0.003) with stressed groups self-administering a significantly higher number of infusions across the binge.

Within infusions across the first 24 hours of the binge, a significant effect was also observed for stress ($F_{„34}$=7.667, p=0.009), but not sex ($F_{1,34}$=0.190, n.s.) or sex x stress interaction ($F_{1,34}$=0.609, n.s., Figure 9b). Here, post hoc analyses yielded a significant effect of stress within males (non-stressed M=283, SEM=67.837; stressed M=481.25, SE<=54.248; Holm-Sidak p=0.026), but not females (non-stressed M=350.912, SEM=62.609; stressed M=462, SEM=47.313; n.s.).

Interestingly, effects of both stress ($F_{1,34}$=21.131, p<0.001) and sex ($F_{1,34}$=5.288, p=0.028) were observed in the time of last infusion, or binge duration, without a sex x stress interaction ($F_{1,34}$=1.550, n.s., Figure 9c). Non-stressed males stopped responding for cocaine significantly earlier than stressed males (non-stressed M=820 min, SEM=243.664; stressed M=1516.625 min, SEM=92.258; p=0.034, Figure 10a). Stressed females also binged significantly longer than non-stressed females (non-stressed M=1039.167, SEM=137.536; stressed M=2253.4, SEM=336.133; p<0.001, Figure 10b). Furthermore, while sex differences were not observed in binge duration of non-stressed rats, a sex difference was observed in stressed rats such that stressed females binged significantly longer than stressed males (p=0.019).

IV. DISCUSSION

This series of experiments has demonstrated that brief, episodic social defeat engenders substantial behavioral and neurochemical changes in rats ten days after

the last defeat experience, and that these changes occur to a much greater degree in females than in males, regardless of circulating ovarian hormones.

No quantitative difference in social defeat in males and females

As this was the first project to use intermittent social defeat stress in female rats, and there is only one reported study of this degree of episodic social defeat in females (Haney et al., 1995), it was crucial to first verify that the defeat experiences between the two sexes were as similar as possible. Admittedly, the aggressive behavior exhibited by males and female Long Evans rats qualitatively differs, in that male aggressors tend to attack the vulnerable neck and shoulders from a downward angle, while females generally bite the face, flank, and tail from a more forward, direct angle (De Almeida & Lucion, 1994).

However, our results, much like those of Haney et al. (1995), show no quantitative differences between males and females in attack latency, number of bites, or total fight duration. While there was a significant difference in the proportion of defeats ending with a submissive supine posture between males and females, the incredibly low proportion of these instances within both of the sexes yields the differences n this behavior virtually negligible. Thus, while the location of bite delivery differs between sexes, the male and female rats in our study were subjected to highly similar defeat experiences in terms of intensity and duration. While the qualitative differences in social defeat cannot be completely disregarded, it is likely that any observed sex differences within socially stressed rats are not a result of a difference in the nature of social defeat.

Stress, sex, and estrous phase differentially affect behavioral sensitization

In agreement with extensive work in males, intermittent social defeat stress in females evokes long lasting behavioral changes. In particular, intermittently stressed females in this experiment display an enhanced locomotor response to a cocaine challenge compared to controls. Additionally, this behavioral sensitization in females is both of a greater magnitude and longer duration than that of similarly stressed males. Previous studies on sex differences in drug-induced behavioral sensitization have also demonstrated a larger, longer lasting sensitization in females compared with males in terms of magnitude and duration of locomotor activation, regardless of circulating estrogens (Becker, Molenda, & Hummer, 2001; Chin et al., 2001; Hu & Becker, 2003; Sircar & Kim, 1999).

Although intermittently socially defeated females of all estrous phases in this experiment displayed behavioral sensitization to a greater degree than males, effects of the estrous cycle were still observed such that females in the estrous phase, with higher levels of estradiol, showed an augmented response compared to non-estrous rats in metestrus or diestrus. This is consistent with extensive studies in females showing a facilitatory effect of estradiol on behavioral sensitization. Despite vast differences in sensitization protocols, strains and ages of rats used, and means of measuring locomotor activity, most studies agree that estradiol augments cocaine-induced behavioral sensitization. For example, although some studies show no behavioral sensitization in OVX females (Puig-Ramos, Santiago, & Segarra, 2008; Sircar & Kim, 1999) while others do (Becker et al., 2001; Hu & Becker, 2003; Sell et al., 2000; Sell, Thomas, & Cunningham, 2002), behavioral sensitization is higher in

OVX females treated with estradiol in each of these reports, regardless of dose or route of administration.

Initially, the behavioral sensitization observed within the control females in estrus was surprising. However, a review of the literature indicated that our results were indeed in line with previous studies. Although relatively few published reports have used intact females, van Haaren and Meyer (1991) assessed behavioral sensitization to cocaine at the same dose and time course as this study (10 mg/kg, ip for both induction and expression, activity recorded for 30 minutes) in both OVX and intact females. While they found a significant behavioral sensitization within intact females that received 9 previous injections of cocaine, they also observed a behavioral sensitization in intact, but not OVX, females that had received chronic saline injections. While they did not account for individual phases of the estrous cycle, they posit that this observed effect of sensitization within cocaine-naïve females was due to an interaction between circulating ovarian hormones and mesocorticolimic DAergic neruons.

In addition to circulating ovarian hormone levels, several other factors have been shown to play a role in the expression of behavioral sensitization in female rats. Sircar and Kim (1999) demonstrated that in female rats, strain significantly affects the degree of gonadal modulation of behavioral sensitization to cocaine. In that study, Sircar and Kim examined Fischer, Lewis (two inbred srains), and Sprague-Dawley (an outbred strain) rats, showing differential effects in the degree of behavioral sensitization in OVX rats receiving estradiol replacement. In general, behavioral sensitization experiments in female rats tend to be performed on

Sprague-Dawley or Wistar rats. To date, no studies of behavioral sensitization in female Long Evans rats have been reported. As such, more work should be done with this strain to compare with other outbred and inbred strains of rats.

Another important factor in the expression of behavioral sensitization for female rats is time of testing. Yang and colleagues (2007) found that females tested at ZT1600, but not ZT2100, exhibited reliable behavioral sensitization. Coincidentally, our expression tests for behavioral sensitization occurred at ZT1700-ZT1900, so it is unlikely that our rats were prone to the behavior suppressing effects Yang et al. showed for the late dark phase.

No effect of sex or stress in dopaminergic tone

No group difference in baseline DA concentration was observed between groups, directly contradicting studies indicating a higher baseline DAergic tone in males compared to females (Castner et al., 1993; Xiao & Becker, 1994), and previous studies indicating a higher DA tone in stressed males relative to non-stressed males (Miczek et al., 2011). However, a trend towards a stress effect was observed, with stressed animals within each sex trending toward a higher DA concentration than non-stressed.

The lack of difference in baseline in this experiment may be a result of inconsistent sensitivity of the HPLC, as baseline DA concentrations tended to decrease in the order which animals were run. In general, data indicated a trend for a sex difference in non-stressed rats, with males showing a slightly higher baseline DA concentration.

Furthermore, due to dynamics of probe recovery, conventional microdialysis measurements of baseline, as performed here, do not allow for a true equilibrium across the membrane. As such, conventional microdialysis only provides information regarding the relative change in neurotransmitter efflux, and does not accurately quantify the true level of neurotransmitters present in the extracellular fluid. The no-net-flux method, on the other hand, allows for dynamic analysis of true neurotransmitter content by direct infusion of known neurotransmitter concentrations and determining the net change in concentration after dialysis, such that the alterations in neurotransmitter clearance do not affect the concentration estimates (Chefer, Thompson, Zapata, & Shippenberg, 2009; Justice, 1993; Parsons & Justice, 1994). Thus, a key next step for this study is performing a no-net-flux experiment on stressed and non-stressed male and female rats to accurately assess any differences in baseline dopaminergic tone.

Differential effects of sex and stress on DA in the NAc after cocaine

Although no effect was observed in baseline DA concentration, the variability within groups was very high, and thus percent change from baseline was used for all analyses. As expected, significant effects of sex, stress, and cocaine were observed, as well as a sex x stress x cocaine interaction. Consistent with previous data, all males showed an increase in DA in response to cocaine, but males previously exposed to intermittent social defeat stress showed a significantly higher percent increase from baseline in extracellular DA than non-stressed males, indicative of neural cross-sensitization to cocaine as a result of intermittent stress(Miczek et al., 2011; Tidey & Miczek, 1996, 1997).

Interestingly, stressed females showed a much different DAergic response to cocaine than stressed males. The percent change in extracellular DA in the NAc occurred significantly earlier in stressed females than all other groups, and while the initial increase was not different than the increase for non-stressed females, the extracellular dopamine remained elevated for the duration of sampling.

One key confound in this study, possibly explaining the lack of heightened DA in the NAc in intermittently stressed females, is the use of isoflurane anesthesia for probe insertion the night before the microdialysis experiment took place. Isoflurane acts by increasing GABA release while simultaneously decreasing glutamate release, resulting in an overall enhancement of inhibition and reduction of excitation (Westphalen & Hemmings, 2006). In the rat striatum in particular, isoflurane-induced GABA increase exerts inhibitory control over both spontaneous and NMDA-evoked DA release (Keita, Henzel-Rouelle, Dupont, Desmonts, & Mantz, 1999). Siegal and Dow-Edwards (2009) noticed in other experiments that their females, but not males, would show a decreased sensitization to repeated cocaine than expected. They then designed an experiment to assess the effects of brief isoflurane anesthesia with no surgery on cocaine-induced behavioral sensitization in male and female Sprague-Dawley rats. Indeed, Siegal and Dow-Edwards found that brief isoflurane anesthesia one day before the expression of behavioral sensitization obliterated effects observed in females, but not males, previously receiving chronic cocaine administration. As behavioral sensitization is purported to be a result of an increase in extracellular DA, it follows that the lack of behavioral sensitization could be due to an influence of isoflurane on GABAergic neurons modulating DA release.

Therefore, it is essential that isoflurane not be used in further studies of psychostimulant action in female rodents.

Intriguingly, stressed females show a significantly faster DAergic response to cocaine than all other groups. While the cardiovascular effects of this particular intermittent social defeat paradigm have clearly not been studied in female rodents, if this type of stress history engenders a long-lasting increase in heart rate in females, it is possible that cocaine could pass through the blood brain barrier more rapidly, resulting in a faster increase in extracellular DA in the NAc. More work should be directed towards assessing cardiovascular activity during social defeat as well as at the time of expression to characterize this novel paradigm in females.

Although enhanced extracellular DA was not initially observed, the DA elevation persisted for stressed but not non-stressed females. This persistent elevation in NAc DA could be a result of either increased firing rate by DA neurons in the VTA, increased amount of DA released from DA neurons in the VTA, or a reduced ability to clear DA from the synapse in the NAc.

While there are limited reports of the effects of intermittent social defeat stress in rats on phasic DA signaling are limited, Anstrom, Miczek, and Budygin (2009) demonstrated an increase in phasic DAergic signaling in rats undergoing social defeat. Additionally, restraint stress results in an increase in firing rate as well as burst firing at least one day following stress exposure (Anstrom & Woodward, 2005). It is possible that these alterations in DA firing activity could cause long lasting neuroadaptations in the DA neurons of the VTA, it is unlikely that these neuroadaptations would lead to a prolonged firing in response to cocaine 105

minutes after injection, as cocaine itself would clearly no longer be exerting an effect. As such, this hypothesis does not seem sufficient to explain the prolonged extracellular DA elevation observed in females.

It seems much more likely that a history of episodic stress modifies the monoamine, particularly DA, reuptake mechanisms in females, but not males. A downregulation of DAT, whether by decrease in mRNA expression or increase in DAT internalization, may explain both the trend towards a heightened baseline DA tone in stressed females as well as prolonged extracellular DA concentration in the NAc. One particularly intriguing future direction from these experiments is to quantitatively assess membrane DAT protein in the NAc via Western blot as well as mRNA expression in the DA cell bodies of the VTA via qPCR. Furthermore, immunofluorescence studies may help to shed some light on the potential role of membrane DAT expression in females with a history of intermittent social defeat.

Limited observed role of estradiol in accumbal DA concentrations

As the number of females in the microdialysis experiment is quite low due to low variability, it was obviously impossible to run statistics comparing the effects of estrous cycle phase, although it was tracked. Interestingly, while there was no observed effect of estrous cycle within the control females, a trend is beginning to emerge in intermittently stressed females. Again, while the number per group is very low, the prolongation of extracellular DA elevation seems to be differentially driven by the amount of circulating estrogens, such that stressed rats in proestrus had the highest sustained elevation, followed by stressed rats in estrus, while

stressed rats in met- or diestrus returned to baseline at a similar timepoint to controls.

Becker (1999) has proposed a model for estradiol mediated increase in DA, which has remained largely uncontested (see figure 11). Estrogens act preferentially on GABAergic neurons within the striatum, decreasing firing rates of collaterals projecting to DA neurons in the VTA, which ultimately serves to disinhibit DA resulting in an increase in DA release (Mermelstein, Becker, & Surmeier, 1996). Furthermore, Becker asserts that the GABA mediated increase in stimulated DA release then causes an enhanced activation of both pre- and post-synaptic DA receptors, ultimately causing enhanced activity of the DAT and down-regulation of presynaptic DA D2 autoreceptors, further enhancing extracellular DA in the NAc (Becker, 1999; Meiergerd, Patterson, & Schenk, 1993).

Thus, it seems likely that an interaction exists between stress exposure and circulating estradiol on DAergic function in female rats. Accordingly, more intact females should be run in this experiment to further elucidate the role of endogenously cycling ovarian hormones, and more causal effects of estradiol should be evaluated in further studies using OVX rats with and without estradiol and/or progesterone treatment.

Sex differences in the effects of intermittent social defeat on cocaine taking

A preliminary experiment evaluated sex differences in the effects of intermittent social defeat stress in a 24 hour binge approximately 6 weeks after the last defeat (results not shown). In that pilot study, no effect of stress was observed in females, contrary to the initial expectations. Both groups of females continued

responding for cocaine for the duration of the 24-hour binge, so it was hypothesized that the lack of stress effect may have been a result of either a ceiling effect in females or a loss of sensitization at that time point. To assess these hypotheses, the current experiment was designed so that the time point of the binge was the same as the behavioral and neural sensitization measures.

In line with previous research at later time points after stress (Boyson et al., 2011; Covington & Miczek, 2001, 2005; Cruz et al., 2011; Miczek et al., 2011; Quadros & Miczek, 2009), stressed male self-administered significantly more cocaine for a significantly longer duration. Cumulative records demonstrate that all both groups of males initially self-administer cocaine at similar rates, but non-stressed males cease responding approximately 12-13 hours into the binge, close to where the light phase would normally begin. Interestingly, as shown by the cumulative record, there was only overlap of one rat between the control and stressed groups in total duration of binge, indicating two discrete groups. Additionally, stressed males cease self-administering cocaine at an average of 25 hours into the binge, indicating that the previously arbitrarily determined 24-hour duration of our standard binge (Boyson et al., 2011; Covington & Miczek, 2001, 2005; Cruz et al., 2011; Miczek et al., 2011; Quadros & Miczek, 2009) was indeed appropriate.

As there are no sex or stress differences in the post-infusion intervals at 0.3 mg/kg/infusion in a variable dose binge paradigm (Holly et al., unpublished data), it was expected that no effects of sex or stress emerge until animals stop responding for cocaine. Indeed, as non-stressed males ended their binges at within the first 24

hours, it is not surprising that effects of stress within males emerged within that time point, and as such, the overall stress effect in the total number of infusions in the first 24 hours was entirely driven by stressed males.

In females, however, the effect of stress was not apparent until after the first 24 hours of the binge, indicating previously described pilot studies in females indeed failed due to a ceiling effect. Notably, there was a much wider range in binge durations of stressed females than any other group, and this effect was not due to levels of circulating gonadal hormones. It is possible that differences in the intensity of social defeat could account for this wide range of binge durations in females. While latency to first bite, total number of attack bites, and total fight duration were recorded, video recordings of the defeats were not made, so post-hoc qualitative analysis of the defeats was not possible. The social defeat procedure has been well established in this and other laboratory for males, but brief episodes of social defeat in females was a novel undertaking for this laboratory, and remains relatively unreported in the literature. As such, while variability is minimal in neural and behavioral sensitization experiments, it may prove important to correlate aspects of social defeat, such as freezing behavior, or other vulnerability/resiliency measures, to cocaine intake during the binge.

Role of ovarian hormones in cocaine taking during the unlimited access binge

While no effect of estrous cycle at the start of the binge was observed for total infusions, infusions within the first 24 hours, or binge duration for either stressed or non-stressed females, the roles of estrogens and progesterone were not accurately assessed within this experiment. Vaginal smears were taken immediately

before the initiation of the session, and animals were not disturbed for the duration of the binge. It should be noted that the proestrous phase typically lasts approximately 6-12 hours, while estrus lasts 18-24 hours, and met- and diestrus encompass the duration of the 4-5 day cycle (Nequin, Alvarez, & Schwartz, 1979). Therefore, while the cocaine self-administration, which has been reported to disrupt the estrous cycle (unreported data from this laboratory, King, Canez, Gaskill, Javors, & Schenken, 1993), fluctuations in circulating ovarian hormones across the binge, which lasted up to three days, were not accounted for. Therefore, while no effects of initial estrous stage were observed in this binge, OVX with estradiol and/or progesterone replacement should be done to accurately determine the role of gonadal hormones in cocaine taking behavior in an unlimited binge protocol.

CONCLUSION

This set of three experiments provides a substantial first look at the interaction between sex and intermittent social defeat stress on the behavioral and dopaminergic response to acute cocaine, as well as cocaine taking and satiation in an unlimited binge protocol, with a particular focus on the role of circulating ovarian hormones in intact, freely-cycling females. Estradiol plays a key facilitatory role in the expression of intermittent social defeat induced behavioral cross-sensitization to cocaine, possibly though modulation of mesocorticolimbic DAergic function. As the role of estrogen and progesterone is only correlational in intact females, more work should be directed at adequately assessing a more causal role of estrogen and progesterone both alone and in combination in all aspects of these experiments.

Furthermore, the mechanism underlying dopaminergic sensitization as a result of intermittent social defat stress appears to differ between males and females, and should be studied further.

Thus far, preclinical and clinical data on the role of ovarian hormones on the cocaine response and cocaine taking/seeking behaviors seem to converge, but preclinical examinations to compare with clinical reports of heightened, estrogen-dependent effects of stress on the response to cocaine in females are lacking. This crucial missing translational evidence needs to be explored further, so that new treatments for cocaine dependence and addiction tailored to females can be pursued.

REFERENCES

Abramson, J., & Wright, E. M. (2009). Structure and function of Na(+)-symporters with inverted repeats. [Research Support, N.I.H., Extramural Review]. *Curr Opin Struct Biol, 19*(4), 425-432. doi: 10.1016/j.sbi.2009.06.002

Ahmed, S. H., & Koob, G. F. (1997). Cocaine- but not food-seeking behavior is reinstated by stress after extinction. *Psychopharmacology (Berl), 132*(3), 289-295.

Akimoto, K., Hamamura, T., & Otsuki, S. (1989). Subchronic cocaine treatment enhances cocaine-induced dopamine efflux, studied by in vivo intracerebral dialysis. *Brain Res, 490*(2), 339-344. doi: 0006-8993(89)90251-5 [pii]

Altar, C. A., Boylan, C. B., Jackson, C., Hershenson, S., Miller, J., Wiegand, S. J., . . . Hyman, C. (1992). Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover in vivo. *Proc Natl Acad Sci U S A, 89*(23), 11347-11351.

Andersen, S. L., Rutstein, M., Benzo, J. M., Hostetter, J. C., & Teicher, M. H. (1997). Sex differences in dopamine receptor overproduction and elimination. *Neuroreport, 8*(6), 1495-1498.

Anker, J. J., & Carroll, M. E. (2010). Sex differences in the effects of allopregnanolone on yohimbine-induced reinstatement of cocaine seeking in rats. *Drug Alcohol Depend, 107*(2-3), 264-267. doi: S0376-8716(09)00409-8 [pii] 10.1016/j.drugalcdep.2009.11.002

Anker, J. J., & Carroll, M. E. (2011). Females are more vulnerable to drug abuse than males: evidence from preclinical studies and the role of ovarian hormones. *Curr Top Behav Neurosci, 8*, 73-96. doi: 10.1007/7854_2010_93

Anker, J. J., Larson, E. B., Gliddon, L. A., & Carroll, M. E. (2007). Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. *Exp Clin Psychopharmacol, 15*(5), 472-480. doi: 2007-14661-007 [pii] 10.1037/1064-1297.15.5.472

Anker, J. J., Perry, J. L., Gliddon, L. A., & Carroll, M. E. (2009). Impulsivity predicts the escalation of cocaine self-administration in rats. *Pharmacol Biochem Behav, 93*(3), 343-348. doi: S0091-3057(09)00164-6 [pii]
10.1016/j.pbb.2009.05.013

Anstrom, K. K., Miczek, K. A., & Budygin, E. A. (2009). Increased phasic dopamine signaling in the mesolimbic pathway during social defeat in rats. [Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. *Neuroscience, 161*(1), 3-12. doi: 10.1016/j.neuroscience.2009.03.023

Anstrom, K. K., & Woodward, D. J. (2005). Restraint increases dopaminergic burst firing in awake rats. [Comparative Study
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Neuropsychopharmacology, 30*(10), 1832-1840. doi: 10.1038/sj.npp.1300730

Atkinson, H. C., & Waddell, B. J. (1997). Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. *Endocrinology, 138*(9), 3842-3848.

Back, S. E., Brady, K. T., Jackson, J. L., Salstrom, S., & Zinzow, H. (2005). Gender differences in stress reactivity among cocaine-dependent individuals. *Psychopharmacology (Berl), 180*(1), 169-176. doi: 10.1007/s00213-004-2129-7

Back, S. E., Hartwell, K., DeSantis, S. M., Saladin, M., McRae-Clark, A. L., Price, K. L., . . . Brady, K. T. (2010). Reactivity to laboratory stress provocation predicts relapse to cocaine. *Drug Alcohol Depend, 106*(1), 21-27. doi: S0376-8716(09)00308-1 [pii]
10.1016/j.drugalcdep.2009.07.016

Back, S. E., Waldrop, A. E., Saladin, M. E., Yeatts, S. D., Simpson, A., McRae, A. L., . . . Brady, K. T. (2008). Effects of gender and cigarette smoking on reactivity to psychological and pharmacological stress provocation. *Psychoneuroendocrinology, 33*(5), 560-568. doi: S0306-4530(08)00035-8 [pii]
10.1016/j.psyneuen.2008.01.012

Barrett, A. E., & Turner, R. J. (2006). Family structure and substance use problems in adolescence and early adulthood: examining explanations for the relationship. *Addiction, 101*(1), 109-120. doi: ADD1296 [pii]
10.1111/j.1360-0443.2005.01296.x

Bazzett, T. J., & Becker, J. B. (1994). Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. *Brain Res, 637*(1-2), 163-172. doi: 0006-8993(94)91229-7 [pii]

Becker, J. B. (1990a). Direct effect of 17 beta-estradiol on striatum: sex differences in dopamine release. *Synapse, 5*(2), 157-164. doi: 10.1002/syn.890050211

Becker, J. B. (1990b). Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. *Neurosci Lett, 118*(2), 169-171.

Becker, J. B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, Non-P.H.S.
Review]. *Pharmacol Biochem Behav, 64*(4), 803-812.

Becker, J. B., & Beer, M. E. (1986). The influence of estrogen on nigrostriatal dopamine activity: behavioral and neurochemical evidence for both pre- and postsynaptic components. *Behav Brain Res, 19*(1), 27-33. doi: 0166-4328(86)90044-6 [pii]

Becker, J. B., Beer, M. E., & Robinson, T. E. (1984). Striatal dopamine release stimulated by amphetamine or potassium: influence of ovarian hormones and the light-dark cycle. *Brain Res, 311*(1), 157-160. doi: 0006-8993(84)91410-0 [pii]

Becker, J. B., & Cha, J. H. (1989). Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. *Behav Brain Res, 35*(2), 117-125.

Becker, J. B., & Hu, M. (2008). Sex differences in drug abuse. *Front Neuroendocrinol, 29*(1), 36-47. doi: S0091-3022(07)00035-0 [pii]
10.1016/j.yfrne.2007.07.003

Becker, J. B., Molenda, H., & Hummer, D. L. (2001). Gender differences in the behavioral responses to cocaine and amphetamine. Implications for mechanisms mediating gender differences in drug abuse. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, Non-P.H.S.]. *Ann N Y Acad Sci, 937*, 172-187.

Becker, J. B., & Ramirez, V. D. (1981a). Experimental studies on the development of sex differences in the release of dopamine from striatal tissue fragments in vitro. *Neuroendocrinology, 32*(3), 168-173.

Becker, J. B., & Ramirez, V. D. (1981b). Sex differences in the amphetamine stimulated release of catecholamines from rat striatal tissue in vitro. *Brain Res, 204*(2), 361-372. doi: 0006-8993(81)90595-3 [pii]

Becker, J. B., & Rudick, C. N. (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*(1), 53-57. doi: S0091-3057(99)00091-X [pii]

Berridge, K. C., & Valenstein, E. S. (1991). What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? [Research Support, U.S. Gov't, P.H.S.]. *Behav Neurosci, 105*(1), 3-14.

Berton, O., McClung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., . . . Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. [Research Support, Non-U.S. Gov't]. *Science, 311*(5762), 864-868. doi: 10.1126/science.1120972

Beuming, T., Kniazeff, J., Bergmann, M. L., Shi, L., Gracia, L., Raniszewska, K., . . . Loland, C. J. (2008). The binding sites for cocaine and dopamine in the dopamine transporter overlap. *Nat Neurosci, 11*(7), 780-789. doi: nn.2146 [pii]
10.1038/nn.2146

Bonate, P. L., Swann, A., & Silverman, P. B. (1997). Context-dependent cross-sensitization between cocaine and amphetamine. *Life Sci, 60*(1), PL1-7.

Boyson, C. O., Miguel, T. T., Quadros, I. M., Debold, J. F., & Miczek, K. A. (2011). Prevention of social stress-escalated cocaine self-administration by CRF-R1 antagonist in the rat VTA. *Psychopharmacology (Berl), 218*(1), 257-269. doi: 10.1007/s00213-011-2266-8

Brack, K. E., Jeffery, S. M., & Lovick, T. A. (2006). Cardiovascular and respiratory responses to a panicogenic agent in anaesthetised female Wistar rats at different stages of the oestrous cycle. *Eur J Neurosci, 23*(12), 3309-3318. doi: EJN4881 [pii]
10.1111/j.1460-9568.2006.04881.x

Bradberry, C. W., & Roth, R. H. (1989). Cocaine increases extracellular dopamine in rat nucleus accumbens and ventral tegmental area as shown by in vivo microdialysis. *Neurosci Lett, 103*(1), 97-102. doi: 0304-3940(89)90492-8 [pii]

Broer, S. (2006). The SLC6 orphans are forming a family of amino acid transporters. [Research Support, Non-U.S. Gov't
Review]. *Neurochem Int, 48*(6-7), 559-567. doi: 10.1016/j.neuint.2005.11.021

Buffalari, D. M., Baldwin, C. K., Feltenstein, M. W., & See, R. E. (2012). Corticotrophin releasing factor (CRF) induced reinstatement of cocaine seeking in male and female rats. [Research Support, N.I.H., Extramural]. *Physiol Behav, 105*(2), 209-214. doi: 10.1016/j.physbeh.2011.08.020

Burgess, L. H., & Handa, R. J. (1992). Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats. *Endocrinology, 131*(3), 1261-1269.

Burke, A. R., Watt, M. J., & Forster, G. L. (2011). Adolescent social defeat increases adult amphetamine conditioned place preference and alters D2 dopamine receptor expression. [Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. *Neuroscience, 197*, 269-279. doi: 10.1016/j.neuroscience.2011.09.008

Caine, S. B., Bowen, C. A., Yu, G., Zuzga, D., Negus, S. S., & Mello, N. K. (2004). Effect of gonadectomy and gonadal hormone replacement on cocaine self-administration in female and male rats. *Neuropsychopharmacology, 29*(5), 929-942. doi: 10.1038/sj.npp.1300387
1300387 [pii]

Caine, S. B., Heinrichs, S. C., Coffin, V. L., & Koob, G. F. (1995). Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. [Research Support, U.S. Gov't, P.H.S.]. *Brain Res, 692*(1-2), 47-56.

Caine, S. B., Thomsen, M., Gabriel, K. I., Berkowitz, J. S., Gold, L. H., Koob, G. F., . . . Xu, M. (2007). Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. [Research Support, N.I.H., Extramural]. *J Neurosci, 27*(48), 13140-13150. doi: 10.1523/JNEUROSCI.2284-07.2007

Carroll, M. E., Anderson, M. M., & Morgan, A. D. (2007). Higher locomotor response to cocaine in female (vs. male) rats selectively bred for high (HiS) and low (LoS) saccharin intake. *Pharmacol Biochem Behav, 88*(1), 94-104. doi: S0091-3057(07)00231-6 [pii]
10.1016/j.pbb.2007.07.010

Carroll, M. E., Morgan, A. D., Lynch, W. J., Campbell, U. C., & Dess, N. K. (2002). Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: phenotype and sex differences. *Psychopharmacology (Berl), 161*(3), 304-313. doi: 10.1007/s00213-002-1030-5

Castner, S. A., Xiao, L., & Becker, J. B. (1993). Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. [Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.]. *Brain Res, 610*(1), 127-134.

Chang, J. Y., Sawyer, S. F., Lee, R. S., & Woodward, D. J. (1994). Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self-administration in freely moving rats. [Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.]. *J Neurosci, 14*(3 Pt 1), 1224-1244.

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response. *Annu Rev Physiol, 67*, 259-284. doi: 10.1146/annurev.physiol.67.040403.120816

Chefer, V. I., Thompson, A. C., Zapata, A., & Shippenberg, T. S. (2009). Overview of brain microdialysis. *Curr Protoc Neurosci, Chapter 7*, Unit7 1. doi: 10.1002/0471142301.ns0701s47

Chen, K., & Kandel, D. (2002). Relationship between extent of cocaine use and dependence among adolescents and adults in the United States. *Drug Alcohol Depend, 68*(1), 65-85. doi: S0376871602000868 [pii]

Chen, N. H., Reith, M. E., & Quick, M. W. (2004). Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. [Research Support, U.S. Gov't, P.H.S.
Review]. *Pflugers Arch, 447*(5), 519-531. doi: 10.1007/s00424-003-1064-5

Chen, R., Tilley, M. R., Wei, H., Zhou, F., Zhou, F. M., Ching, S., . . . Gu, H. H. (2006). Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. *Proc Natl Acad Sci U S A, 103*(24), 9333-9338. doi: 0600905103 [pii]
10.1073/pnas.0600905103

Chin, J., Sternin, O., Wu, H. B., Burrell, S., Lu, D., Jenab, S., . . . Quinones-Jenab, V. (2002). Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration. *Brain Res, 945*(1), 123-130. doi: S000689930202807X [pii]

Chin, J., Sternin, O., Wu, H. B., Fletcher, H., Perrotti, L. I., Jenab, S., & Quinones-Jenab, V. (2001). Sex differences in cocaine-induced behavioral sensitization. *Cell Mol Biol (Noisy-le-grand), 47*(6), 1089-1095.

Cordeira, J. W., Frank, L., Sena-Esteves, M., Pothos, E. N., & Rios, M. (2010). Brain-derived neurotrophic factor regulates hedonic feeding by acting on the mesolimbic dopamine system. [In Vitro
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. *J Neurosci, 30*(7), 2533-2541. doi: 10.1523/JNEUROSCI.5768-09.2010

Corominas, M., Roncero, C., & Casas, M. (2010). Corticotropin releasing factor and neuroplasticity in cocaine addiction. [Research Support, Non-U.S. Gov't Review]. *Life Sci, 86*(1-2), 1-9. doi: 10.1016/j.lfs.2009.11.005

Council, N. R. (1996). *Guide for the care and use of laboratory animals*. Washington, DC: National Academy Press.

Covington, H. E., 3rd, Kikusui, T., Goodhue, J., Nikulina, E. M., Hammer, R. P., Jr., & Miczek, K. A. (2005). Brief social defeat stress: long lasting effects on cocaine taking during a binge and zif268 mRNA expression in the amygdala and prefrontal cortex. *Neuropsychopharmacology, 30*(2), 310-321. doi: 1300587 [pii]
10.1038/sj.npp.1300587

Covington, H. E., 3rd, & Miczek, K. A. (2001). Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology (Berl), 158*(4), 388-398. doi: 10.1007/s002130100858

Covington, H. E., 3rd, & Miczek, K. A. (2005). Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation. *Psychopharmacology (Berl), 183*(3), 331-340. doi: 10.1007/s00213-005-0190-5

Covington, H. E., 3rd, Tropea, T. F., Rajadhyaksha, A. M., Kosofsky, B. E., & Miczek, K. A. (2008). NMDA receptors in the rat VTA: a critical site for social stress to intensify cocaine taking. *Psychopharmacology (Berl), 197*(2), 203-216. doi: 10.1007/s00213-007-1024-4

Cruz, F. C., Quadros, I. M., Hogenelst, K., Planeta, C. S., & Miczek, K. A. (2011). Social defeat stress in rats: escalation of cocaine and "speedball" binge self-administration, but not heroin. *Psychopharmacology (Berl), 215*(1), 165-175. doi: 10.1007/s00213-010-2139-6

Cummings, J. A., Gowl, B. A., Westenbroek, C., Clinton, S. M., Akil, H., & Becker, J. B. (2011). Effects of a selectively bred novelty-seeking phenotype on the motivation to take cocaine in male and female rats. *Biol Sex Differ, 2*, 3. doi: 2042-6410-2-3 [pii]
10.1186/2042-6410-2-3

Cunningham, K. A., & Callahan, P. M. (1991). Monoamine reuptake inhibitors enhance the discriminative state induced by cocaine in the rat. *Psychopharmacology (Berl), 104*(2), 177-180.

Czoty, P. W., Riddick, N. V., Gage, H. D., Sandridge, M., Nader, S. H., Garg, S., . . . Nader, M. A. (2009). Effect of menstrual cycle phase on dopamine D2 receptor availability in female cynomolgus monkeys. *Neuropsychopharmacology, 34*(3), 548-554. doi: npp20083 [pii]
10.1038/npp.2008.3

Dallman, M. F., Akana, S. F., Strack, A. M., Scribner, K. S., Pecoraro, N., La Fleur, S. E., . . . Gomez, F. (2004). Chronic stress-induced effects of corticosterone on brain: direct and indirect. *Ann N Y Acad Sci, 1018*, 141-150. doi: 10.1196/annals.1296.017
1018/1/141 [pii]

Dazzi, L., Seu, E., Cherchi, G., Barbieri, P. P., Matzeu, A., & Biggio, G. (2007). Estrous cycle-dependent changes in basal and ethanol-induced activity of cortical dopaminergic neurons in the rat. *Neuropsychopharmacology, 32*(4), 892-901. doi: 1301150 [pii]
10.1038/sj.npp.1301150

De Almeida, R. M., & Lucion, A. B. (1994). Effects of intracerebroventricular administration of 5-HT receptor agonists on the maternal aggression of rats. [Comparative Study
Research Support, Non-U.S. Gov't]. *Eur J Pharmacol, 264*(3), 445-448.

de Jong, J. G., Wasilewski, M., van der Vegt, B. J., Buwalda, B., & Koolhaas, J. M. (2005). A single social defeat induces short-lasting behavioral sensitization to amphetamine. [Research Support, Non-U.S. Gov't]. *Physiol Behav, 83*(5), 805-811. doi: 10.1016/j.physbeh.2004.10.002

Di Chiara, G. (1992). Reinforcing drug seeking. [Comment

Letter]. *Trends Pharmacol Sci, 13*(12), 428-429.

Di Paolo, T., Falardeau, P., & Morissette, M. (1988). Striatal D-2 dopamine agonist binding sites fluctuate during the rat estrous cycle. *Life Sci, 43*(8), 665-672.

Di Paolo, T., Rouillard, C., & Bedard, P. (1985). 17 beta-Estradiol at a physiological dose acutely increases dopamine turnover in rat brain. *Eur J Pharmacol, 117*(2), 197-203. doi: 0014-2999(85)90604-1 [pii]

Dietz, D. M., Dietz, K. C., Moore, S., Ouimet, C. C., & Kabbaj, M. (2008). Repeated social defeat stress-induced sensitization to the locomotor activating effects of d-amphetamine: role of individual differences. *Psychopharmacology (Berl), 198*(1), 51-62. doi: 10.1007/s00213-008-1078-y

Diller, J. W., Patros, C. H., & Prentice, P. R. (2011). Temporal discounting and heart rate reactivity to stress. [Research Support, Non-U.S. Gov't]. *Behav Processes, 87*(3), 306-309. doi: 10.1016/j.beproc.2011.05.001

Dow-Edwards, D. (2010). Sex differences in the effects of cocaine abuse across the life span. *Physiol Behav, 100*(3), 208-215. doi: S0031-9384(09)00403-X [pii]
10.1016/j.physbeh.2009.12.017

Elman, I., Karlsgodt, K. H., & Gastfriend, D. R. (2001). Gender differences in cocaine craving among non-treatment-seeking individuals with cocaine dependence. *Am J Drug Alcohol Abuse, 27*(2), 193-202.

Erb, S., Shaham, Y., & Stewart, J. (1996). Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. *Psychopharmacology (Berl), 128*(4), 408-412.

Ettenberg, A., Pettit, H. O., Bloom, F. E., & Koob, G. F. (1982). Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology (Berl), 78*(3), 204-209.

Evans, S. M., & Foltin, R. W. (2006). Exogenous progesterone attenuates the subjective effects of smoked cocaine in women, but not in men. *Neuropsychopharmacology, 31*(3), 659-674. doi: 1300887 [pii]
10.1038/sj.npp.1300887

Evans, S. M., Haney, M., & Foltin, R. W. (2002). The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology (Berl), 159*(4), 397-406. doi: 10.1007/s00213-001-0944-7

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci, 8*(11), 1481-1489. doi: nn1579 [pii]
10.1038/nn1579

Farde, L., Hall, H., Pauli, S., & Halldin, C. (1995). Variability in D2-dopamine receptor density and affinity: a PET study with [11C]raclopride in man. *Synapse, 20*(3), 200-208. doi: 10.1002/syn.890200303

Feltenstein, M. W., Byrd, E. A., Henderson, A. R., & See, R. E. (2009). Attenuation of cocaine-seeking by progesterone treatment in female rats. *Psychoneuroendocrinology, 34*(3), 343-352. doi: S0306-4530(08)00255-2 [pii]
10.1016/j.psyneuen.2008.09.014

Feltenstein, M. W., Henderson, A. R., & See, R. E. (2011). Enhancement of cue-induced reinstatement of cocaine-seeking in rats by yohimbine: sex differences and the role of the estrous cycle. [Research Support, N.I.H., Extramural]. *Psychopharmacology (Berl), 216*(1), 53-62. doi: 10.1007/s00213-011-2187-6

Feltenstein, M. W., & See, R. E. (2007). Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. *Drug Alcohol Depend, 89*(2-3), 183-189. doi: S0376-8716(06)00468-6 [pii]
10.1016/j.drugalcdep.2006.12.017

Festa, E. D., & Quinones-Jenab, V. (2004). Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. *Horm Behav, 46*(5), 509-519. doi: S0018-506X(04)00135-7 [pii]
10.1016/j.yhbeh.2004.04.009

Fox, H. C., Hong, K. I., Siedlarz, K., & Sinha, R. (2008). Enhanced sensitivity to stress and drug/alcohol craving in abstinent cocaine-dependent individuals compared to social drinkers. *Neuropsychopharmacology, 33*(4), 796-805. doi: 1301470 [pii]
10.1038/sj.npp.1301470

Fox, H. C., Hong, K. I., Siedlarz, K. M., Bergquist, K., Anderson, G., Kreek, M. J., & Sinha, R. (2009). Sex-specific dissociations in autonomic and HPA responses to stress and cues in alcohol-dependent patients with cocaine abuse. *Alcohol Alcohol, 44*(6), 575-585. doi: agp060 [pii]
10.1093/alcalc/agp060

Fox, H. C., & Sinha, R. (2009). Sex differences in drug-related stress-system changes: implications for treatment in substance-abusing women. *Harv Rev Psychiatry, 17*(2), 103-119. doi: 910510562 [pii]
10.1080/10673220902899680

Fuchs, R. A., Evans, K. A., Mehta, R. H., Case, J. M., & See, R. E. (2005). Influence of sex and estrous cyclicity on conditioned cue-induced reinstatement of cocaine-seeking behavior in rats. *Psychopharmacology (Berl), 179*(3), 662-672. doi: 10.1007/s00213-004-2080-7

Gallop, R. J., Crits-Christoph, P., Ten Have, T. R., Barber, J. P., Frank, A., Griffin, M. L., & Thase, M. E. (2007). Differential transitions between cocaine use and abstinence for men and women. *J Consult Clin Psychol, 75*(1), 95-103. doi: 2007-00916-010 [pii]
10.1037/0022-006X.75.1.95

Gether, U., Andersen, P. H., Larsson, O. M., & Schousboe, A. (2006). Neurotransmitter transporters: molecular function of important drug targets. *Trends Pharmacol Sci, 27*(7), 375-383. doi: S0165-6147(06)00127-1 [pii]
10.1016/j.tips.2006.05.003

Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., & Caron, M. G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature, 379*(6566), 606-612. doi: 10.1038/379606a0

Goeders, N. E., & Guerin, G. F. (1994). Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. *Psychopharmacology (Berl), 114*(1), 63-70.

Goeders, N. E., & Guerin, G. F. (1996). Role of corticosterone in intravenous cocaine self-administration in rats. *Neuroendocrinology, 64*(5), 337-348.

Graham, D. L., Edwards, S., Bachtell, R. K., DiLeone, R. J., Rios, M., & Self, D. W. (2007). Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Nat Neurosci, 10*(8), 1029-1037. doi: 10.1038/nn1929

Grimm, J. W., Lu, L., Hayashi, T., Hope, B. T., Su, T. P., & Shaham, Y. (2003). Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. [Research Support, U.S. Gov't, P.H.S.]. *J Neurosci, 23*(3), 742-747.

Gulley, J. M., Hoover, B. R., Larson, G. A., & Zahniser, N. R. (2003). Individual differences in cocaine-induced locomotor activity in rats: behavioral characteristics, cocaine pharmacokinetics, and the dopamine transporter. *Neuropsychopharmacology, 28*(12), 2089-2101. doi: 10.1038/sj.npp.1300279 1300279 [pii]

Handa, R. J., Burgess, L. H., Kerr, J. E., & O'Keefe, J. A. (1994). Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Horm Behav, 28*(4), 464-476. doi: S0018-506X(84)71044-0 [pii] 10.1006/hbeh.1994.1044

Haney, M., Maccari, S., Le Moal, M., Simon, H., & Piazza, P. V. (1995). Social stress increases the acquisition of cocaine self-administration in male and female rats. *Brain Res, 698*(1-2), 46-52.

Hatzinger, M., Brand, S., Herzig, N., & Holsboer-Trachsler, E. (2011). In healthy young and elderly adults, hypothalamic-pituitary-adrenocortical axis reactivity (HPA AR) varies with increasing pharmacological challenge and with age, but not with gender. *J Psychiatr Res, 45*(10), 1373-1380. doi: 10.1016/j.jpsychires.2011.05.006

Heinsbroek, R. P., van Haaren, F., Feenstra, M. G., Boon, P., & van de Poll, N. E. (1991). Controllable and uncontrollable footshock and monoaminergic activity in the frontal cortex of male and female rats. *Brain Res, 551*(1-2), 247-255. doi: 0006-8993(91)90939-S [pii]

Hiroi, R., & Neumaier, J. F. (2006). Differential effects of ovarian steroids on anxiety versus fear as measured by open field test and fear-potentiated startle. *Behav Brain Res, 166*(1), 93-100. doi: S0166-4328(05)00288-3 [pii]
10.1016/j.bbr.2005.07.021

Hoglund, P. J., Adzic, D., Scicluna, S. J., Lindblom, J., & Fredriksson, R. (2005). The repertoire of solute carriers of family 6: identification of new human and rodent genes. [Research Support, Non-U.S. Gov't]. *Biochem Biophys Res Commun, 336*(1), 175-189. doi: 10.1016/j.bbrc.2005.08.048

Hruska, R. E., Ludmer, L. M., Pitman, K. T., De Ryck, M., & Silbergeld, E. K. (1982). Effects of Estrogen on Striatal Dopamine receptor function in male and female rats. *Pharmacol Biochem Behav, 16*(2), 285-291.

Hu, M., & Becker, J. B. (2003). Effects of sex and estrogen on behavioral sensitization to cocaine in rats. *J Neurosci, 23*(2), 693-699. doi: 23/2/693 [pii]

Hu, M., Crombag, H. S., Robinson, T. E., & Becker, J. B. (2004). Biological basis of sex differences in the propensity to self-administer cocaine. *Neuropsychopharmacology, 29*(1), 81-85. doi: 10.1038/sj.npp.1300301
1300301 [pii]

Huang, X., Gu, H. H., & Zhan, C. G. (2009). Mechanism for cocaine blocking the transport of dopamine: insights from molecular modeling and dynamics simulations. [Research Support, N.I.H., Extramural]. *J Phys Chem B, 113*(45), 15057-15066. doi: 10.1021/jp900963n

Hyman, S. M., Paliwal, P., Chaplin, T. M., Mazure, C. M., Rounsaville, B. J., & Sinha, R. (2008). Severity of childhood trauma is predictive of cocaine relapse outcomes in women but not men. *Drug Alcohol Depend, 92*(1-3), 208-216. doi: S0376-8716(07)00296-7 [pii]
10.1016/j.drugalcdep.2007.08.006

Ignjatova, L., & Raleva, M. (2009). Gender difference in the treatment outcome of patients served in the mixed-gender program. *Bratisl Lek Listy, 110*(5), 285-289.

Iversen, L. (2006). Neurotransmitter transporters and their impact on the development of psychopharmacology. *Br J Pharmacol, 147 Suppl 1*, S82-88. doi: 0706428 [pii]
10.1038/sj.bjp.0706428

Jackson, L. R., Robinson, T. E., & Becker, J. B. (2006). Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. *Neuropsychopharmacology, 31*(1), 129-138. doi: 1300778 [pii]
10.1038/sj.npp.1300778

Jones, D. C., & Miller, G. W. (2008). The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction. *Biochem Pharmacol, 76*(5), 569-581. doi: S0006-2952(08)00304-3 [pii]
10.1016/j.bcp.2008.05.010

Jones, S. R., Gainetdinov, R. R., Hu, X. T., Cooper, D. C., Wightman, R. M., White, F. J., & Caron, M. G. (1999). Loss of autoreceptor functions in mice lacking the dopamine transporter. [In Vitro
Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Nat Neurosci, 2*(7), 649-655. doi: 10.1038/10204

Jones, S. R., Gainetdinov, R. R., Jaber, M., Giros, B., Wightman, R. M., & Caron, M. G. (1998). Profound neuronal plasticity in response to inactivation of the dopamine transporter. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Proc Natl Acad Sci U S A, 95*(7), 4029-4034.

Justice, J. B., Jr. (1993). Quantitative microdialysis of neurotransmitters. [Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.
Review]. *J Neurosci Methods, 48*(3), 263-276.

Kabbaj, M., Isgor, C., Watson, S. J., & Akil, H. (2002). Stress during adolescence alters behavioral sensitization to amphetamine. [Research Support, U.S. Gov't, P.H.S.]. *Neuroscience, 113*(2), 395-400.

Kalivas, P. W. (1995). Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. [Research Support, U.S. Gov't, P.H.S.
Review]. *Drug Alcohol Depend, 37*(2), 95-100.

Kalivas, P. W., & Duffy, P. (1990). Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse, 5*(1), 48-58. doi: 10.1002/syn.890050104

Kalivas, P. W., & Duffy, P. (1993). Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine perikarya. *J Neurosci, 13*(1), 276-284.

Kalivas, P. W., & O'Brien, C. (2008). Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology, 33*(1), 166-180. doi: 1301564 [pii]
10.1038/sj.npp.1301564

Kalivas, P. W., Richardson-Carlson, R., & Van Orden, G. (1986). Cross-sensitization between foot shock stress and enkephalin-induced motor activity. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Biol Psychiatry, 21*(10), 939-950.

Kalivas, P. W., & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev, 16*(3), 223-244.

Karlsgodt, K. H., Lukas, S. E., & Elman, I. (2003). Psychosocial stress and the duration of cocaine use in non-treatment seeking individuals with cocaine dependence. *Am J Drug Alcohol Abuse, 29*(3), 539-551.

Kaufman, M. J., Levin, J. M., Maas, L. C., Kukes, T. J., Villafuerte, R. A., Dostal, K., . . . Renshaw, P. F. (2001). Cocaine-induced cerebral vasoconstriction differs as a function of sex and menstrual cycle phase. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Biol Psychiatry, 49*(9), 774-781.

Keita, H., Henzel-Rouelle, D., Dupont, H., Desmonts, J. M., & Mantz, J. (1999). Halothane and isoflurane increase spontaneous but reduce the N-methyl-D-aspartate-evoked dopamine release in rat striatal slices: evidence for direct presynaptic effects. [In Vitro
Research Support, Non-U.S. Gov't]. *Anesthesiology, 91*(6), 1788-1797.

Kerstetter, K. A., Aguilar, V. R., Parrish, A. B., & Kippin, T. E. (2008). Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. *Psychopharmacology (Berl), 198*(1), 63-75. doi: 10.1007/s00213-008-1089-8

King, T. S., Canez, M. S., Gaskill, S., Javors, M. A., & Schenken, R. S. (1993). Chronic cocaine disruption of estrous cyclicity in the rat: dose-dependent effects. [Comparative Study
Research Support, U.S. Gov't, P.H.S.]. *J Pharmacol Exp Ther, 264*(1), 29-34.

Kippin, T. E., Fuchs, R. A., Mehta, R. H., Case, J. M., Parker, M. P., Bimonte-Nelson, H. A., & See, R. E. (2005). Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. *Psychopharmacology (Berl), 182*(2), 245-252. doi: 10.1007/s00213-005-0071-y

Kleven, M. S., Anthony, E. W., & Woolverton, W. L. (1990). Pharmacological characterization of the discriminative stimulus effects of cocaine in rhesus monkeys. *J Pharmacol Exp Ther, 254*(1), 312-317.

Kleven, M. S., & Koek, W. (1998). Discriminative stimulus properties of cocaine: enhancement by monoamine reuptake blockers. *J Pharmacol Exp Ther, 284*(3), 1015-1025.

Koob, G. F., & Le Moal, M. (1997). Drug abuse: hedonic homeostatic dysregulation. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Science, 278*(5335), 52-58.

Koob, G. F., Sanna, P. P., & Bloom, F. E. (1998). Neuroscience of addiction. [Research Support, U.S. Gov't, P.H.S.
Review]. *Neuron, 21*(3), 467-476.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology, 35*(1), 217-238. doi: npp2009110 [pii]
10.1038/npp.2009.110

Koolhaas, J. M., De Boer, S. F., De Rutter, A. J., Meerlo, P., & Sgoifo, A. (1997). Social stress in rats and mice. [Review]. *Acta Physiol Scand Suppl, 640*, 69-72.

Kosten, T. A., Gawin, F. H., Kosten, T. R., & Rounsaville, B. J. (1993). Gender differences in cocaine use and treatment response. *J Subst Abuse Treat, 10*(1), 63-66. doi: 0740-5472(93)90100-G [pii]

Kouri, E. M., Lundahl, L. H., Borden, K. N., McNeil, J. F., & Lukas, S. E. (2002). Effects of oral contraceptives on acute cocaine response in female volunteers. [Clinical Trial
Randomized Controlled Trial
Research Support, U.S. Gov't, P.H.S.]. *Pharmacol Biochem Behav, 74*(1), 173-180.

Larson, E. B., Anker, J. J., Gliddon, L. A., Fons, K. S., & Carroll, M. E. (2007). Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. *Exp Clin Psychopharmacol, 15*(5), 461-471. doi: 2007-14661-006 [pii]
10.1037/1064-1297.15.5.461

Larson, E. B., & Carroll, M. E. (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*(6), 1334-1345. doi: 1301249 [pii]
10.1038/sj.npp.1301249

Larson, E. B., Roth, M. E., Anker, J. J., & Carroll, M. E. (2005). Effect of short- vs. long-term estrogen on reinstatement of cocaine-seeking behavior in female rats. *Pharmacol Biochem Behav, 82*(1), 98-108. doi: S0091-3057(05)00249-2 [pii]
10.1016/j.pbb.2005.07.015

Levesque, D., & Di Paolo, T. (1988). Rapid conversion of high into low striatal D2-dopamine receptor agonist binding states after an acute physiological dose of 17 beta-estradiol. *Neurosci Lett, 88*(1), 113-118.

Levesque, D., & Di Paolo, T. (1993). Modulation by estradiol and progesterone of the GTP effect on striatal D-2 dopamine receptors. *Biochem Pharmacol, 45*(3), 723-733. doi: 0006-2952(93)90148-P [pii]

Levesque, K., Moskowitz, D. S., Tardif, J. C., Dupuis, G., & D'Antono, B. (2010). Physiological stress responses in defensive individuals: age and sex matter. [Research Support, Non-U.S. Gov't]. *Psychophysiology, 47*(2), 332-341. doi: 10.1111/j.1469-8986.2009.00943.x

Liu, Y., Morgan, D., & Roberts, D. C. (2007). Cross-sensitization of the reinforcing effects of cocaine and amphetamine in rats. [Research Support, N.I.H., Extramural]. *Psychopharmacology (Berl), 195*(3), 369-375. doi: 10.1007/s00213-007-0909-6

Lodge, D. J., & Grace, A. A. (2005). Acute and chronic corticotropin-releasing factor 1 receptor blockade inhibits cocaine-induced dopamine release: correlation with dopamine neuron activity. *J Pharmacol Exp Ther, 314*(1), 201-206. doi: jpet.105.084913 [pii]
10.1124/jpet.105.084913

Loh, E. A., & Roberts, D. C. (1990). Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. *Psychopharmacology (Berl), 101*(2), 262-266.

Lukas, S. E., Sholar, M., Lundahl, L. H., Lamas, X., Kouri, E., Wines, J. D., . . . Mendelson, J. H. (1996). Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers. *Psychopharmacology (Berl), 125*(4), 346-354.

Lynch, W. J. (2008). Acquisition and maintenance of cocaine self-administration in adolescent rats: effects of sex and gonadal hormones. *Psychopharmacology (Berl), 197*(2), 237-246. doi: 10.1007/s00213-007-1028-0

Lynch, W. J., Arizzi, M. N., & Carroll, M. E. (2000). Effects of sex and the estrous cycle on regulation of intravenously self-administered cocaine in rats. *Psychopharmacology (Berl), 152*(2), 132-139.

Lynch, W. J., & Carroll, M. E. (1999). Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. *Psychopharmacology (Berl), 144*(1), 77-82.

Lynch, W. J., & Carroll, M. E. (2000). Reinstatement of cocaine self-administration in rats: sex differences. *Psychopharmacology (Berl), 148*(2), 196-200. doi: 01480196.213 [pii]

Lynch, W. J., Roth, M. E., Mickelberg, J. L., & Carroll, M. E. (2001). Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. *Pharmacol Biochem Behav, 68*(4), 641-646. doi: S0091-3057(01)00455-5 [pii]

Lynch, W. J., & Taylor, J. R. (2004). Sex differences in the behavioral effects of 24-h/day access to cocaine under a discrete trial procedure. *Neuropsychopharmacology, 29*(5), 943-951. doi: 10.1038/sj.npp.1300389 1300389 [pii]

Lynch, W. J., & Taylor, J. R. (2005). Persistent changes in motivation to self-administer cocaine following modulation of cyclic AMP-dependent protein kinase A (PKA) activity in the nucleus accumbens. *Eur J Neurosci, 22*(5), 1214-1220. doi: EJN4305 [pii] 10.1111/j.1460-9568.2005.04305.x

Mantsch, J. R., & Goeders, N. E. (1998). Generalization of a restraint-induced discriminative stimulus to cocaine in rats. *Psychopharmacology (Berl), 135*(4), 423-426.

Mantsch, J. R., & Goeders, N. E. (1999). Ketoconazole blocks the stress-induced reinstatement of cocaine-seeking behavior in rats: relationship to the discriminative stimulus effects of cocaine. *Psychopharmacology (Berl), 142*(4), 399-407.

Mantsch, J. R., Saphier, D., & Goeders, N. E. (1998). Corticosterone facilitates the acquisition of cocaine self-administration in rats: opposite effects of the type II glucocorticoid receptor agonist dexamethasone. *J Pharmacol Exp Ther, 287*(1), 72-80.

Marrow, L. P., Overton, P. G., & Brain, P. F. (1999). A re-evaluation of social defeat as an animal model of depression. [Research Support, Non-U.S. Gov't]. *J Psychopharmacol, 13*(2), 115-121.

Maus, M., Bertrand, P., Drouva, S., Rasolonjanahary, R., Kordon, C., Glowinski, J., . . . Enjalbert, A. (1989). Differential modulation of D1 and D2 dopamine-sensitive adenylate cyclases by 17 beta-estradiol in cultured striatal neurons and anterior pituitary cells. *J Neurochem, 52*(2), 410-418.

Maus, M., Cordier, J., Glowinski, J., & Premont, J. (1989). 17-beta Oestradiol Pretreatment of Mouse Striatal Neurons in Culture Enhances the Responses of Adenylate Cyclase Sensitive to Biogenic Amines. *Eur J Neurosci, 1*(2), 154-161. doi: ejn_01020154 [pii]

McCance-Katz, E. F., Carroll, K. M., & Rounsaville, B. J. (1999). Gender differences in treatment-seeking cocaine abusers--implications for treatment and prognosis. *Am J Addict, 8*(4), 300-311.

McCormick, C. M., & Mathews, I. Z. (2007). HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacol Biochem Behav, 86*(2), 220-233. doi: S0091-3057(06)00222-X [pii]
10.1016/j.pbb.2006.07.012

McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annu Rev Neurosci, 22*, 105-122. doi: 10.1146/annurev.neuro.22.1.105

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev, 87*(3), 873-904. doi: 87/3/873 [pii]
10.1152/physrev.00041.2006

McEwen, B. S., & Alves, S. E. (1999). Estrogen actions in the central nervous system. *Endocr Rev, 20*(3), 279-307.

McGregor, A., & Roberts, D. C. (1993). Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Brain Res, 624*(1-2), 245-252.

McKay, J. R., Rutherford, M. J., Cacciola, J. S., Kabasakalian-McKay, R., & Alterman, A. I. (1996). Gender differences in the relapse experiences of cocaine patients. *J Nerv Ment Dis, 184*(10), 616-622.

McLaughlin, J. P., Li, S., Valdez, J., Chavkin, T. A., & Chavkin, C. (2006). Social defeat stress-induced behavioral responses are mediated by the endogenous kappa opioid system. [Comparative Study
Research Support, N.I.H., Extramural

Research Support, Non-U.S. Gov't]. *Neuropsychopharmacology, 31*(6), 1241-1248. doi: 10.1038/sj.npp.1300872

Meiergerd, S. M., Patterson, T. A., & Schenk, J. O. (1993). D2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *J Neurochem, 61*(2), 764-767.

Mendelson, J. H., Sholar, M. B., Siegel, A. J., & Mello, N. K. (2001). Effects of cocaine on luteinizing hormone in women during the follicular and luteal phases of the menstrual cycle and in men. [Clinical Trial
Comparative Study
Controlled Clinical Trial
Research Support, U.S. Gov't, P.H.S.]. *J Pharmacol Exp Ther, 296*(3), 972-979.

Menendez-Delmestre, R., & Segarra, A. C. (2011). Testosterone is essential for cocaine sensitization in male rats. *Physiol Behav, 102*(1), 96-104. doi: S0031-9384(10)00353-7 [pii]
10.1016/j.physbeh.2010.09.025

Mermelstein, P. G., Becker, J. B., & Surmeier, D. J. (1996). Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. [Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.]. *J Neurosci, 16*(2), 595-604.

Miczek, K. A. (1979). A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology (Berl), 60*(3), 253-259.

Miczek, K. A., & Mutschler, N. H. (1996). Activational effects of social stress on IV cocaine self-administration in rats. *Psychopharmacology (Berl), 128*(3), 256-264.

Miczek, K. A., Mutschler, N. H., van Erp, A. M., Blank, A. D., & McInerney, S. C. (1999). d-amphetamine "cue" generalizes to social defeat stress: behavioral sensitization and attenuated accumbens dopamine. [Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology (Berl), 147*(2), 190-199.

Miczek, K. A., Nikulina, E. M., Shimamoto, A., & Covington, H. E., 3rd. (2011). Escalated or suppressed cocaine reward, tegmental BDNF, and accumbal

dopamine caused by episodic versus continuous social stress in rats. *J Neurosci, 31*(27), 9848-9857. doi: 31/27/9848 [pii]
10.1523/JNEUROSCI.0637-11.2011

Minerly, A. E., Russo, S. J., Kemen, L. M., Nazarian, A., Wu, H. B., Weierstall, K. M., . . . Quinones-Jenab, V. (2008). Testosterone plays a limited role in cocaine-induced conditioned place preference and locomotor activity in male rats. *Ethn Dis, 18*(2 Suppl 2), S2-200-204.

Minerly, A. E., Wu, H. B., Weierstall, K. M., Niyomchai, T., Kemen, L., Jenab, S., & Quinones-Jenab, V. (2010). Testosterone differentially alters cocaine-induced ambulatory and rearing behavioral responses in adult and adolescent rats. *Pharmacol Biochem Behav, 94*(3), 404-409. doi: S0091-3057(09)00292-5 [pii]
10.1016/j.pbb.2009.10.001

Munro, C. A., McCaul, M. E., Wong, D. F., Oswald, L. M., Zhou, Y., Brasic, J., . . . Wand, G. S. (2006). Sex differences in striatal dopamine release in healthy adults. *Biol Psychiatry, 59*(10), 966-974. doi: S0006-3223(06)00133-8 [pii]
10.1016/j.biopsych.2006.01.008

Narita, M., Aoki, K., Takagi, M., Yajima, Y., & Suzuki, T. (2003). Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. [Research Support, Non-U.S. Gov't]. *Neuroscience, 119*(3), 767-775.

Nequin, L. G., Alvarez, J., & Schwartz, N. B. (1979). Measurement of serum steroid and gonadotropin levels and uterine and ovarian variables throughout 4 day and 5 day estrous cycles in the rat. [Research Support, U.S. Gov't, P.H.S.]. *Biol Reprod, 20*(3), 659-670.

Nestler, E. J. (2001). Molecular neurobiology of addiction. [Research Support, U.S. Gov't, P.H.S.
Review]. *Am J Addict, 10*(3), 201-217.

Nestler, E. J. (2002). Common molecular and cellular substrates of addiction and memory. *Neurobiol Learn Mem, 78*(3), 637-647. doi: S1074742702940840 [pii]

Newcomb, M. D., & Bentler, P. M. (1988). Impact of adolescent drug use and social support on problems of young adults: a longitudinal study. *J Abnorm Psychol, 97*(1), 64-75.

Newcomb, M. D., & Harlow, L. L. (1986). Life events and substance use among adolescents: mediating effects of perceived loss of control and meaninglessness in life. *J Pers Soc Psychol, 51*(3), 564-577.

Newhouse, P. A., Dumas, J., Hancur-Bucci, C., Naylor, M., Sites, C. K., Benkelfat, C., & Young, S. N. (2008). Estrogen administration negatively alters mood following monoaminergic depletion and psychosocial stress in postmenopausal women. [Randomized Controlled Trial
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. *Neuropsychopharmacology, 33*(7), 1514-1527. doi: 10.1038/sj.npp.1301530

Nikulina, E. M., Covington, H. E., 3rd, Ganschow, L., Hammer, R. P., Jr., & Miczek, K. A. (2004). Long-term behavioral and neuronal cross-sensitization to amphetamine induced by repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. *Neuroscience, 123*(4), 857-865. doi: S0306452203008352 [pii]

O'Brien, M. S., & Anthony, J. C. (2005). Risk of becoming cocaine dependent: epidemiological estimates for the United States, 2000-2001. *Neuropsychopharmacology, 30*(5), 1006-1018. doi: 1300681 [pii]
10.1038/sj.npp.1300681

Parsons, L. H., & Justice, J. B., Jr. (1993). Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area, and dorsal raphe nucleus following repeated cocaine administration. *J Neurochem, 61*(5), 1611-1619.

Parsons, L. H., & Justice, J. B., Jr. (1994). Quantitative approaches to in vivo brain microdialysis. [Review]. *Crit Rev Neurobiol, 8*(3), 189-220.

Patchev, V. K., Hayashi, S., Orikasa, C., & Almeida, O. F. (1995). Implications of estrogen-dependent brain organization for gender differences in hypothalamo-pituitary-adrenal regulation. *FASEB J, 9*(5), 419-423.

Paulson, P. E., & Robinson, T. E. (1995). Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral

striatum: a microdialysis study in behaving rats. *Synapse, 19*(1), 56-65. doi: 10.1002/syn.890190108

Paxinos, G., & Watson, C. (1997). *The rat brain in stereotaxic coordinates* (3 ed.). San Diego: Academic.

Pazos, A., Stoeckel, M. E., Hindelang, C., & Palacios, J. M. (1985). Autoradiographic studies on dopamine D2 receptors in rat pituitary: influence of hormonal states. *Neurosci Lett, 59*(1), 1-7. doi: 0304-3940(85)90206-X [pii]

Perrotti, L. I., Russo, S. J., Fletcher, H., Chin, J., Webb, T., Jenab, S., & Quinones-Jenab, V. (2001). Ovarian hormones modulate cocaine-induced locomotor and stereotypic activity. *Ann N Y Acad Sci, 937*, 202-216.

Perry, J. L., Nelson, S. E., & Carroll, M. E. (2008). Impulsive choice as a predictor of acquisition of IV cocaine self- administration and reinstatement of cocaine-seeking behavior in male and female rats. *Exp Clin Psychopharmacol, 16*(2), 165-177. doi: 2008-03846-007 [pii]
10.1037/1064-1297.16.2.165

Piazza, P. V., Deminiere, J. M., le Moal, M., & Simon, H. (1990). Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. *Brain Res, 514*(1), 22-26. doi: 0006-8993(90)90431-A [pii]

Piazza, P. V., & Le Moal, M. (1997). Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. *Brain Res Brain Res Rev, 25*(3), 359-372. doi: S0165017397000258 [pii]

Piazza, P. V., & Le Moal, M. L. (1996). Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu Rev Pharmacol Toxicol, 36*, 359-378. doi: 10.1146/annurev.pa.36.040196.002043

Pich, E. M., Heinrichs, S. C., Rivier, C., Miczek, K. A., Fisher, D. A., & Koob, G. F. (1993). Blockade of pituitary-adrenal axis activation induced by peripheral

immunoneutralization of corticotropin-releasing factor does not affect the behavioral response to social defeat stress in rats. [Research Support, U.S. Gov't, P.H.S.]. *Psychoneuroendocrinology, 18*(7), 495-507.

Pierce, R. C., Duffy, P., & Kalivas, P. W. (1995). Sensitization to cocaine and dopamine autoreceptor subsensitivity in the nucleus accumbens. *Synapse, 20*(1), 33-36. doi: 10.1002/syn.890200106

Prasad, B. M., Ulibarri, C., & Sorg, B. A. (1998). Stress-induced cross-sensitization to cocaine: effect of adrenalectomy and corticosterone after short- and long-term withdrawal. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology (Berl), 136*(1), 24-33.

Prus, A. J., James, J. R., & Rosecrans, J. A. (2009). Conditioned Place Preference. In J. J. Buccafusco (Ed.), *Methods of Behavior Analysis in Neuroscience* (2nd ed.). Boca Raton (FL).

Puig-Ramos, A., Santiago, G. S., & Segarra, A. C. (2008). U-69593, a kappa opioid receptor agonist, decreases cocaine-induced behavioral sensitization in female rats. [Research Support, N.I.H., Extramural]. *Behav Neurosci, 122*(1), 151-160. doi: 10.1037/0735-7044.122.1.151

Quadros, I. M., & Miczek, K. A. (2009). Two modes of intense cocaine bingeing: increased persistence after social defeat stress and increased rate of intake due to extended access conditions in rats. *Psychopharmacology (Berl), 206*(1), 109-120. doi: 10.1007/s00213-009-1584-6

Quinones-Jenab, V., Perrotti, L. I., Mc Monagle, J., Ho, A., & Kreek, M. J. (2000). Ovarian hormone replacement affects cocaine-induced behaviors in ovariectomized female rats. *Pharmacol Biochem Behav, 67*(3), 417-422. doi: S0091-3057(00)00381-6 [pii]

Ramsey, N. F., & Van Ree, J. M. (1993). Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naive rats. *Brain Res, 608*(2), 216-222. doi: 0006-8993(93)91461-Z [pii]

Reith, M. E., Kim, S. S., & Lajtha, A. (1986). Structural requirements for cocaine congeners to interact with [3H]batrachotoxinin A 20-alpha-benzoate binding sites on sodium channels in mouse brain synaptosomes. *J Biol Chem, 261*(16), 7300-7305.

Ribeiro Do Couto, B., Aguilar, M. A., Lluch, J., Rodriguez-Arias, M., & Minarro, J. (2009). Social experiences affect reinstatement of cocaine-induced place preference in mice. [Research Support, Non-U.S. Gov't]. *Psychopharmacology (Berl), 207*(3), 485-498. doi: 10.1007/s00213-009-1678-1

Ribeiro Do Couto, B., Aguilar, M. A., Manzanedo, C., Rodriguez-Arias, M., Armario, A., & Minarro, J. (2006). Social stress is as effective as physical stress in reinstating morphine-induced place preference in mice. [Research Support, Non-U.S. Gov't]. *Psychopharmacology (Berl), 185*(4), 459-470. doi: 10.1007/s00213-006-0345-z

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237*(4819), 1219-1223.

Rivier, C. (1999). Gender, sex steroids, corticotropin-releasing factor, nitric oxide, and the HPA response to stress. *Pharmacol Biochem Behav, 64*(4), 739-751.

Robbins, S. J., Ehrman, R. N., Childress, A. R., & O'Brien, C. P. (1999). Comparing levels of cocaine cue reactivity in male and female outpatients. *Drug Alcohol Depend, 53*(3), 223-230. doi: S0376871698001355 [pii]

Roberts, D. C. (2010). Self-Administration of Drugs. In I. P. Stolerman (Ed.), *Encyclopedia of psychopharmacology*. New York: Springer.

Roberts, D. C., Bennett, S. A., & Vickers, G. J. (1989). The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. *Psychopharmacology (Berl), 98*(3), 408-411.

Roberts, D. C., Corcoran, M. E., & Fibiger, H. C. (1977). On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav, 6*(6), 615-620.

Roberts, D. C., & Koob, G. F. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. [Research Support, Non-U.S. Gov't]. *Pharmacol Biochem Behav, 17*(5), 901-904.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev, 18*(3), 247-291.

Robinson, T. E., Jurson, P. A., Bennett, J. A., & Bentgen, K. M. (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*(2), 211-222. doi: 0006-8993(88)90549-5 [pii]

Rocha, B. A., Fumagalli, F., Gainetdinov, R. R., Jones, S. R., Ator, R., Giros, B., . . . Caron, M. G. (1998). Cocaine self-administration in dopamine-transporter knockout mice. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Nat Neurosci, 1*(2), 132-137. doi: 10.1038/381

Rodaros, D., Caruana, D. A., Amir, S., & Stewart, J. (2007). Corticotropin-releasing factor projections from limbic forebrain and paraventricular nucleus of the hypothalamus to the region of the ventral tegmental area. *Neuroscience, 150*(1), 8-13. doi: S0306-4522(07)01098-6 [pii]
10.1016/j.neuroscience.2007.09.043

Roth, M. E., & Carroll, M. E. (2004). Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. *Pharmacol Biochem Behav, 78*(2), 199-207. doi: 10.1016/j.pbb.2004.03.018
S0091305704001029 [pii]

Russo, S. J., Festa, E. D., Fabian, S. J., Gazi, F. M., Kraish, M., Jenab, S., & Quinones-Jenab, V. (2003). Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. *Neuroscience, 120*(2), 523-533. doi: S0306452203003178 [pii]

Russo, S. J., Jenab, S., Fabian, S. J., Festa, E. D., Kemen, L. M., & Quinones-Jenab, V. (2003). Sex differences in the conditioned rewarding effects of cocaine. *Brain Res, 970*(1-2), 214-220. doi: S0006899303023461 [pii]

SAMHSA. (2010). *Results from the 2010 National Survey on Drug Use and Health: National Findings*. (DHHS pub. no SMA 09-4434). Rockville, MD: SAMHSA.

Sarnyai, Z., Mello, N. K., Mendelson, J. H., Eros-Sarnyai, M., & Mercer, G. (1996). Effects of cocaine on pulsatile activity of hypothalamic-pituitary-adrenal axis in male rhesus monkeys: neuroendocrine and behavioral correlates. *J Pharmacol Exp Ther, 277*(1), 225-234.

Schenk, S., Lacelle, G., Gorman, K., & Amit, Z. (1987). Cocaine self-administration in rats influenced by environmental conditions: implications for the etiology of drug abuse. *Neurosci Lett, 81*(1-2), 227-231.

Schmaus, B. J., Laubmeier, K. K., Boquiren, V. M., Herzer, M., & Zakowski, S. G. (2008). Gender and stress: differential psychophysiological reactivity to stress reexposure in the laboratory. [Comparative Study]. *Int J Psychophysiol, 69*(2), 101-106. doi: 10.1016/j.ijpsycho.2008.03.006

Segal, D. S., & Mandell, A. J. (1974). Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmacol Biochem Behav, 2*(2), 249-255.

Segarra, A. C., Agosto-Rivera, J. L., Febo, M., Lugo-Escobar, N., Menendez-Delmestre, R., Puig-Ramos, A., & Torres-Diaz, Y. M. (2010). Estradiol: a key biological substrate mediating the response to cocaine in female rats. *Horm Behav, 58*(1), 33-43. doi: S0018-506X(09)00258-X [pii]
10.1016/j.yhbeh.2009.12.003

Self, D. W., Barnhart, W. J., Lehman, D. A., & Nestler, E. J. (1996). Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. *Science, 271*(5255), 1586-1589.

Sell, S. L., Scalzitti, J. M., Thomas, M. L., & Cunningham, K. A. (2000). Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. *J Pharmacol Exp Ther, 293*(3), 879-886.

Sell, S. L., Thomas, M. L., & Cunningham, K. A. (2002). Influence of estrous cycle and estradiol on behavioral sensitization to cocaine in female rats. [Research Support, Non-U.S. Gov't

Research Support, U.S. Gov't, P.H.S.]. *Drug Alcohol Depend, 67*(3), 281-290.

Shaham, Y., Erb, S., & Stewart, J. (2000). Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res Brain Res Rev, 33*(1), 13-33. doi: S0165017300000242 [pii]

Shimamoto, A., Debold, J. F., Holly, E. N., & Miczek, K. A. (2011). Blunted accumbal dopamine response to cocaine following chronic social stress in female rats: exploring a link between depression and drug abuse. *Psychopharmacology (Berl), 218*(1), 271-279. doi: 10.1007/s00213-011-2364-7

Siegal, N., & Dow-Edwards, D. (2009). Isoflurane anesthesia interferes with the expression of cocaine-induced sensitization in female rats. [Comparative Study]. *Neurosci Lett, 464*(1), 52-56. doi: 10.1016/j.neulet.2009.07.088

Sinha, R. (2001). How does stress increase risk of drug abuse and relapse? *Psychopharmacology (Berl), 158*(4), 343-359. doi: 10.1007/s002130100917

Sinha, R. (2008). Chronic stress, drug use, and vulnerability to addiction. *Ann N Y Acad Sci, 1141*, 105-130. doi: NYAS1141030 [pii]
10.1196/annals.1441.030

Sinha, R. (2009). Modeling stress and drug craving in the laboratory: implications for addiction treatment development. [Research Support, N.I.H., Extramural Review]. *Addict Biol, 14*(1), 84-98. doi: 10.1111/j.1369-1600.2008.00134.x

Sinha, R., Catapano, D., & O'Malley, S. (1999). Stress-induced craving and stress response in cocaine dependent individuals. *Psychopharmacology (Berl), 142*(4), 343-351.

Sinha, R., Fox, H., Hong, K. I., Sofuoglu, M., Morgan, P. T., & Bergquist, K. T. (2007). Sex steroid hormones, stress response, and drug craving in cocaine-dependent women: implications for relapse susceptibility. *Exp Clin Psychopharmacol, 15*(5), 445-452. doi: 2007-14661-004 [pii]
10.1037/1064-1297.15.5.445

Sinha, R., Fuse, T., Aubin, L. R., & O'Malley, S. S. (2000). Psychological stress, drug-related cues and cocaine craving. *Psychopharmacology (Berl), 152*(2), 140-148.

Sinha, R., Garcia, M., Paliwal, P., Kreek, M. J., & Rounsaville, B. J. (2006). Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. *Arch Gen Psychiatry, 63*(3), 324-331. doi: 63/3/324 [pii]
10.1001/archpsyc.63.3.324

Sinha, R., & Li, C. S. (2007). Imaging stress- and cue-induced drug and alcohol craving: association with relapse and clinical implications. *Drug Alcohol Rev, 26*(1), 25-31. doi: 769824468 [pii]
10.1080/09595230601036960

Sinha, R., Talih, M., Malison, R., Cooney, N., Anderson, G. M., & Kreek, M. J. (2003). Hypothalamic-pituitary-adrenal axis and sympatho-adreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. *Psychopharmacology (Berl), 170*(1), 62-72. doi: 10.1007/s00213-003-1525-8

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*(1), 54-65.

Sofuoglu, M., Babb, D. A., & Hatsukami, D. K. (2002). Effects of progesterone treatment on smoked cocaine response in women. *Pharmacol Biochem Behav, 72*(1-2), 431-435. doi: S0091305702007165 [pii]

Sofuoglu, M., Dudish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K. (1999). Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. *Exp Clin Psychopharmacol, 7*(3), 274-283.

Sora, I., Wichems, C., Takahashi, N., Li, X. F., Zeng, Z., Revay, R., . . . Uhl, G. R. (1998). Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Proc Natl Acad Sci U S A, 95*(13), 7699-7704.

Sorg, B. A. (1992). Mesocorticolimbic dopamine systems: cross-sensitization between stress and cocaine. *Ann N Y Acad Sci, 654*, 136-144.

Sorg, B. A., & Kalivas, P. W. (1991). Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Brain Res, 559*(1), 29-36.

Sorg, B. A., & Steketee, J. D. (1992). Mechanisms of cocaine-induced sensitization. *Prog Neuropsychopharmacol Biol Psychiatry, 16*(6), 1003-1012. doi: 0278-5846(92)90117-W [pii]

Staples, R. E., & Geils, H. D. (1965). An Observation on the Vaginal Smear of the Rat. *J Endocrinol, 32*, 263-264.

Stoney, C. M., Davis, M. C., & Matthews, K. A. (1987). Sex differences in physiological responses to stress and in coronary heart disease: a causal link? [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Psychophysiology, 24*(2), 127-131.

Swanson, L. W., Sawchenko, P. E., Rivier, J., & Vale, W. W. (1983). Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology, 36*(3), 165-186.

Swanson, L. W., & Simmons, D. M. (1989). Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J Comp Neurol, 285*(4), 413-435. doi: 10.1002/cne.902850402

Tersman, Z., Collins, A., & Eneroth, P. (1991). Cardiovascular responses to psychological and physiological stressors during the menstrual cycle. [Research Support, Non-U.S. Gov't]. *Psychosom Med, 53*(2), 185-197.

Thomas, M. J., Kalivas, P. W., & Shaham, Y. (2008). Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. *Br J Pharmacol, 154*(2), 327-342. doi: bjp200877 [pii]
10.1038/bjp.2008.77

Thompson, T. L. (1999). Attenuation of dopamine uptake in vivo following priming with estradiol benzoate. *Brain Res, 834*(1-2), 164-167. doi: S0006-8993(99)01508-5 [pii]

Thompson, T. L., Moore, C. C., & Smith, B. (2000). Estrogen priming modulates autoreceptor-mediated potentiation of dopamine uptake. *Eur J Pharmacol, 401*(3), 357-363. doi: S0014299900004325 [pii]

Thompson, T. L., & Moss, R. L. (1995). In vivo stimulated dopamine release in the nucleus accumbens: modulation by the prefrontal cortex. *Brain Res, 686*(1), 93-98. doi: 0006-8993(95)00429-T [pii]

Thomsen, M., Hall, F. S., Uhl, G. R., & Caine, S. B. (2009). Dramatically decreased cocaine self-administration in dopamine but not serotonin transporter knock-out mice. [Research Support, N.I.H., Extramural
Research Support, N.I.H., Intramural]. *J Neurosci, 29*(4), 1087-1092. doi: 10.1523/JNEUROSCI.4037-08.2009

Thomsen, M., Han, D. D., Gu, H. H., & Caine, S. B. (2009). Lack of cocaine self-administration in mice expressing a cocaine-insensitive dopamine transporter. [Comparative Study
Research Support, N.I.H., Extramural]. *J Pharmacol Exp Ther, 331*(1), 204-211. doi: 10.1124/jpet.109.156265

Tidey, J. W., & Miczek, K. A. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain Res, 721*(1-2), 140-149. doi: 0006-8993(96)00159-X [pii]

Tidey, J. W., & Miczek, K. A. (1997). Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. [Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology (Berl), 130*(3), 203-212.

Tornatzky, W., & Miczek, K. A. (1995). Alcohol, anxiolytics and social stress in rats. *Psychopharmacology (Berl), 121*(1), 135-144.

Torres, G. E., & Amara, S. G. (2007). Glutamate and monoamine transporters: new visions of form and function. *Curr Opin Neurobiol, 17*(3), 304-312. doi: S0959-4388(07)00068-2 [pii]
10.1016/j.conb.2007.05.002

Toufexis, D. J., Davis, C., Hammond, A., & Davis, M. (2004). Progesterone attenuates corticotropin-releasing factor-enhanced but not fear-potentiated startle via the activity of its neuroactive metabolite, allopregnanolone. *J Neurosci, 24*(45), 10280-10287. doi: 24/45/10280 [pii]
10.1523/JNEUROSCI.1386-04.2004

Trzcinska, M., Bergh, J., DeLeon, K., Stellar, J. R., & Melloni, R. H., Jr. (2002). Social stress does not alter the expression of sensitization to cocaine. *Physiol Behav, 76*(4-5), 457-463.

Uchimura, N., & North, R. A. (1990). Actions of cocaine on rat nucleus accumbens neurones in vitro. *Br J Pharmacol, 99*(4), 736-740.

UNODC. (2011). World Drug Report.

van Haaren, F., & Meyer, M. E. (1991). Sex differences in locomotor activity after acute and chronic cocaine administration. *Pharmacol Biochem Behav, 39*(4), 923-927. doi: 0091-3057(91)90054-6 [pii]

Vanderschuren, L. J., & Kalivas, P. W. (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl), 151*(2-3), 99-120.

Veit, R., Brody, S., & Rau, H. (1997). Four-year stability of cardiovascular reactivity to psychological stress. [Clinical Trial
Controlled Clinical Trial
Research Support, Non-U.S. Gov't]. *J Behav Med, 20*(5), 447-460.

Volkow, N. D., Wang, G. J., Fischman, M. W., Foltin, R. W., Fowler, J. S., Abumrad, N. N., . . . Shea, C. E. (1997). Relationship between subjective effects of cocaine and dopamine transporter occupancy. [Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.]. *Nature, 386*(6627), 827-830. doi: 10.1038/386827a0

Waldrop, A. E., Price, K. L., Desantis, S. M., Simpson, A. N., Back, S. E., McRae, A. L., . . . Brady, K. T. (2010). Community-dwelling cocaine-dependent men and women respond differently to social stressors versus cocaine cues. [Comparative Study
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. *Psychoneuroendocrinology, 35*(6), 798-806. doi: 10.1016/j.psyneuen.2009.11.005

Walker, Q. D., Ray, R., & Kuhn, C. M. (2006). Sex differences in neurochemical effects of dopaminergic drugs in rat striatum. *Neuropsychopharmacology, 31*(6), 1193-1202. doi: 1300915 [pii]
10.1038/sj.npp.1300915

Walker, Q. D., Rooney, M. B., Wightman, R. M., & Kuhn, C. M. (2000). Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. *Neuroscience, 95*(4), 1061-1070. doi: S030645229900500X [pii]

Wanat, M. J., Hopf, F. W., Stuber, G. D., Phillips, P. E., & Bonci, A. (2008). Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. *J Physiol, 586*(8), 2157-2170. doi: jphysiol.2007.150078 [pii]
10.1113/jphysiol.2007.150078

Weeks, J. R. (1962). Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. *Science, 138*(3537), 143-144.

Westphalen, R. I., & Hemmings, H. C., Jr. (2006). Volatile anesthetic effects on glutamate versus GABA release from isolated rat cortical nerve terminals: basal release. [Comparative Study
In Vitro
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. *J Pharmacol Exp Ther, 316*(1), 208-215. doi: 10.1124/jpet.105.090647

Wills, T. A., & Cleary, S. D. (1996). How are social support effects mediated? A test with parental support and adolescent substance use. *J Pers Soc Psychol, 71*(5), 937-952.

Wills, T. A., Vaccaro, D., & McNamara, G. (1992). The role of life events, family support, and competence in adolescent substance use: a test of vulnerability and protective factors. *Am J Community Psychol, 20*(3), 349-374.

Wise, R. A. (1996). Neurobiology of addiction. [Research Support, Non-U.S. Gov't Review]. *Curr Opin Neurobiol, 6*(2), 243-251.

Wuo-Silva, R., Fukushiro, D. F., Borcoi, A. R., Fernandes, H. A., Procopio-Souza, R., Hollais, A. W., . . . Frussa-Filho, R. (2011). Addictive potential of modafinil and cross-sensitization with cocaine: a pre-clinical study. [Research Support, Non-U.S. Gov't]. *Addict Biol, 16*(4), 565-579. doi: 10.1111/j.1369-1600.2011.00341.x

Xiao, L., & Becker, J. B. (1994). Quantitative microdialysis determination of extracellular striatal dopamine concentration in male and female rats: effects of estrous cycle and gonadectomy. *Neurosci Lett, 180*(2), 155-158.

Yamashita, A., Singh, S. K., Kawate, T., Jin, Y., & Gouaux, E. (2005). Crystal structure of a bacterial homologue of Na+/Cl--dependent neurotransmitter transporters. [Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. *Nature, 437*(7056), 215-223. doi: 10.1038/nature03978

Yang, H., Zhao, W., Hu, M., & Becker, J. B. (2007). Interactions among ovarian hormones and time of testing on behavioral sensitization and cocaine self-administration. *Behav Brain Res, 184*(2), 174-184. doi: S0166-4328(07)00355-5 [pii]
10.1016/j.bbr.2007.07.005

Yap, J. J., Covington, H. E., 3rd, Gale, M. C., Datta, R., & Miczek, K. A. (2005). Behavioral sensitization due to social defeat stress in mice: antagonism at mGluR5 and NMDA receptors. [Research Support, N.I.H., Extramural
Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology (Berl), 179*(1), 230-239. doi: 10.1007/s00213-004-2023-3

Yap, J. J., & Miczek, K. A. (2007). Social defeat stress, sensitization, and intravenous cocaine self-administration in mice. [Research Support, N.I.H., Extramural]. *Psychopharmacology (Berl), 192*(2), 261-273. doi: 10.1007/s00213-007-0712-4

Yoshimura, S., Sakamoto, S., Kudo, H., Sassa, S., Kumai, A., & Okamoto, R. (2003). Sex-differences in adrenocortical responsiveness during development in rats. *Steroids, 68*(5), 439-445. doi: S0039128X0300045X [pii]

Zhang, D., Yang, S., Yang, C., Jin, G., & Zhen, X. (2008). Estrogen regulates responses of dopamine neurons in the ventral tegmental area to cocaine. *Psychopharmacology (Berl), 199*(4), 625-635. doi: 10.1007/s00213-008-1188-6

Zhou, W., Cunningham, K. A., & Thomas, M. L. (2002). Estrogen regulation of gene expression in the brain: a possible mechanism altering the response to psychostimulants in female rats. *Brain Res Mol Brain Res, 100*(1-2), 75-83. doi: S0169328X02001341 [pii]

FIGURES AND TABLES

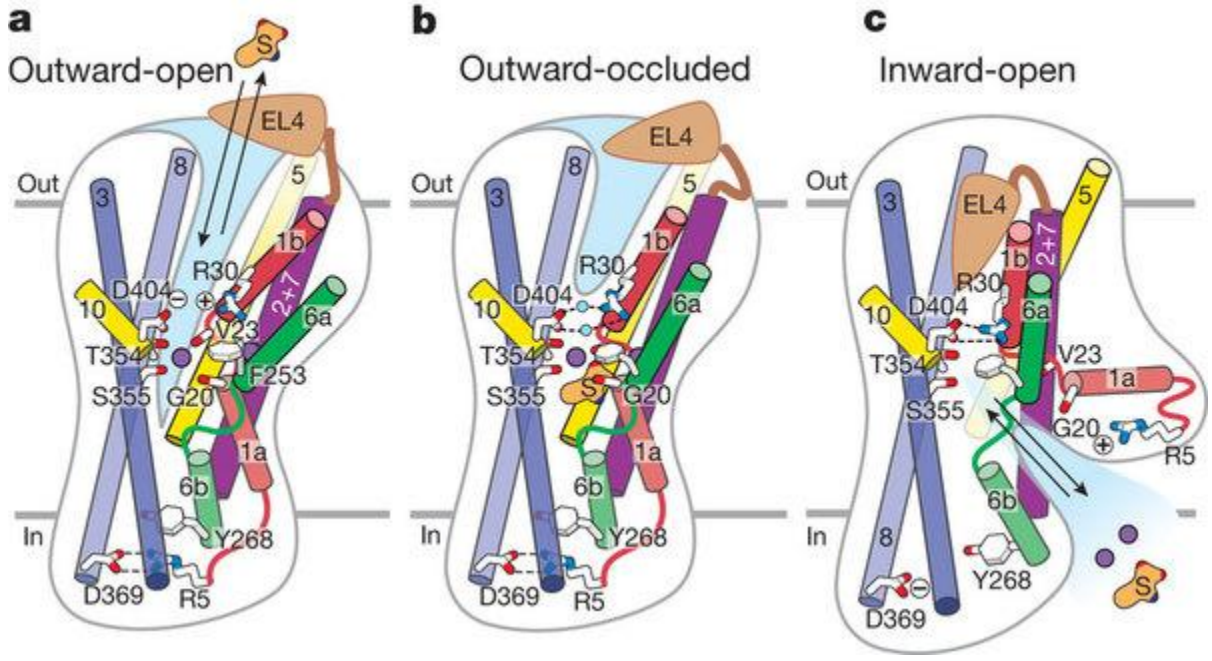

Figure 1: ***Mechanism of DAT function*** In the (a) outward-open conformation, the substrate (s), in this case DA, is able to freely move in and out of the outwardly exposed DA. After two Na^+ ions (purple circle) bind, the extracellular loop 4 (ECL4 or EL4) moves to form the (b) outward-occluded conformation. In this conformation, Na^+ can dissociate, causing further conformational changes, ultimately resulting in the (c) inward-open conformation, in which the substrate and the two Na^+ ions are released. Figure from Krishnamurthy and Gouaux, 2012.

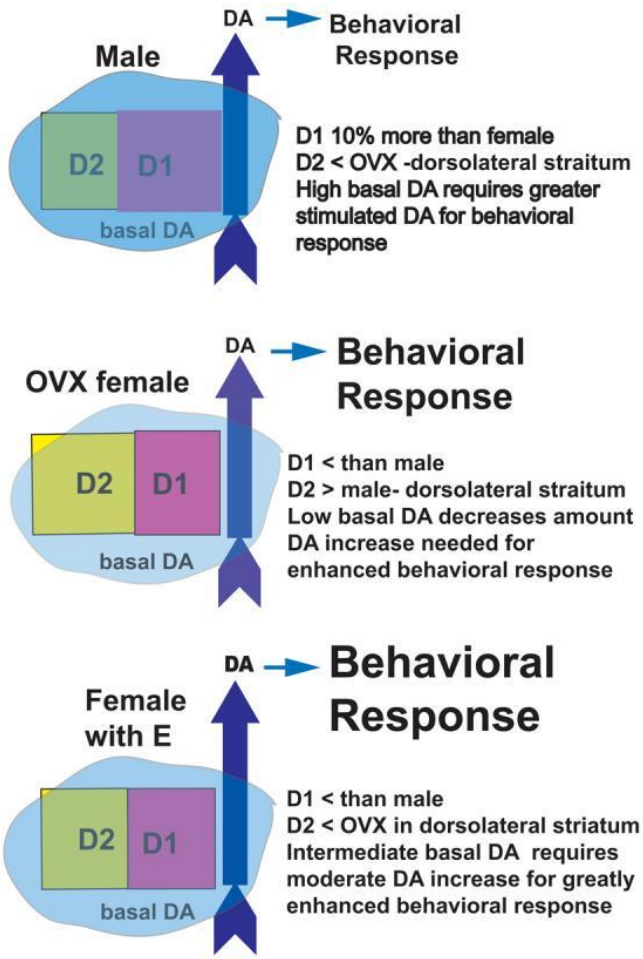

Figure 2: ***Schematic of sex differences and effects of estradiol (E) on striatal and accumbal DA system*** The pink and yellow squares represent the relative ratio of D1 to D2 DA receptors (respectively). The blue overlay represents the basal DAergic tone, where the intensity of color represents quantity of basal DA. Stimulated DA release is represented by the blue arrows, with darkness of color proportional to amount of release. Finally, the consequence of the coordinated receptor activation and DA stimulation is represented by the font size of the behavioral response. Figure from Becker and Hu, 2008.

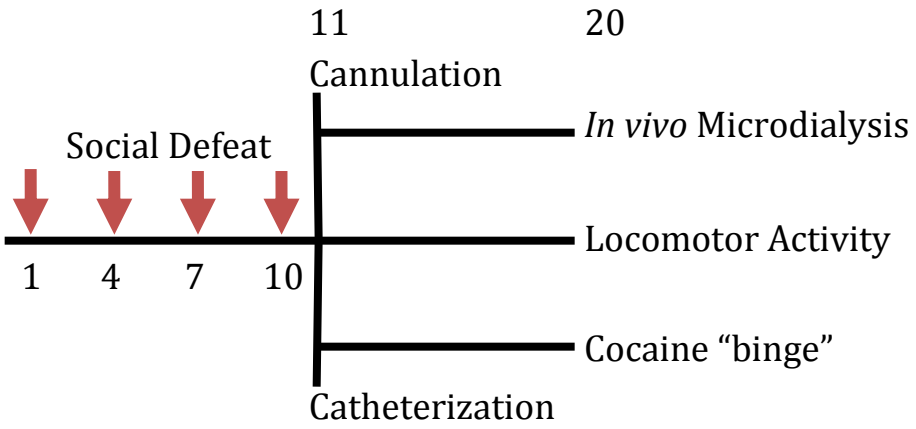

Figure 3: ***Experimental Design*** Social defeats (indicated by red arrows) occurred on days 1, 4, 7, and 10. One cohort of rats was implanted with intracerebral cannulas on day 11, and underwent *in vivo* microdialysis to determine neural sensitization on day 20. A separate cohort was assessed for behavioral sensitization by locomotor activity in response to acute cocaine on day 20. A final cohort was catheterized on day 11 and allowed to self-administer cocaine in an unlimited "binge" on day 20.

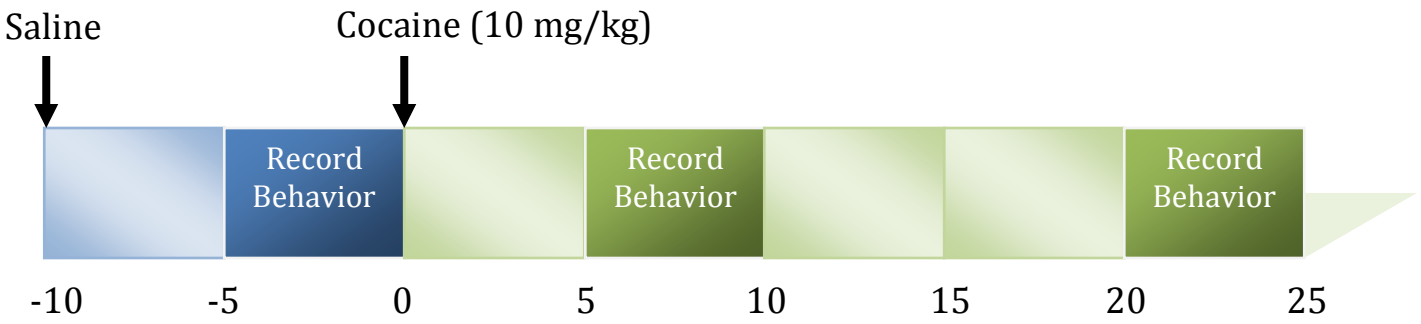

Figure 4: ***Protocol for assessing behavioral sensitization*** Boxes represent 5 minute bins. Five minute segments of behavior were videotaped five minutes after saline and five and twenty minutes after cocaine (10 mg/kg, ip) and subsequently scored for walking duration and frequency.

	Males	Females
Latency	28.2 ± 2.6	29.2 ± 3.2
Duration	8.6 ± 0.1	8.9 ± 0.1
Bites	170.8 ± 7.7	185.8 ± 9.8
Submission	0.11 ± 0.02	0.0047 ± 0.0047***

Table 1: ***Quantitative analysis of sex differences in social defeat*** Latency to first attack bite, total duration of fight, total number of bites received, and proportion of defeats ending in submission, defined as being held in supine position for six seconds or more by the resident. All values are expressed as means ± SEM; ***=p<0.001

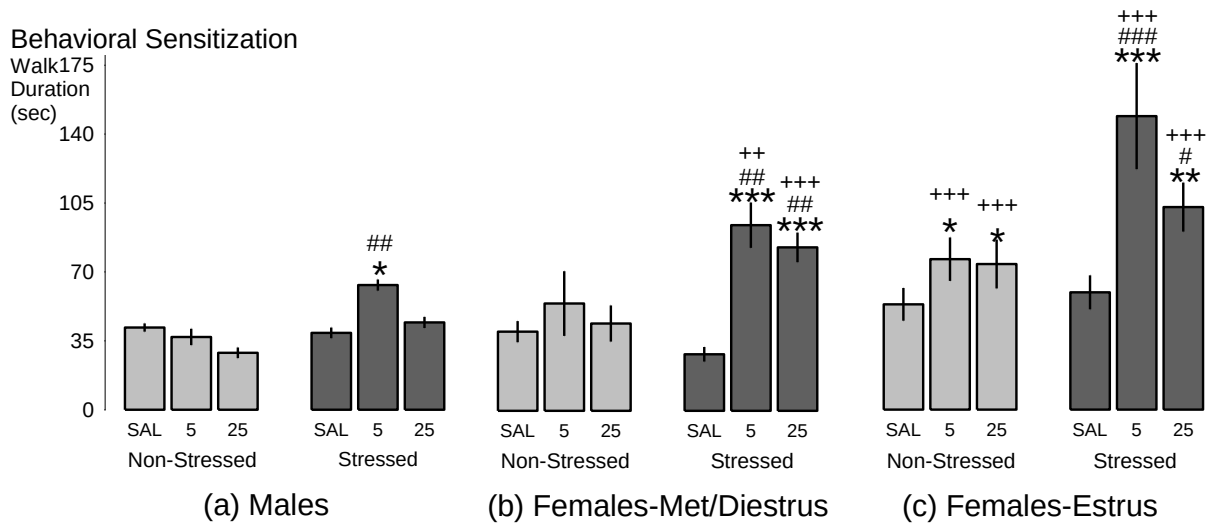

Figure 5: ***Behavioral Sensitization*** Walking duration (seconds) 5-10 minutes after saline (SAL) and 5-10 (5) and 25-30 (25) minutes after cocaine (10 mg/kg, ip) in (a) males and (b) non-estrous and (c) estrous females. All values are expressed as means ± SEM; *=p<0.05, **=p<0.01, ***=p<0.001 vs. within group SAL; #=p<0.05, ##=p<0.01, ###=p<0.001 vs same sex/estrous phase control; ++=p<0.01, +++=p<0.001 vs same stress group male.

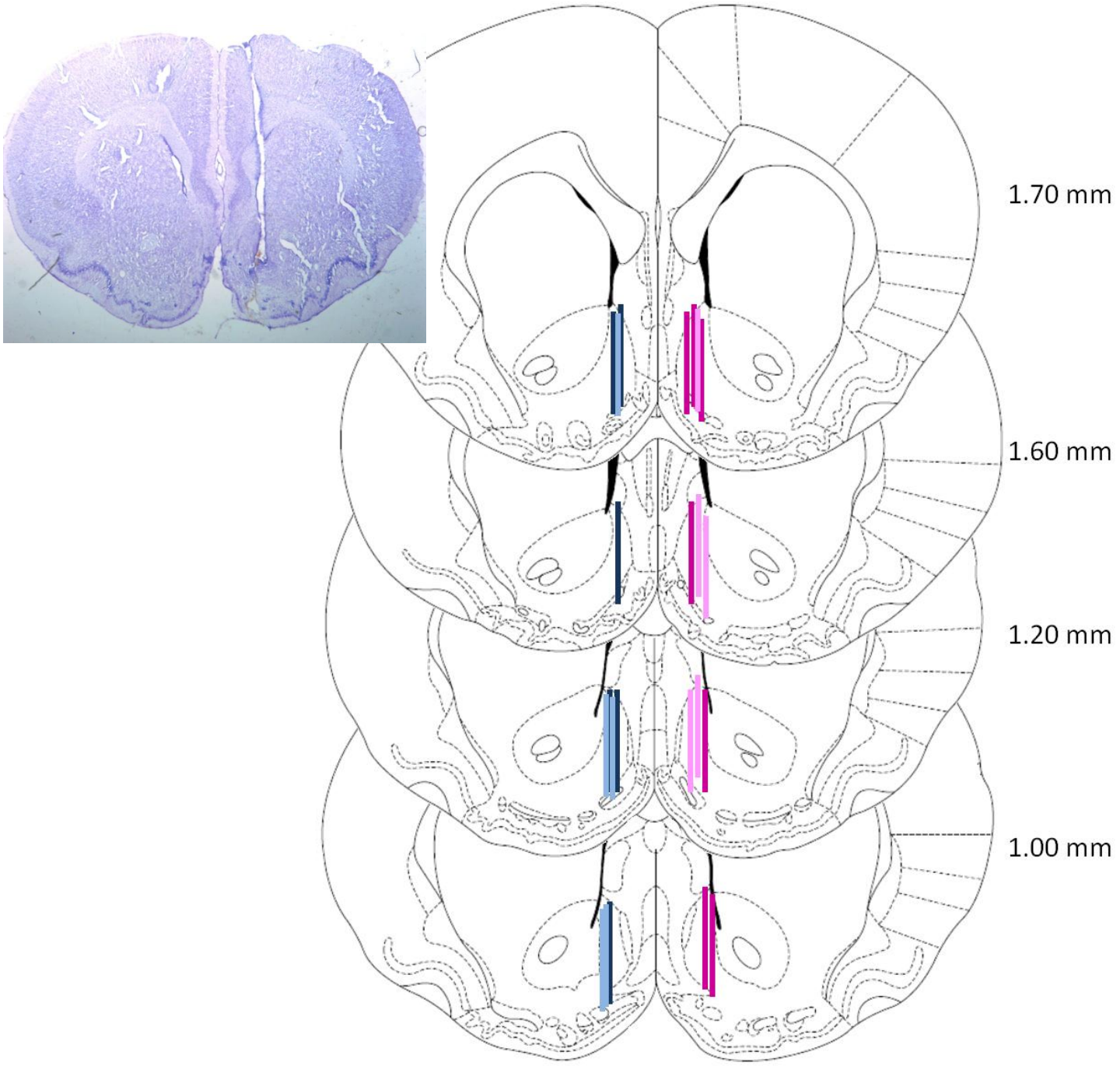

Figure 6: ***Histological verification of probe placements*** (left) is a representative photograph of staining and probe placement. (right) shows placements for all male control (light blue) and stressed (dark blue) on the left and female control (light pink) and stressed (dark pink) on the right. Although all cannulae and probes were implanted on the right side, male placements are depicted on the opposite side for the purposes of this figure.

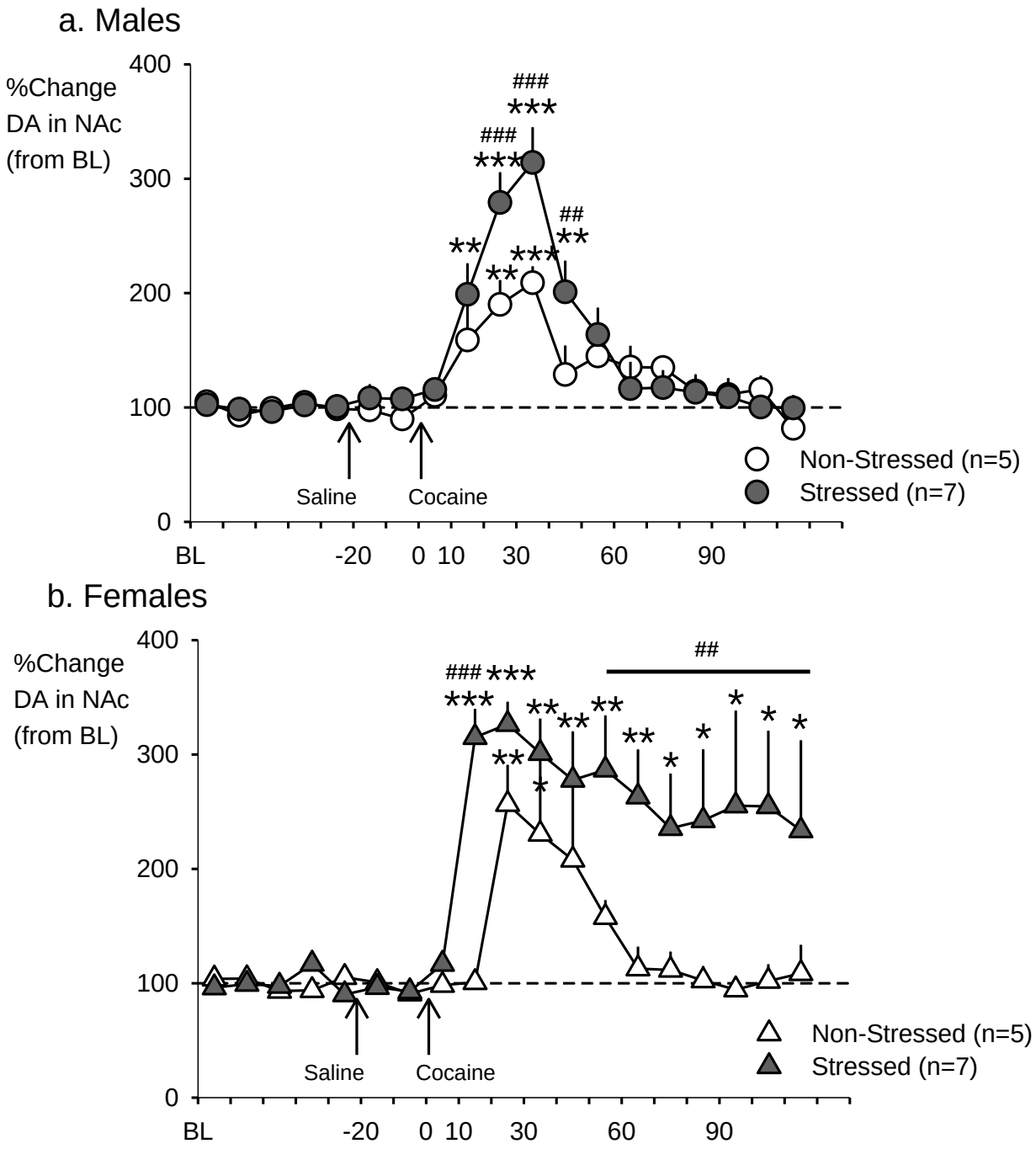

Figure 7: ***Dopaminergic Sensitization*** DA levels in the NAc expressed as percent change from baseline in the same animals in (a) intermittently stressed (n=7, filled circles) or control (n=5, open circles) male rats and (b) intermittently stressed (n=7, filled triangles) or control (n=5, open triangles) female rats. All values are means ± SEM; *=p<0.05, **=p<0.01, ***=p<0.001 compared to within-group baseline and ##=p<0.01, ###=p<0.001 compared to same-sex control.

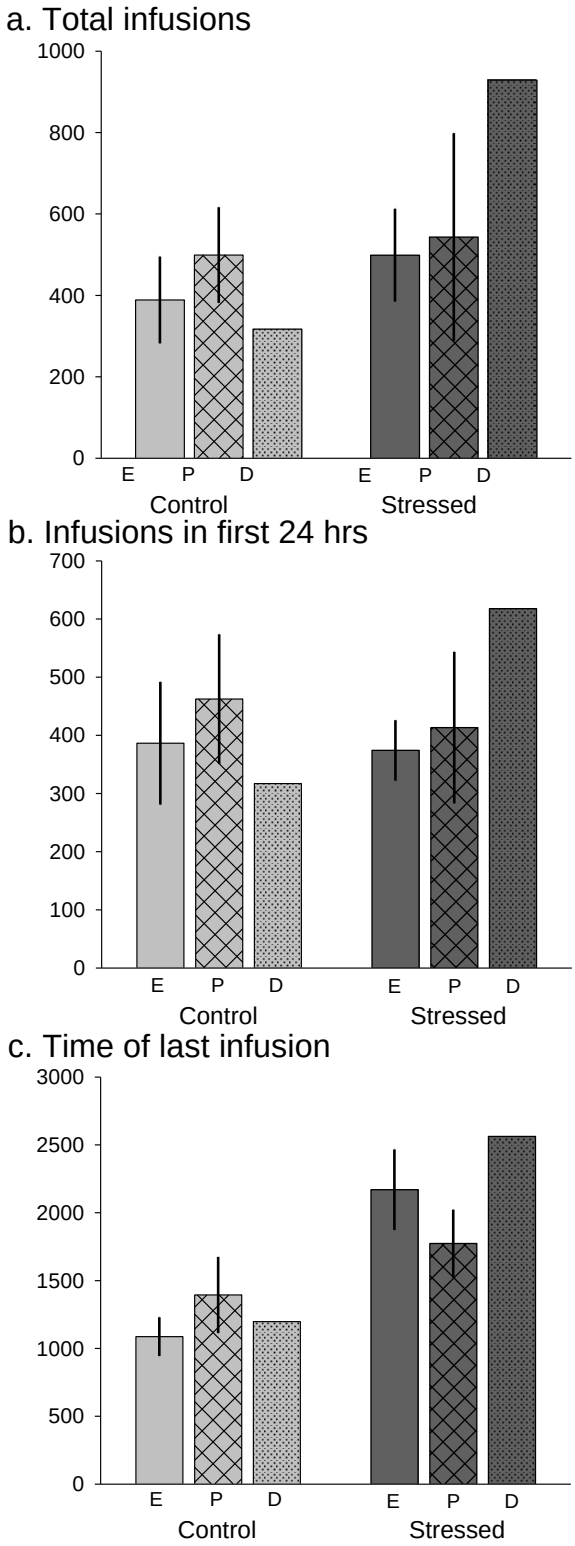

Figure 8: ***Effect of ovarian hormones on "binge"*** No differences were observed in (a) total number of infusions, (b) infusions within the first 24 hours, and (c) binge duration across the estrous cycle of control (light grey bars) and stressed (dark grey bars) females. All values expressed as means ± SEM. E=Estrus (control n=6, stressed n=6), P=Proestrus (control n=6, stressed n=3), D=Met/Diestrus (control n=1, stressed n=1).

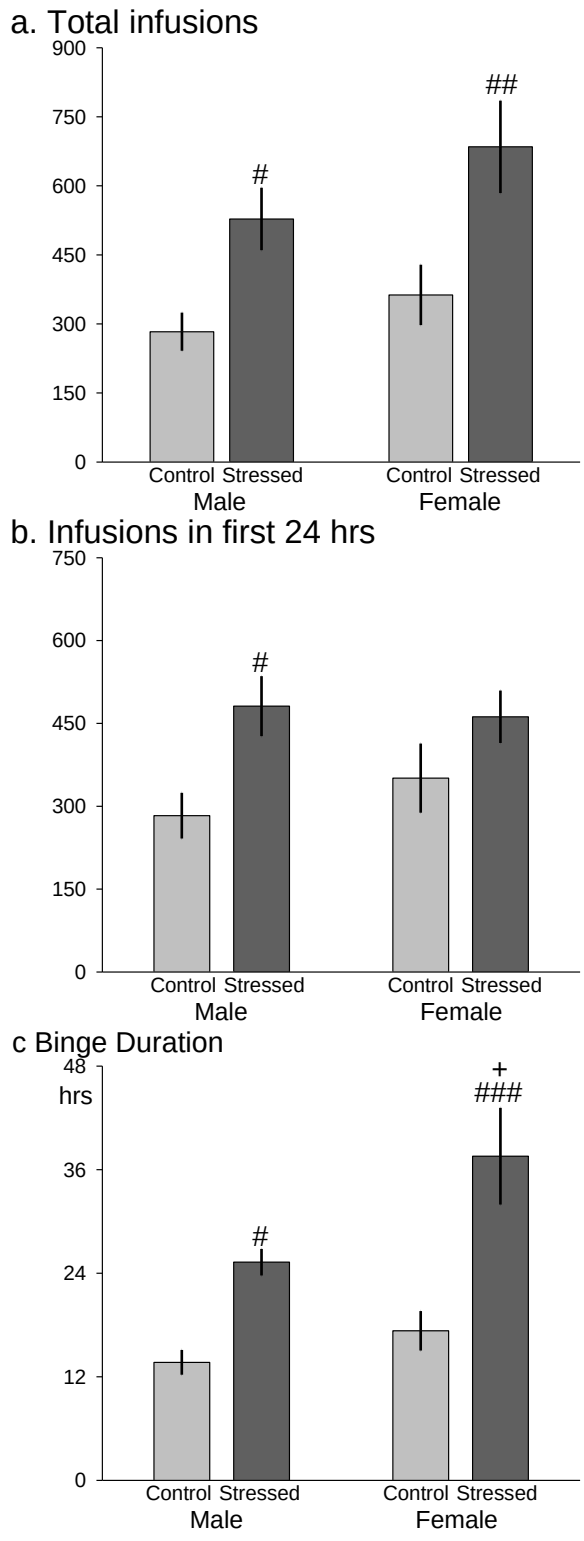

Figure 9: ***Effects of stress and sex on the "binge"*** Effects were observed in (a) total infusions, (b) infusions within the first 24 hours, and (c) binge duration in an unlimited access cocaine self-administration (0.3 mg/kg/infusion, FR1) binge in male (control n=8, stressed n=8) and female (control n=12, stressed n=10) rats. Binge terminated after 120 minutes without a cocaine infusion. Values are expressed as mean ± SEM; #=p<0.05, ##=p<0.01, ###=p<0.001 vs same-sex control; +=p<0.05 vs stressed male

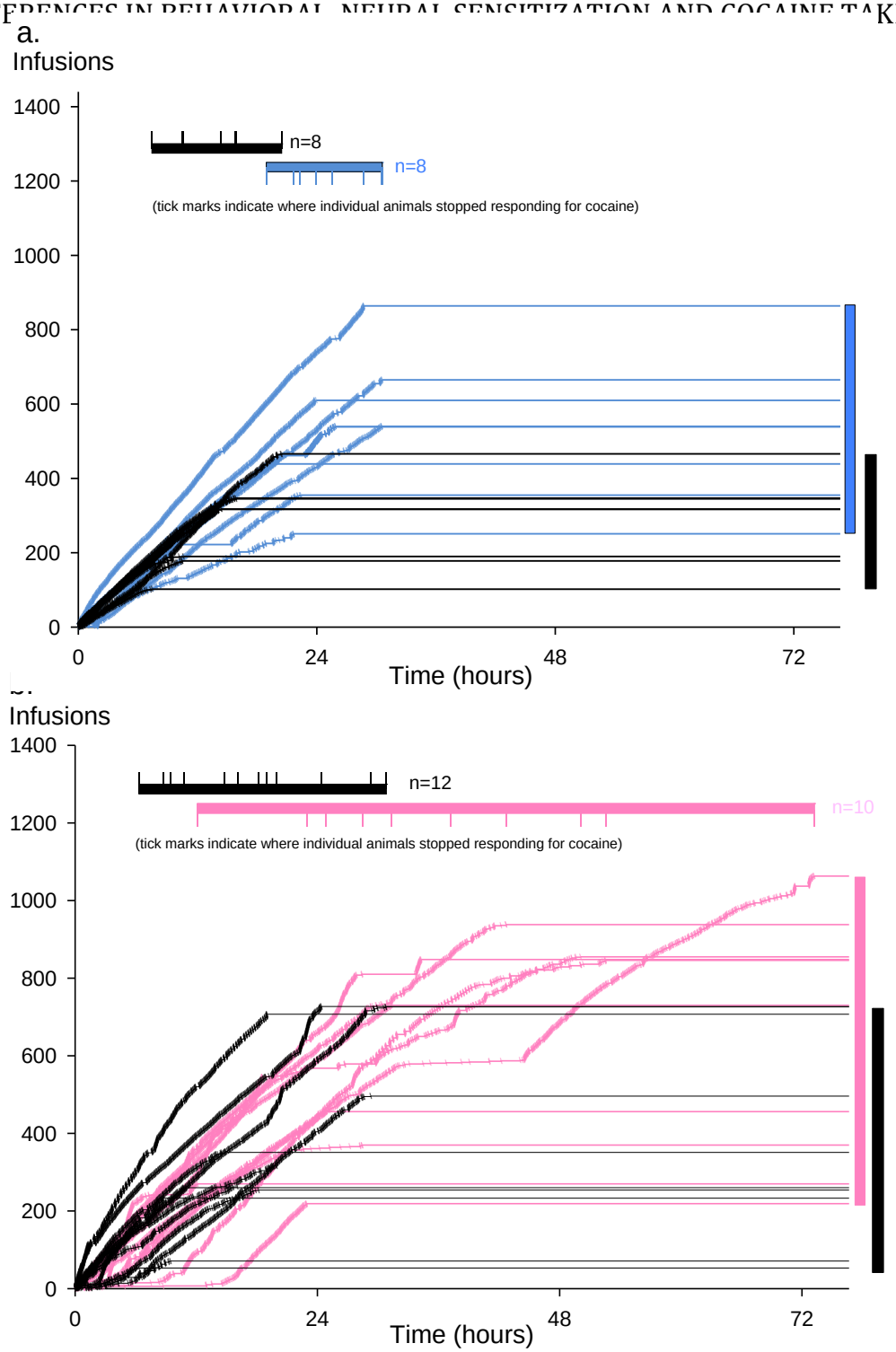

Figure 10: ***Cumulative records of unlimited access "binge"*** Cumulative records for (a) males and (b) females for the unlimited access binge are shown with colored records denoting intermittently stressed animals and black records representing non-stressed controls. Horizontal bars across the top indicate the range of binge duration, with the time of last infusion for each animal noted by a tick. Vertical bars on the right represent the range of total infusions across the binge.

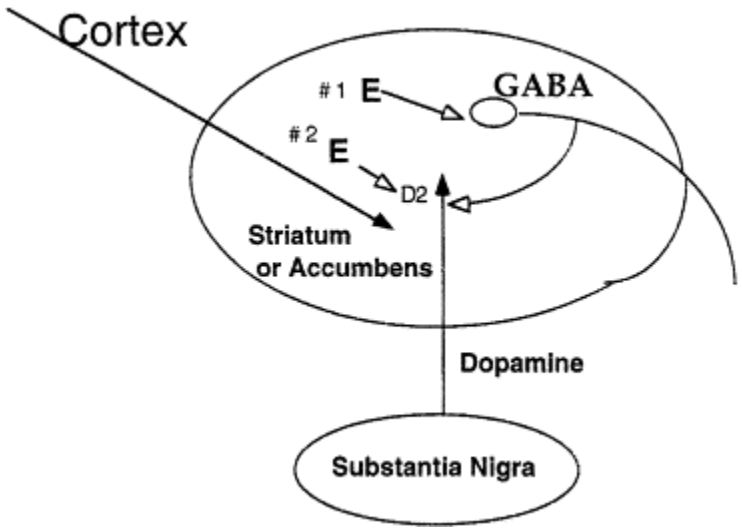

Figure 11: ***Mechanisms proposed to drive estradiol's effects on DA release*** As posited by Becker (1999), there are two hypothesized mechanisms contributing to the effect of estradiol (E) on stimulated DA release. Mechanism #1 is that E directly inhibits GABA neurons that have recurrent collaterals projecting to DA neurons, and this disinhibition leads to an increase in DA release. Mechanism #2 is that E directly acts on DA terminals in the striatum or accumbens to downregulate presynaptic D2 receptor function, ultimately enhancing DA release. Figure from Becker, 1999.