MECHANISMS MEDIATING SEX DIFFERENCES IN THE EFFECTS OF COCAINE

by

Wei Zhao

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Doctoral Committee:

Professor Jill Becker, Chair
Professor Kent C. Berridge
Professor Margaret E. Gnegy
Professor Terry E. Robinson

…… to the memory of my dear grandmother Mrs. Daxun Yang who gave me the first and best lessons of scientific thinking and introduced me to the world of science when I was young. Her encouragement until the last moment of her life, and her memories ever since, have been the best source of motivation for completion of this work. To the memory of a great teacher and women whose thoughts were always ahead of her time…….

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CHAPTER I

INTRODUCTION

Cocaine abuse is an important social health concern of people around the world. Cocaine has the second highest level of dependence among all illicit drugs in 2006 (Marijuana was the most abused drug) and has accounted for more emergency room visits than any other illegal drug in United States (CEWG 2006; DAWN 2004). In the United States, among people aged 12 or older, around 6 million people used cocaine and the average age of onset of cocaine use was 20 in 2006 according to the National Surveys on Drug Use and Health (SAMHSA 2007). Problems associated with cocaine addiction range from psychological and physical health problems to social problems including HIV infection, AIDS and associated crimes. In the last decade, extensive research has been conducted to understand the neural mechanisms underlying cocaine use and cocaine addiction. The ascending midbrain dopamine (DA) system, in particular, plays a critical role in mediating the effects of cocaine, and neuroadaptations of this system with chronic cocaine use have been suggested to contribute to cocaine addiction (Thomas et al. 2008).

Over the last thirty years, the patterns of cocaine use differ between men and women (Wetherington 2007). Clinical studies suggest a role of ovarian hormones in modulating subjective response to cocaine in humans (Evans 2007). Both intrinsic sex differences that are independent of gonadal hormones, as well as sex differences that are induced by ovarian hormones contribute to sex differences in the effects of cocaine as shown in

preclinical studies (Becker and Hu 2008; Lynch et al. 2002). This dissertation is designed to further characterize the effect of ovarian hormones on long term effects of cocaine. Additionally, an effort has been made to understand how the DA system is influenced by sex or hormones to mediate the differences in cocaine-induced responses. Even though the effect of cocaine is the major focus of this dissertation, cocaine and amphetamine (AMPH) are psychostimulants that produce very similar effects through similar mechanisms. Therefore, this background discussion is based on current preclinical and clinical studies with psychostimulant drugs that include both cocaine and AMPH.

First, I will review the effect of cocaine and AMPH on the central nervous system (CNS) and discuss the role of DA in cocaine-related effects based on preclinical studies and human imaging studies. Second, I will review evidence demonstrating sex differences in psychostimulant-related effects in both humans and animals, which suggests that females are more vulnerable than males to the effect of cocaine and AMPH. The differences are due to both intrinsic sex differences independent of gonadal hormones and effects of gonadal hormones on a sexually dimorphic brain. Finally, the influences of sex and hormones in the brain, in particular the midbrain dopaminergic system, will be discussed in relation to the neural mechanism that may be mediating sex differences in response to psychostimulants. This introduction will conclude with the working hypothesis and specific aims of the studies that comprise the dissertation.

1. Neural mechanisms mediating effects of cocaine and AMPH

1.1 Effects of cocaine and AMPH in humans

Cocaine and AMPH are potent psychomotor stimulant drugs. At low doses, they

enhance hyperactivity, alertness, concentration, restlessness, motivation to work, self-confidence, sexual interest and pleasure, and feelings of well being, and reduce social inhibition and anxiety (Fischman and Schuster 1980; Gold 1984; Swerdlow et al. 1986). In the peripheral nervous system, they cause an increase in heart rate (Evans et al. 2002; Knuepfer and Branch 1992), blood pressure (Kollins and Rush 2002; Sofuoglu et al. 2001), and respiratory rate (Foltin et al. 2003; Javaid et al. 1978). With higher doses, the drugs can produce itching, tachycardia, hallucinations, and paranoid delusions. Overdose produces a life-threatening elevation of blood pressure and hyperthermia (Callaway and Clark 1994; Sulzer et al. 2005).

With repeated use of cocaine, episodic and prolonged binge patterns of cocaine use often occur. During the binge use of cocaine, users experience intense and extreme euphoria and often develop uncontrolled dosage escalation or switch to rapid and higher intensity administration routes such as smoking or intravenous administration. Immediately following such a cocaine binge, the user suffers from severe depressive symptoms mixed with irritability, anxiety and cocaine craving, which is called a “crash”. Anhedonia emerges after a crash, and is the major characteristic of the withdrawal phase. This anhedonic dysphoria often induces severe cocaine cravings and results in further cocaine binges, especially when they are presented with a conditioned cue. Abstinence occurs when addicts have resolved the withdrawal anhedonia. In this stage, conditioned cocaine craving can recure even years after the last cocaine use, which often results in relapse. Unlike other addictive drugs, such as alcohol and opiates, cocaine produces no severe physiological withdrawal symptoms (Gawin 1991).

Similarly, addiction to AMPH is also associated with tolerance to the medication,

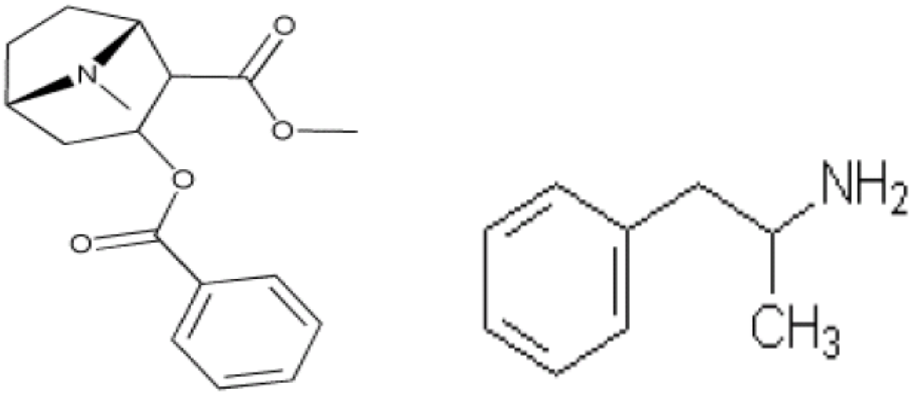
withdrawal symptoms, such as excessive sleeping, increased appetite, depression, anxiety and craving. Heavy use of AMPH often leads to psychosis that includes hallucinations, behavioral disorganization and delusions. With the increasing use of AMPH, the incidence of psychotic reaction to this drug is growing. Finally, this psychotic reaction may undergo spontaneous recurrences known as “flashbacks” even when the users have been abstinent from the drug for a very long period (Ellinwood et al. 2000; Meyer and Quenzer 2005a).

In the clinic, cocaine is a topical anesthetic used in eye, throat and nose surgery. AMPH is commonly used to treat attention-deficit hyperactivity disorder (ADHD) in adults and children, the daytime drowsiness symptoms of narcolepsy and chronic fatigue syndrome (Ellinwood et al. 2000; Meyer and Quenzer 2005a).

Since psychostimulants do not produce severe physiological withdrawal symptoms, subjective experiences or symptoms other than physiological discomfort are thought to be crucial in psychostimulant addiction (Gawin 1991). Thus, understanding the effect of psychostimulants in the brain is of particular interest to us and will be reviewed below.

1.2 Basic function of psychomotor stimulants

Cocaine (Figure 1.1) is a crystalline tropane alkaloid and was first derived from the coca plant and related species. Now lots of synthesized cocainelike drug are available. It acts primarily as an inhibitor of monoamine (DA, serotonin and norepinephrine) uptake transporters. It binds to transporter proteins, blocks the reuptake of monoamines, and results in accumulation of monoamines in the extracellular space (Meyer and Quenzer 2005b). Cocaine can also block voltage-gated sodium channels to produce local anesthetic effects (Yasuda et al. 1984).



Cocaine ($C_{17}H_{21}NO_4$) **AMPH ($C_9H_{13}N$)**

Figure 1.1 Chemical structure of cocaine and AMPH

AMPH (Figure 1.1) was first synthesized in 1887 by Lazar Edeleanu at the University of Berlin. Unlike cocaine, AMPH binds to transporter proteins and is subsequently transported into the cytoplasm of the nerve terminal, resulting in increasing cytoplasmic levels of monoamines by interfering with vesicular storage. The increased cytoplasmic concentration of monoamines and the direct action of monoamine on transporter leads to the reversal of the transporter and monoamine efflux from the intracellular to the extracellular space (Howell and Kimmel 2008; Sulzer et al. 2005).

1.3 Neuroanatomy of ascending midbrain DA system

Even though cocaine and AMPH influence DA, serotonin and norepinephrine neurotransmission in the brain, the psychostimulant and reinforcing properties of those drugs have been thought to be mediated mainly through DA. Thus, for the convenience of the discussion that follows, the ascending midbrain DA system will be briefly reviewed below.

Most DA neurons in the brain originate from the ventral tegmental area (VTA) and

substantia nigra pars compacta (SNc). VTA DA neurons mainly project to the limbic cortex (prefrontal cortex, orbitofrontal cortex, anterior cingulate) and other limbic structures (such as ventral pallidum, amygdala, hippocampus, nucleus accumbens), which have been termed mesocortical and mesolimbic DA systems, respectively. The mesocorticolimbic system has been extensively studied for its role in the rewarding properties of both natural stimuli and addictive drugs (Wise 1996). The dopaminergic projection from SNc to the striatum is called nigrostriatal DA system. This pathway is a major part of basal ganglia and plays an important role in the coordination of motor activity. Degeneration of nigrostriatal DA neurons leads to Parkinson's disease (Hornykiewicz 2001).

Approximately 90% of the neurons in the nucleus accumbens (NAc) and striatum are medium spiny GABAergic neurons. In addition to DA projections, striatum also receives glutamatergic inputs from the neocortex and thalamus while NAc receives glutamatergic inputs mainly from the amygdala, hippocampus, prefrontal cortex and thalamus (Sesack et al. 2003; Smith and Bolam 1990; Smith et al. 2004). The glutamatergic inputs play an important role in addiction (Kalivas et al. 2005; Wolf 1998).

1.4 DA and locomotor stimulating and reinforcing effects of psychostimulants

Several animal behavioral models, such as conditioned place preference, rotational behavior, locomotor activity, stereotypy, sensitization and self-administration, have been widely used to examine and understand the neural mechanisms mediating the effects of psychostimulants. The following section will review the current understanding of neural mechanism mediating the acute response to psychomotor stimulants and the reinforcing effects of cocaine and AMPH based on preclinical studies.

DA and psychomotor stimulating effect

Relevant to the hyperactivity produced in humans, psychostimulants also produce behavioral activating effects in animals. Psychostimulant-induced hyperactivity is often evaluated by quantifying locomotion, stereotypy, or rotational behavior exhibited by animals. After an acute injection of a low dose of cocaine or AMPH, animals will show a profound increase in locomotor activity or rotational behavior (another indication of locomotor activation due to endogenous- or lesion produced- imbalance of the nigrostriatal DA system). Higher doses of these drugs produce stereotyped activity that consists of repetitive, invariant motor responses without purpose or goal (Kelley 2001). Locomotor activity and stereotypy are associated with the activation of mesolimbic and nigrostriatal dopaminergic pathways, respectively (Amalric and Koob 1993; Castall et al. 1977; Costall et al. 1977; Creese and Iversen 1975; Kelly et al. 1975), suggesting a primary dopaminergic activating effect of psychostimulants. Specifically, the intra-accumbens infusion of either cocaine or AMPH produces a locomotor stimulant effect (Delfs et al. 1990; Kelley et al. 1989), and the behavioral activation induced by psychostimulants is inhibited by intra-accumbens administration of DA antagonists or by destruction of mesolimbic DA neurons by 6-OHDA lesion (Joyce and Koob 1981; Kelly and Iversen 1976; Vaccarino et al. 1986). Overexpression of DA transporters in mutant mice enhances AMPH-induced hyperactivity (Salahpour et al. 2008). Both DA D1-like or D2-like receptor antagonists inhibit psychostimulant-induced behavioral activation (Delfs and Kelley 1990). The above evidence suggests that the psychomotor stimulant effect of cocaine and AMPH is mediated through increased DA neurotransmission in the mesolimbic and nigrostratial DA system (Swerdlow et al. 1986). However, caution

should be taken in that stimulant-induced locomotion or stereotype do not quantitively associated with DA response in either NAc or dorsal striatum (Kuczenski et al. 1991). Therefore, the appearance of specific behavior activation, especially the quantitive characteristic, is not solely dependent on the quantitative features of the caudate or accumbens DA response. Other neurochemical systems and mechanisms might be involved as well.

Reinforcing effect of DA

Of particular relevance to the subjective and addictive properties of psychostimulants are their reinforcing effects. Conditional place preference (CPP) is a test that is often used to evaluate preferences for environmental stimuli that have been associated with a positive or negative stimulus as a measure of reinforcing property. In the CPP test, animals will spend more time in the place where they have ever received cocaine or AMPH administration compared to the place paired with saline (Spyraki et al. 1982a; b). Because drug use is a voluntary behavior of humans, a self-administration model is often used to measure self-regulated drug taking in animals. Cocaine and AMPH are both self-administered by rodents (Ettenberg et al. 1982; Lyness et al. 1979; Pettit et al. 1984; Roberts et al. 1977) and non-human primates (Johanson et al. 1976) as by humans. Furthermore, intracranial self-stimulation (ICSS) reward thresholds in rats are lowered immediately after low dose of cocaine self-administration, which indicates a facilitation of rewarding effects of the ICSS (Kenny et al. 2003). Collectively, this evidence suggests a reinforcing effect of cocaine and AMPH in animals as well.

The ability of cocaine-like compounds to bind to the DA transporter closely correlates with their potency to induce conditioned place preference or to maintain self-

administration (Ritz et al. 1990; Ritz et al. 1987). Pharmacological blockade of DA transmission by DA antagonists also attenuates cocaine self-administration in both rodents and non-human primates (Bergman et al. 1990; De Wit and Wise 1977; Wilson and Schuster 1972; Yokel and Wise 1976). The above evidence suggests that the reinforcing effect of psychostimulants is also mediated by DA. Moreover, destruction of dopaminergic nerve terminals in the NAc attenuates the reinforcing effects of cocaine and AMPH, shown by decreased self-administration behavior and CPP (Lyness et al. 1979; Pettit et al. 1984; Roberts et al. 1977; Roberts and Koob 1982; Roberts et al. 1980; Spyraki et al. 1982b), suggesting the mesolimbic DA system is the key circuit underlying the reinforcing effect of psychostimulants (Pierce and Kumaresan 2006). Furthermore, studies with D1-like and D2-like agonists or antagonists suggest that both D1-like and D2-like DA receptors are involved as well (Bachtell et al. 2005; Mello and Negus 1996; Platt et al. 2002). Another reason that the DA system has been the major target of research to understand the addictive property of psychostimulants is that the reinforcing effects of other addictive drugs (opiates, ethanol, cannabinoids and nicotine) are also partly due to increased DA transmission in limbic regions of the brain, even though they may have different primary sites and mechanisms of action (Pierce and Kumaresan 2006). Collectively, the mesolimbic DA system is critically involved in the reinforcing effect of psychostimulants and it is suggested that animals self-administer the drugs to maintain elevated extracellular DA levels (Ranaldi et al. 1999; Wise et al. 1995).

To summarize, even though serotonin (Dukat et al. 2007; Muller et al. 2007; Neumaier et al. 2002; Parsons et al. 1996; 1999; Parsons et al. 1998; Shippenberg et al. 2000) and norepinephrine systems (Drouin et al. 2002a; Drouin et al. 2002b; Ventura et al.

2003) modulate the behavioral and subjective effects of psychostimulants, current knowledge suggests that the ascending midbrain DA projections play a primary role in mediating both locomotor stimulant effects and reinforcing properties of psychostimulants (Kalivas and Nakamura 1999; Koob 1998; Pierce and Kumaresan 2006). Recently, the role of NAc core vs. shell has also been investigated as they may serve different roles in the rewarding and locomotor stimulant effect of psychomotor stimulant drugs (Heimer et al. 1997; Sellings and Clarke 2003).

1.5 DA and behavioral sensitization

The psychomotor stimulant effect can increase with repeated administration of cocaine or AMPH such that lower doses of drug will produce an equivalent behavioral effect as that previously produced by higher doses of drug. This is called behavioral sensitization (Robinson and Berridge 1993). It is often produced by intermittent administration of psychomotor stimulants. In addition to experimenter-administered drug, drug self-administration can also induce behavioral sensitization (Hooks et al. 1994; Phillips and Di Ciano 1996). Behavioral sensitization persists from weeks to months after withdrawal from the drug. Heightened behavioral response produced by AMPH administration can even last for a year (Paulson et al. 1991; Robinson and Becker 1986b). Since behavioral sensitization persists for very long time, and more importantly, the same neural substrates mediate both locomotor stimulant and reinforcing effects of psychostimulants, the neural mechanisms mediating behavioral sensitization are hypothesized to contribute to addiction as well (Robinson and Berridge 1993; 2000a; 2001).

Recent studies suggest that the VTA is a critical site for the initiation of psychomotor sensitization (Vanderschuren and Kalivas 2000; Vezina 2004). Direct injection of AMPH into the VTA has no acute locomotor stimulant effect, but repeated intra-VTA administration results in sensitized locomotor response to systemic injection of AMPH and cocaine. In contrast, intra-NAc AMPH injections do not lead to sensitization, but elicit a sensitized response in rats that received repeated AMPH injections (Cador et al. 1995; Hooks et al. 1992; Kalivas and Weber 1988; Paulson and Robinson 1991; Perugini and Vezina 1994; Stewart and Vezina 1989; Vezina 1996; Vezina and Stewart 1990). Studies with D1 antagonists and agonists have established a critical role for D1 DA receptors in induction of AMPH sensitization, but its role in cocaine sensitization is not conclusive (Vanderschuren and Kalivas 2000). Repeated administration of cocaine or AMPH induces subsensitivity of DA autoreceptors within the VTA or SNc (Ackerman and White 1990; Henry et al. 1989). This subsensitivity, however, does not persist and can be observed only for 1 to 4 days after discontinuation of cocaine injections (Ackerman and White 1990). The time course is consistent with the period when basal levels of extracellular DA and DA transporter binding are enhanced in NAc (Parsons et al. 1991; Pilotte et al. 1994; Weiss et al. 1992). Considering the role of the VTA in the initiation of behavioral sensitization, the transient subsensitivity of VTA DA autoreceptors and the increases in basal DA transmission in the NAc may be important for triggering persistent alterations in DA terminal areas (Hammer 1995).

Compared to the initiation of sensitization, neuroadaptations that occur among circuits innervated by DA neurons, especially the NAc and striatum, are more likely to mediate the expression of sensitization (Pierce and Kalivas 1997). As reviewed above, a

direct effect of cocaine and AMPH is to enhance extracelullar DA levels in NAc and striatum, which is thought to be responsible for their psychomotor stimulant and rewarding effects. Related to this idea, augmented overflow of DA in NAc and striatum induced by psychomotor stimulants among cocaine- or AMPH- pretreated rats has been reported (Heidbreder et al. 1996; Kalivas and Duffy 1990; 1993; Paulson and Robinson 1995; Torregrossa and Kalivas 2007)}. This dopaminergic neuroadaptation, however, has been only consistently observed after a withdrawal of more than 14 days. Reduced, unaltered or enhanced ability of psychostimulants to enhance DA overflow have been reported at early withdrawal (Heidbreder et al. 1996; Hurd et al. 1989; Kalivas and Duffy 1993; Paulson and Robinson 1995; Segal and Kuczenski 1992a; b; Weiss et al. 1992; Wolf et al. 1993). At the same time, the activity of the DA transporter is up-regulated during early withdrawal (Cass et al. 1993; Ng et al. 1991) and a reduction of transporter density in the NAc has been observed after a longer withdrawal (Pilotte et al. 1994; Sharpe et al. 1991; Wilson et al. 1994). The changes of DA transporter function may contribute to the above dynamic change of DA transmission.

Besides those presynaptic changes, changes of postsynaptic function have been observed as well. In NAc, DA inhibits glutamate-evoked neural activity, and this effect is enhanced in psychostimulant-pretreated rats (Henry et al. 1989; Higashi et al. 1989; White et al. 1995), which may be related to the enhanced sensitivity of D1 receptors in NAc (Henry and White 1991). Interestingly, D1 receptor supersensitivity persists up to a month, which parallels the time course of increases in cocaine-induced inhibition of NAc neurons as well as behavioral sensitization induced by the same treatment regimen (Hammer 1995). Further more, D1 receptor antagonists abolish the expression of

behavioral sensitization, suggesting D1 receptors play an important role in the expression of sensitized behavior (Pierce et al. 1996; Pierce and Kalivas 1997). Studies examining D2 receptor binding after sensitization in rodents have mixed results with enhanced, reduced or unaltered D2 receptors binding being reported (Dwoskin et al. 1988; Kleven et al. 1990; Peris et al. 1990). Dopamine D2 receptors, however, exist in two interconvertible affinity states: high vs. low affinity state. Rats that have been sensisitzed to AMPH or self-administered cocaine have increased dopamine D2-high receptors (Briand et al. 2008; Seeman et al. 2004). Consistently, sensitized rats are usually hypersensitive to the psychomotor effects of D2 agonists (De Vries et al. 2002; Edwards et al. 2007; Ujike et al. 1990). Thus, changes in D2-high receptors may also contribute to the expression of behavioral sensitization.

Taken together, repeated treatments of psychostimulants are associated with neuroadaptations in ascending midbrain DA system (Figure 1.2). Transient subsensitivity of autoreceptors in cell body region may contribute to the enhanced basal activity of DA neurons and transient increases in basal DA transmission in DA terminal regions. These transient responses might be important for initiating more persistent changes in DA terminal areas, including both presynaptic and postsynaptic neuroadaptations, which contribute to the expression of behavioral sensitization. Since behavioral sensitization develops early while different neuroplasticity occurs at early vs late stage after withdrawal, different mechanisms might be responsible for behavior sensitization seen during early withdrawal vs. late in the withdrawal period.

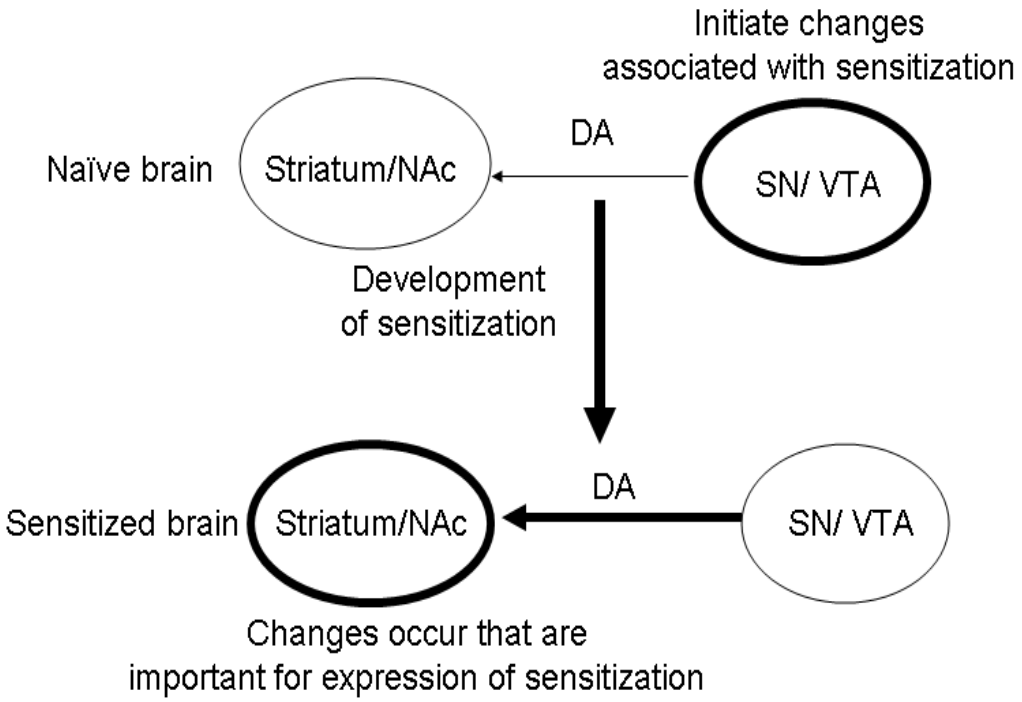


Figure 1.2 Schematic representation of the key neural mechanism associated with cocaine sensitizaiton

1.6 DA and drug-taking

As reviewed above, an extensive body of literature demonstrates that enhanced DA transmission in the NAc critically contributes to the reinforcing effects of cocaine. Consistent with this idea, DA levels in accumbens are enhanced during cocaine self-administration in rats and monkey (Czoty et al. 2000; Hurd et al. 1989; Pettit and Justice 1989). Interestingly, the lever pressing for cocaine is initiated when accumbal DA concentrations decrease to a threshold level during the cocaine self-administration session, suggesting that rats self-administer cocaine to maintain DA levels in NAc (Kiyatkin and Stein 1995; Ranaldi et al. 1999; Wise et al. 1995). Additional support comes from the evidence showing that DA receptor antagonists and agonists always modulate cocaine self-administration (Britton et al. 1991; Caine and Koob 1994; Hubner and Moreton

1991; Woolverton 1986).

In addition, a role for DA in the reinstatement of cocaine-seeking behavior has also been demonstrated. Most factors that trigger drug craving and induce reinstatement, such as drug priming, stress or drug associated cues, increase DA neuron firing or DA release (Nicola et al. 2005; Schultz 1998; Shaham et al. 2003). DA reuptake inhibitors or D2-like DA receptor agonists reinstate cocaine seeking whereas D2-like DA receptor antagonists attenuate cocaine priming-induced drug-seeking behavior (De Vries et al. 2002; De Vries et al. 1999; Khroyan et al. 2000; Schenk and Partridge 1999; Self et al. 1996; Spealman et al. 1999; Wise et al. 1990), suggesting that a role for DA in reinstatement of cocaine-seeking is mediated by D2-like DA receptors. Moreover, reinstatement of cocaine self-administration is promoted by direct stimulation of DA cell bodies in the VTA or application of DA in the NAc (Cornish and Kalivas 2000; Stewart 1984), suggesting a critical role for mesolimbic pathway in mediating cocaine-seeking behavior.

Recently, more attention has been given to the role of the dorsal striatum in cocaine seeking behavior because psychostimulant administration produces significant changes in gene expression in the dorsal striatum (Curran et al. 1996; Graybiel et al. 1990). Extracellular DA is increased in the dorsal striatum rather than the NAc during responding for cocaine-associated cue after chronic cocaine self-administration (Ito et al. 2000; Ito et al. 2002). Moreover, inactivation of the dorsal striatum attenuates cocaine seeking after forced abstinence whereas inactivation of NAc failed to do so (Fuchs et al. 2006; See et al. 2007). Consistent with this idea, infusion of a DA receptor antagonist into the dorsal striatum decreases cocaine seeking under a second-order schedule of reinforcement (Vanderschuren et al. 2005). Recent development of neuroimaging

techniques provides additional clinical evidence. In cocaine-dependent subjects, DA release in response to cocaine-associated cues has also only been observed in the dorsal striatum and this increase has been positively associated with cocaine craving (Volkow et al. 2006; Wong et al. 2006). All of the above evidence points to a critical role of dorsal striatum DA in relapse.

1.7 Sensitization and addiction

In addition to behavioral sensitization, repeated exposure to psychostimulants results in an enhancement of the reinforcing effect of psychostimulants. For example, prior repeated drug treatment shifts the dose-effect curve for CPP to the left (Shippenberg and Heidbreder 1995; Shippenberg et al. 1996), shortens the latency or decreases the dose necessary for rats to acquire self-administration (Horger et al. 1992; Horger et al. 1990; Piazza et al. 1989; 1990). Moreover, sensitized rats reach higher "breaking point" in progressive ratio schedules, indicating a stronger motivation to obtain drug (Lorrain et al. 2000; Mendrek et al. 1998).

A relationship between sensitization and drug seeking has also been suggested based on the evidence that all of the drugs that elicit sensitized locomotor responses in drug pretreated rats cause reinstatement of previously extinguished drug seeking behavior (Vanderschuren et al. 1999b). Behavioral sensitization is very sensitive to the environment surrounding drug administration (Robinson et al. 1998). It is more readily to be induced when the drug is administered in a novel environement compared to home cages (Badiani et al. 1995). When a drug is repeatedly administered to rats in one environment, robust sensitized response is produced when the drug is subsequently administered in the same drug-paired environment. But sensitization may not be

expressed if the drug is administered in a non-paired environment (Anagnostaras and Robinson 1996; Mattson et al. 2008). Coincidently, reinstatement of drug self-administration is reliably induced by drug-paired cues, including discrete cues, discriminative cues and contextual cues, after extinction of operant responding (Caprioli et al. 2007). Therefore, sensitization not only enhances the reinforcing effect of a drug itself, but also functionally recruits context and drug associated cues in drug abuse to powerfully modulate drug-taking behavior. It may account for the critical role of context in precipitating relapse in humans (Robinson and Berridge 1993; 2000a; 2003).

The incentive-sensitization theory of addiction refers to an enhanced incentive salience-"wanting" of a drug after repeated drug exposure as incentive sensitization. In addition to drug, context may have also acquired some incentive salience in this process. Both context and drug result in excessive incentive motivation for drugs and lead to a shift from drug "liking" to "wanting" underlying compulsive drug use (Robinson and Berridge 1993; 2000a). Another popular view suggests that sensitization is a process to facilitate some forms of learning between drug and context, which results in habitual and compulsive drug use in addiction (Everitt et al. 1999; Everitt and Robbins 2005). Dysfunction of the frontocortical system, associated with the sensitization process, may occur and impair normal cognition and inhibitory control over behavior and contribute to irrationally compulsive drug pursuit (Jentsch and Taylor 1999). No matter what psychological function has been changed, persistent drug-induced neuroadaptations accompanying the sensitization processes are thought to be critical in the transition to addiction (Robinson and Berridge 2003).

1.8 DA and rewarding effects of psychomotor stimulants -insight from clinical imaging studies

The majority of existing knowledge about the neural mechanisms mediating effects of psychostimulants has been built on preclinical studies; very little is known about humans. The recent development of neuroimaging techniques allows us to look at the effect of psychostimulants in the human brain directly and adds to our knowledge gained from animals. PET scan combined with DA type 2 and 3 receptor radiolabeled antagonist [$_{11}$C] raclopride can be used to measure changes in extracellular DA in the human brain (Laruelle 2000). In humans, acute administration of AMPH significantly induced DA release in the limbic (ventral striatum) and sensorimotor (postcommissural putamen) region of striatum. Furthermore, the greater DA release in ventral striatum was associated with the increase of subjects' reported euphoria (Drevets et al. 2001; Martinez et al. 2003).

AMPH sensitization and the associated increased DA release in the ventral striatum has been modeled and observed in healthy human subjects in a laboratory study (Boileau et al. 2006). The subjects were administered oral AMPH (0.3 mg/kg) three times. When AMPH was given again 14 and 365 days after the last dose of AMPH, there was a greater psychomotor response and increased DA release relative to the initial dose in the ventral striatum. Moreover, cues associated with AMPH also increased DA release to the same magnitude as did AMPH in the ventral striatum (Boileau et al. 2007), supporting the idea that sensitization is associated with enhanced DA transmission, and this neuroadaptation is also modulated by the environment.

Intriguingly, a reduction of methylphenidate- or AMPH- induced DA release in striatum has been reported in cocaine-dependent humans, which suggests an alteration in

presynaptic DA function in those dependent on cocaine (Martinez et al. 2007; Volkow et al. 1997). Participants with greater reduction in DA release in the anterior caudate and ventral striatum were more likely to choose cocaine over an alternative reinforcer in the self-administration session (Martinez et al. 2004). The absence of DA sensitization with extensive exposure to psychostimulants indicated by indirect measures of D2 ligand displacement in clinical imaging studies are supported by studies directly measuring DA release in monkeys (Bradberry and Rubino 2006), suggesting different mechanism may exist between non-human primates and humans as well as rodents. In terms of postsynaptic changes, decreased D2 receptor avaiablity in the striatum has been observed in cocaine addicts (Martinez et al. 2004; Volkow et al. 2004; Volkow et al. 1990). But until now, human imaging studies cannot differentiate D2 low vs. high affinity state, thus it is not clear if D2-high receptors has been upregulated in cocaine addicts.

Collectively, the above clinical imaging studies confirm that an acute effect of psychomotor stiumulants is to enhance extracellular DA levels in the mesolimbic DA system, and the changes of DA level is associated with changes of subjective effect induced by the drug. The relationship of the neuroadaptations associated with repeated use of drugs and addiction in humans still remains open for further investigation.

2. Sex differences in cocaine and AMPH abuse

In the above section, I have reviewed the current understanding of the major neural mechanism mediating the psychomotor activating and reinforcing effect of psychomotor stimulants, and the neuroadaptations associated with repeated drug exposure, as well as

possible role of those circuits in drug abuse and addiction. Another characteristic we must pay attention to is individual differences in susceptibility to drug abuse and addiction. Recent studies suggest gender is an important factor influencing cocaine abuse (Becker and Hu 2008).

Cocaine abuse had been traditionally considered a male issue because epidemiological data shows greater prevalence of drug use and dependence among males before. In recent years, many male-female differences, however, have gradually disappeared or even reversed especially when opportunity to use is considered and when drug dependence rates are calculated only among users (Van Etten et al. 1999). Recent epidemiological data reveal that women are three to four times more likely than men to become addicted to cocaine within 24 months from the first time of cocaine use (O'Brien and Anthony 2005). Furthermore, there is a significantly a shorter time from initiation of cocaine use to abuse and dependence (Ridenour et al. 2005), greater cocaine use, and more dependence on cocaine in women than men (Lejuez et al., 2007). This topic has drawn attention nation wide (Wetherington 2007).

2.1 Gender difference in response to cocaine and AMPH -clinical evidence

Cocaine

Clinical studies suggest gender differences in response to cocaine because cocaine cues induce more drug craving in female than male addicts (Robbins et al. 1999). Women report less pleasure and greater anxiety after acute intranasal cocaine administration

(Kosten et al. 1996; Lukas et al. 1996). Repeated administrations of intranasal or intravenous cocaine produce a greater increase in “Feel Good” and greater cardiovascular effects in cocaine-dependent women (Haney et al. 1998; McCance-Katz et al. 2005).

The effect of cocaine changes over the menstrual cycle.The human menstrual cycle (Figure 1.3) can be separated into two typical phases, which are follicular and luteal phases. In the follicular phase (day 1-14), estradiol levels are low at first, gradually rise and reach peak later, while progesterone levels are always low. The luteal phase (day 15-28) is characterized by moderate estradiol and high progesterone levels (Becker and Breedlove 2002).

Female subjects report more positive subjective effects of smoked or intravenous cocaine administration during the follicular phase than during the luteal phase (Evans and Foltin 2006; Evans et al. 2002; Mendelson et al. 1999b; Sofuoglu et al. 1999). The cardiovascular effects of smoked cocaine also change over the menstrual cycle (Evans and Foltin 2006; Evans et al. 2002). There were no menstrual cycle phase or sex differences, however, in cocaine plasma levels after intravenous or smoked cocaine (Evans and Foltin 2006; Evans et al. 2002; Mendelson et al. 1999b; Sofuoglu et al. 1999). Therefore, the differences of subjective and cardiovascular effects could not be accounted for by differences in plasma cocaine levels.

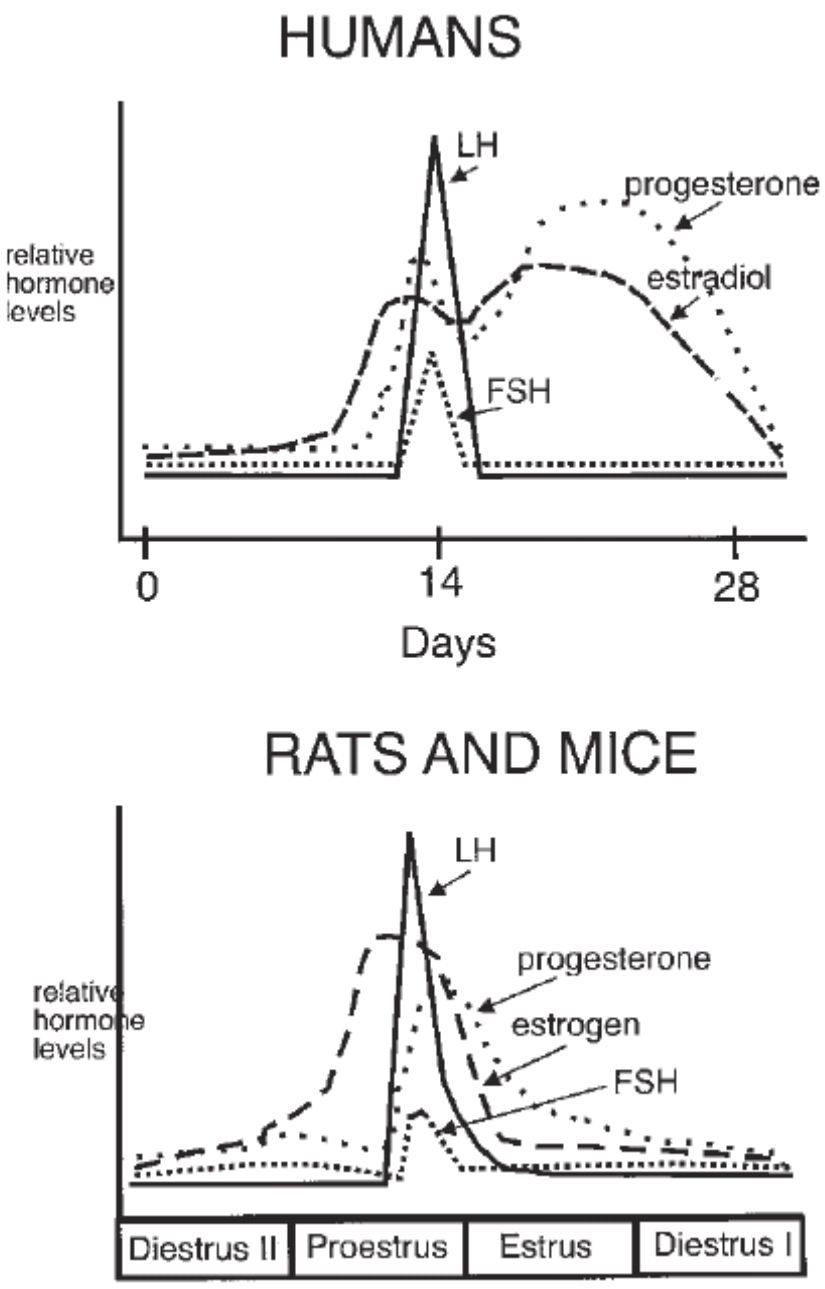


Figure 1.3 Schematic representation of changing hormonal profiles across the cycle in humans and rodents. Adapted from McCarthy and Becker, 2002 (Becker and Breedlove 2002)

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The above evidence suggests that female gonadal hormones may modulate the

response to cocaine. It is possible that positive drug effects of cocaine are either enhanced by the presence of estradiol during the follicular phase, or decreased by the presence of progesterone in the luteal phase, or modulated by both processes. Clinical studies using exogenous estradiol or progesterone have found that administration of progesterone during the follicular phase in women attenuates the positive subjective effects of smoked cocaine and cocaine-induced diastolic blood pressure (Evans and Foltin 2006; Sofuoglu et al. 2002; Sofuoglu et al. 2004). The effect of progesterone in men is not conclusive, as the same inhibitory effect was evident in one study (Sofuoglu et al. 2004), but was not observed in another (Evans and Foltin 2006). In clinical studies, there has been very limited evidence suggesting that estradiol modulates the subjective response to cocaine (Evans 2007; Evans and Foltin 2006).

AMPH

Gender differences and the effect of menstrual cycle have also been reported in the effect of AMPH (Evans 2007; Terner and de Wit 2006). A study using a drug choice procedure found that women are more likely to choose 10 mg oral AMPH over placebo than do men (Gabbay 2003). In contrast, one recent neuroimaging study reported that men have greater DA release and greater positive subjective responses to an intravenous dose of AMPH (0.3 mg/kg) compared to women (Munro et al. 2006). A retrospective study, which has analyzed data from 6 studies from their lab, suggests men experience greater “high” and “sluggish” and less “nausea” than women (Vansickel et al. 2007). Unfortunately, this analysis also failed to take into consideration the phase of menstrual cycle in women.

Studies examining the effect of menstrual cycle show that females feel more “high”

and “euphoria” after AMPH when they are in the follicular phase compared to the luteal phase. Greater AMPH-induced subjective effect during the follicular phase positively correlates with estradiol levels and negatively correlates with progesterone levels (Justice and de Wit 1999; White et al. 2002). In White’s study, the effect of AMPH has also been compared between genders. Men reported greater stimulation than women who are in the luteal phase (White et al. 2002). When the women were in the follicular phase, ratings of stimulation after oral AMPH were not different between men and women (Gabbay 2005). The above evidence suggests that estradiol and progesterone may modulate the subjective response of AMPH as it does on cocaine.

A subsequent study assessing the subjective effect of AMPH during the early (low estradiol level) and late follicular (high estradiol level) phases of menstrual cycle found that women reported greater unpleasant stimulation and less unpleasant sedation during the late follicular phase than during the early follicular phase (Justice and De Wit 2000a). In another study, exogenous administration of estradiol was found to increase the magnitude of the effects of AMPH on subjective ratings of “pleasant stimulation’ and decrease ratings of ‘want more’ (Justice and de Wit 2000b). Taken togethet, it suggests the subjective effect of AMPH is modulated by estradiol. Until now, the effect of progesterone on the response to AMPH has not been examined.

In summary, the current research suggests that there are gender differences in subjective response to both cocaine and AMPH. Since the subjective response to cocaine and AMPH in women fluctuates over menstrual cycle, the reported gender differences depend on when women are tested. However, one consistent finding is women tend to have greater euphoria experience to both cocaine and AMPH during follicular phase with

high estradiol and low progesterone level, than during luteal phase characterized by moderate estradiol and high progesterone. Furthermore, exogenous estradiol enhances some subjective effects of AMPH. In contrast, progesterone attenuates subjective effect of cocaine in women. More experiments are needed to further verify their specific role on psychostimulants-related response. Lastly, caution should be taken when interpreting results from clinical studies since most studies examining the effect of AMPH have been conducted on healthy people whereas cocaine's effect have been examined in cocaine addicts.

2.2 Sex differences in the effects of psychomotor stimulants- preclinical studies

There has been an increase in preclinical research addressing sex differences in drugs of abuse, particularly on psychostimulants like AMPH and cocaine. This topic has been summarized recently in several reviews (Becker et al. 2001; Carroll et al. 2004; Festa and Quinones-Jenab 2004; Lynch et al. 2002; Mendelson et al. 2002; Roth et al. 2004). Clinical studies gave us some clue in terms of variables to consider when we examine sex difference in response to psychomotor stimulants. Animal models allow us to manipulate and control all those variables and test the specific role of each. Current body of knowledge gained from preclinical studies will be summarized below.

Sex differences in the effects of psychostimulants were first reported in the AMPH-stimulated behavioral response in rats (Beatty and Holzer 1978; Meyer and Lytle 1978). Female rats show greater and prolonged locomotor behavior, stereotypy and rotational behavior in response to acute AMPH than do male rats (Beatty and Holzer 1978; Becker et al. 1982; Bisagno et al. 2003; Camp et al. 1986; Milesi-Halle et al. 2007). Similar observations in the effect of cocaine have also been reported in rodents, including

cocaine-induced locomotor activity, conditioned rewarding effect as well as cocaine self-administration (Becker and Hu 2008; Becker et al. 2001; Bowman and Kuhn 1996; Caine et al. 2004; Dalton et al. 1986; Glick et al. 1983; Hu et al. 2004; Lynch and Taylor 2005; Russo et al. 2003b; Thompson et al. 1984; van Haaren and Meyer 1991; Walker et al. 2001). Females show substantially greater cocaine-induced locomotor behavior than males (Glick et al. 1983; Hu et al. 2004; van Haaren and Meyer 1991), require less pairing sessions to develop cocaine CPP (Russo et al. 2003b), acquire self-administration more rapidly and self-administered more cocaine (Lynch and Carroll 1999), and are more sensitive to cocaine priming during the reinstatement of cocaine seeking (Lynch and Carroll 2000).

Besides acute effect of psychomotor stimulants, sex differences have also been reported after repeated administration of psychostimulants. Female rats exhibit greater behavioral sensitization in response to AMPH and cocaine than do male rats (Camp and Robinson 1988a; b; Chin et al. 2002; Chin et al. 2001; Forgie and Stewart 1994; Robinson 1984; Robinson et al. 1982; van Haaren and Meyer 1991). Besides those quantitative differences of behavioral response, males and females also exhibit qualitative differences in drug-taking behavior. Using drug self-administration model, females exhibit greater reinstatement in the drug-primed reinstatement procedure (Lynch and Carroll 2000). In contrast, males exhibit greater reinstatement in the cue-induced reinstatement procedure (Fuchs et al. 2005). It suggests that the factors precipitating relapse might be different for males and females.

Several factors may contribute to the sex differences in response to cocaine and AMPH. First of all, due to both genetic differences and organizational effects of

hormones, fetuses go through sexual differentiation during development to form sexually dimorphic brain. This intrinsic sex differences independent of circulating gonadal hormones in adulthood may directly lead to sex differences in response to psychostimulants. Secondly, males and females have different gonadal hormones as adults. Estradiol and progesterone are produced by ovaries in females, and testosterone is produced by testes in males. The activational effect of these hormones may make males and females show sexually dimorphic behavioral response to psychostimulants. Thirdly, the above two processes may interact and work together to contribute to sex differences in response to psychostimulants (Figure 1.4). The three hypotheses are discussed below.

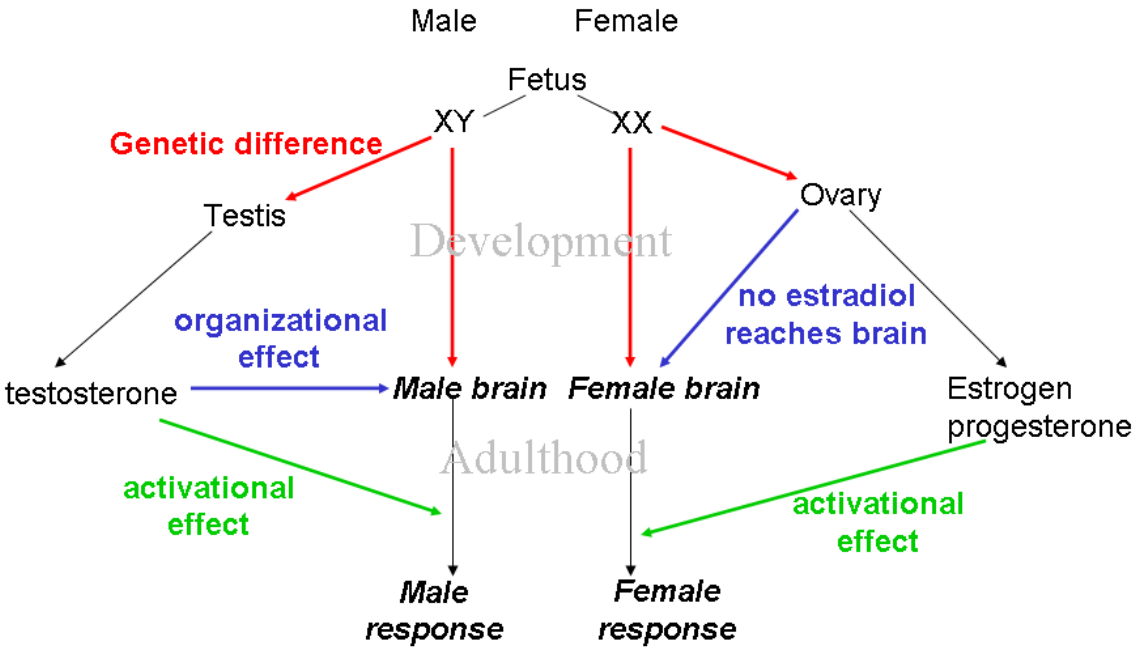


Figure 1.4 Factors influencing sex differences in the effects of psychostimulants

2.2.1 Sex differences independent of gonadal hormones

Castrated male rats (CAST) and ovariectomized (OVX) female rats are often used to test whether there is a sex difference in brain that is independent of gonadal hormones. It has been shown that OVX female rats exhibit greater behavioral sensitization to cocaine, acquire cocaine self-administration behavior more rapidly, and self-administer more cocaine at a faster rate than CAST male rats (Becker et al. 2001; Hu and Becker 2003; Hu et al. 2004). These data suggest that there is sex difference in the effect of cocaine and AMPH even without circulating gonadal hormones.

2.2.2 Effect of Testosterone

Experiments comparing CAST males and intact males found that castration has no effect on locomotor activity induced by acute cocaine (van Haaren and Meyer 1991) or rotational behavioral induced by acute AMPH or electrical stimulation (Camp et al. 1986; Robinson et al. 1981), or on cocaine self-administration (Hu et al. 2004). Collectively, it suggests testosterone does not modulate the acute response to psychostimulants.

In addition to the acute response, castration has no effect on sensitization of locomotor activity, stereotyped activity and rotational behavioral after repeated injections of cocaine (Becker et al. 2001; Hu and Becker 2003; van Haaren and Meyer 1991). Intriguingly, the effect of testosterone on behavioral sensitization to AMPH is not conclusive. Some studies have shown that CAST male rats exhibit greater sensitization to AMPH than do intact male rats (Camp and Robinson 1988a; b; Robinson 1984). But others have reported neither an effect of castration at enhancing behavioral sensitization nor a suppressing effect of exogenous testosterone on CAST male rats (Forgie and

Stewart 1994).

2.2.3 Effect of Estradiol

Female rats have a 4-5 day estrous cycle with four stages (Figure 1.3), that are referred to as diestrus (diestrus II), metestrus, proestrus and estrus (diestrus I). Estradiol is low during diestrus and metestrus. It rises gradually until approximately noon on proestrus, when there is a surge of estradiol that triggers ovulation 12 hours later (Becker and Breedlove 2002).

There is estrous cycle-dependent variation in behavior induced by AMPH or cocaine with greater behavior exhibited in estrus (Becker and Cha 1989; Becker et al. 1982). Females' self-administration behavior fluctuates over estrous cycle as well with higher motivation to take cocaine during estrus (Lynch et al. 2000; Roberts et al. 1989). Following high-dose cocaine priming, females in estrus exhibit higher reinstatement than either males or non-estrus females (Kippin et al. 2005).

The effect of estradiol on the behavioral response to cocaine after first administration is not conclusive depending on the specific estradiol treatment. An acute effect of estradiol on locomotor activity after a first injection of cocaine has not been observed in the studies when estradiol was administered just prior to cocaine injections (Hu and Becker 2003; Peris et al. 1991; Perrotti et al. 2000; Sircar and Kim 1999; Yang et al. 2007). A role for estradiol, however, has been reported in several other studies when estradiol benzoate-filled silastic capsules were implanted some time before the testing (Perrotti et al. 2001; Quinones-Jenab et al. 2000; Sell et al. 2000). The differential effect may be due to short vs. long exposure to estradiol. Unlike cocaine, the behavioral responses to AMPH are enhanced rapidly by estradiol (Becker 1990b; Becker and Beer

1986). As reviewed previously, AMPH both releases DA and blocks DA reuptakes, which makes AMPH more efficacious than cocaine. The greater efficacy may make AMPH-induced behavioral activation more sensitive to the regulation of estradiol (Hu and Becker 2003).

In terms of the behavioral response to repeated drug administrations, OVX females sometimes do not show behavioral sensitization to cocaine at time when intact females or gonadal hormone-treated females do (Peris et al. 1991; Sircar and Kim 1999; van Haaren and Meyer 1991). Hormone replacement experiments demonstrate that estradiol enhances behavioral sensitization to cocaine in female rats in a variety of behavioral paradigms, such as locomotor behavior, stereotypy, rotational behavior (Becker et al. 2001; Chin et al. 2002; Hu and Becker 2003; Peris et al. 1991; Perrotti et al. 2001; Sell et al. 2002; Sircar and Kim 1999; Yang et al. 2007; Zhen et al. 2007). Interestingly, most studies find that ovariectomy in female rats does not affect AMPH sensitization (Camp and Robinson 1988a; b; Forgie and Stewart 1994; Robinson 1984; Robinson et al. 1982).

In addition to the psychomotor stimulant effect, estradiol also powerfully modulates cocaine-taking behavior. In female rats estradiol enhances the acquisition of cocaine self-administration (Hu et al. 2004; Jackson et al. 2006; Lynch et al. 2001), cocaine-induced reinstatement (Larson and Carroll 2007; Larson et al. 2005) and females' motivation to take cocaine as exhibited by a higher breaking point on a progressive ratio schedule (Becker and Hu 2008). In an extended drug seeking model, estradiol also promotes escalation of cocaine self-administration when rats have access to cocaine for more than 6 hours per day (Larson et al. 2007).

Collectively, the above results strongly suggest that estradiol facilitates the

behavioral response to psychomotor stimulants. Moreover, the effect of estradiol is sexually dimorphic as it has no effect on cocaine sensitization or cocaine self-administration on CAST male rats (Becker et al. 2001; Jackson et al. 2006)

2.2.4 Effect of Progesterone

Progesterone is suggested as an agent that may attenuate the cocaine response in women in clinical studies (Evans and Foltin 2006; Sofuoglu et al. 2002; Sofuoglu et al. 2004). Consistent with this idea, a recent preclinical study monitoring plasma progesterone and estradiol level as well as cocaine seeking behavior in cycling female rats have found that the highest progesterone levels occur at the time of lowest cocaine-seeking and lowest levels occur at the time of highest cocaine-seeking (Feltenstein and See 2007). The inhibitory effect of progesterone is also suggested by the studies showing that progesterone treatment blocks the conditioned place preference for cocaine in mice (Romieu et al. 2003) and rats (Russo et al. 2003a; Russo et al. 2008), suppresses cocaine-induced reinstatement of cocaine-seeking (Anker et al. 2007), and inhibits the escalation of cocaine self-administration in an extended drug seeking model (Larson et al. 2007). In contrast to the reinforcement effect, cocaine-induced psychomotor activation seems to be invulnerable to the regulation of progesterone, as progesterone has no effect on cocaine-induced locomotive acitivity (Perrotti et al. 2001; Sell et al. 2000; Sircar and Kim 1999). The effect of progesterone is dose dependent because low and high doses inhibit, whereas intermediate doses stimulate, rearing responses (Niyomchai et al. 2005). One caution to be kept in mind is that the above experiments have also employed different administration routes, such as progesterone replacement via silastic capsules vs. subcutaneous injections. Thus, the effect of progesterone is dose and route dependent.

Different mechanisms might be involved and have yet to be determined.

In addition to an independent role of progesterone, an interaction between progesterone and estradiol has been suggested as well. For example, administration of estradiol and progesterone suppressed cocaine-induced locomotion and cocaine self-administration when compared to estradiol-treated animals (Jackson et al. 2006; Quinones-Jenab et al. 2000). An acute administration of progesterone, however, enhances cocaine-induced locomotor activity in estradiol-primed OVX rats (Perrotti et al. 2001). Moreover, OVX rats implanted with silastic capsules containing both estradiol and progesterone exhibit greater magnitude of cocaine conditioned place preference (Russo et al. 2003a). Due to this conflicting evidence, the effect of progesterone still remains to be determined. It is possible that progesterone plays a role in the facilitation or inhibition of estradiol's effect on cocaine-induced behavior depending on when it is administered.

To summarize this part, preclinical studies suggest that males and females have intrinsically different brains that make females more vulnerable to the effect of psychomotor stimulants than males. In addition, preclinical studies strongly suggest an important role of estradiol on the response to cocaine and AMPH. The facilitative effect of estradiol is evident on females rather than males suggesting that it works on sexually dimorphic brain. Progesterone can either facilitate or antagonize the effect of estradiol depending on when it is administered. Furthermore, most evidence supports the notion that there is no effect of testosterone on the response to psychostimulants.

3. Neural mechanisms mediating sex differences on psychotimulant-related effects

3.1 Sex differences in pharmacokinetics of psychomotor stimulants

Sex differences exits in the metabolism of AMPH. AMPH is metabolized more rapidly in males than in female rats so that brain levels are lower in males than in females if they receive the same systemic dose of AMPH (Kato 1974; Meyer and Lytle 1978). It may explain some discrepancy among studies. However, studies administering different dose of AMPH to achieve equivalent brain concentration of AMPH still found an greater behavioral response to AMPH in females (Becker et al. 1982; Bisagno et al. 2003; Camp et al. 1986).

There are fewer problems with studies investigating sex differences in the effects of cocaine, since there is neither a sex difference nor an effect of gonadal hormones on plasma or brain cocaine levels after acute cocaine injection in rodents (Bowman et al. 1999). Similar results have been obtained in humans and rhesus monkeys as well (Mendelson et al. 1999a; Mendelson et al. 1999b). A recent study found lower brain levels of cocaine 30 minutes after cocaine administration in estradiol-treated rats, but those changes play very limited role in mediating behavior responses to cocaine especially considering that estradiol-treated rats exhibit a greater behavioral response to cocaine (Niyomchai et al. 2006).

Therefore, sex differences in response to psychomotor stimulants cannot be explained by differences in pharmacokinetics of cocaine or AMPH, even though sex

differences in AMPH metabolism exist and may partly contribute to some of the reported differences.

3.2 Sex differences in the brain

Recent clinical imaging studies have demonstrated that cocaine-dependent men and women exhibit different brain responses. For example, regional cerebral blood flow (rCBF) and perfusion abnormalities on cocaine-dependent men and women have been found to exhibit gender-specific patterns (Adinoff et al. 2006; Tucker et al. 2004). Gender differences in the functional anatomy of cue-induced cocaine craving have been reported as well (Kilts et al. 2004). Conditioned cocaine craving in women is associated with less activation of amygdala, insula, orbitofrontal cortex, and ventral cingulated cortex and greater activation of the central sulcus and frontal cortical area as compared to men (Kilts et al. 2004). Moreover, anticipation of uncertain reward activates ventral putamen more in man than women (Dreher et al. 2007). All of the above evidence suggests that different neural mechanisms may exist in cocaine-dependent men and women.

Further preclinical studies have shown that the striatum and NAc are sexually dimorphic. For example, male rats have more D1 DA receptors (Hruska et al. 1982; Levesque and Di Paolo 1988). Female rats, however, exhibit greater sensitivity to the effect of D1 receptor antagonist at attenuating cocaine-induced psychomotor activation. Moreover, the striatal D1 binding level is reduced by acute cocaine administration only in male rats (Festa et al. 2006). Thus, D1 receptor may be an important substrate in regulating sex differences in cocaine-induced psychomotor activation.

The basal DA as well as AMPH-stimulated striatal DA release are higher in CAST male rats than in OVX female rats (Becker and Ramirez 1981b; Xiao and Becker 1994).

In addition, male rats have a lower density of DA transporter mRNA in striatum than do intact or OVX female rats (Bosse et al. 1997). This suggests that there is an intrinsic sex difference in the organization of the striatum. Furthermore, there is a significantly greater percentage of AMPH-stimulated immediately early gene expression in the middle and caudal dorsal striatum in proestrous female rats than in female rats in any other stage, CAST male or intact male rats (Castner and Becker 1996), suggesting that gonadal hormones, in addition to an intrinsic sex difference, may also work on the ascending midbrain DA system to contribute to the sex differences in response to psychostimulants.

Related to sex differences in long term effect of cocaine, a very recent study comparing the PKA-regulated signaling in striatum and NAc, which may be critical in the development of addiction, have reported that females had higher phosphorylated DA and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) at the PKA site in the striatum compared to males. Moreover, increased phorphorylation of DARPP-32 as a result of cocaine self-administration was observed in NAc after 10-days abstinence period in females and after 0-days abstenance period in males, suggesting gender-specific patterns may exist in the development of cocaine addiction (Lynch et al. 2007).

Unfortunately, since gonadal hormone levels have not been monitored or controlled well in clinical and preclinical studies, and furthermore, cocaine use directly disrupts reproductive endocrine functions (King et al. 1993; King et al. 1990; Siegel 1982), it is hard to determine if the observations are due to pure intrinsic brain difference, an effect of gonadal hormones, or a combination of the two.

3.3 Ovarian hormones and ascending midbrain DA system

As predicted by behavioral studies, gonadal hormones, in particular estradiol and progesterone, have a tremendous effect on the nigrostriatal and mesolimbic dopaminergic system (Becker 1999). The first line of evidence comes from the studies showing natural fluctuations of DA neural activity throughout the estrous cycle. Basal extracellular DA concentration in the striatum is higher when the rats are in estrus and proestrus with high endogenous estradiol (Xiao and Becker 1994). Besides basal DA, AMPH-induced increases in extracellular DA in striatum show estrous cycle-dependent variation with significantly greater AMPH-induced increase during estrus both in vivo and in vitro (Becker and Cha 1989; Becker and Ramirez 1981b). Moreover, the striatal DA uptake site density and DA receptors vary across estrous cycle (Di Paolo et al. 1988; Levesque et al. 1989; Morissette and Di Paolo 1993b). DA turnover and potassium-stimulated DA release and reuptake fluctuate over estrous cycle in the NAc as well (Shimizu and Bray 1993; Thompson and Moss 1997). Interestingly, a very recent clinical study using fMRI found that reward-related neural functions in women were modulated by menstrual cycle. Reward delivery activates the midbrain striatum more in women in the follicular phase than during the luteal phase, which is consistent with menstrual cycle fluctuation of subjective effect of cocaine and AMPH (Dreher et al. 2007).

The second line of evidence comes from gonadectomy studies. OVX decreases DA levels in VTA (Russo et al. 2003a), basal extracellular DA concentrations in striatum (Xiao and Becker 1994), AMPH-stimulated striatal DA release (Becker and Ramirez 1981a; b), striatal DA transporter density (Bosse et al. 1997), striatal D1 DA receptor density (Levesque et al. 1989) and DA-stimulated adenylate cyclase activity in striatum

and NAc (Kumakura et al. 1979). The function of D2 DA receptors, however, is upregulated after OVX (Hruska et al. 1982).

Taken together, these studies suggest that female gonadal hormones play a role in modulating the functions of DA system, which is probably the mechanism mediating hormone's effect on psychostimulants-related effects. Extensive hormone replacement studies have been done to address specific role of estradiol and progesterone on DA system.

3.3.1 Estradiol and DA

Estradiol enters the cell to induce protein synthesis through its actions on nuclear receptors, which is termed genomic effect. Genomic effects are characterized by slow onset and prolonged duration. In addition, estradiol also has direct effects on the cell membrane, resulting in changes in electrical potential and the release of neurotransmitters. This nongenomic effect has a rapid onset and is short in duration (McEwen and Alves 1999). Both pro- and antidopmainergic effects of estradiol have been reported depending on the dose and time course of estradiol treatment (Di Paolo 1994). Different effect of estradiol on dopaminergic function might be due to either genomic effects or nongenomic effects or a combination.

Some evidence suggests a rapid effect of estradiol on the DA neurotransmission in the striatum of female rats. For example, acute estrogen treatment rapidly induces an increase in striatal DA turnover (Di Paolo et al. 1985), upregulates striatal DA uptake site density (Morissette et al. 1990), and decreases D2 class DA receptors (Bazzett and Becker 1994). Furthermore, direct application of estradiol to the striatal tissue from OVX rats enhances AMPH-induced DA release in vitro (Becker 1990a). Moreover, an acute

administration of estradiol to OVX rats induced a rapid increase in AMPH- or cocaine-induced DA release in striatum in vivo (Becker 1990b; Becker and Rudick 1999; Hu and Becker 2006). The rapid effect of estrogen on striatal DA release is antagonized by the pure estrogen receptor antagonist rather than a nonsteroid agent with antiestrogenic property, suggesting that the steroidal feature is critical for the estrogen-binding site in the striatum (Xiao et al. 2003). Electrophysiological experiments using whole-cell clamp recording demonstrated that a low dose of 17β-estradiol inhibits the L-type calcium current within milliseconds through a G-protein-coupled process (Mermelstein et al. 1996). Furthermore, bovine serum albumin conjugated-, membrane-impermeable estradiol mimics the effect of estradiol suggesting that estradiol works in the striatum through a non-genomic activation on extracellular cell membrane. Similarly, direct infusion of estradiol into the NAc also potentiated K^+-stimulated DA release (Thompson and Moss 1994), and attenuated autoreceptor-mediated potentiation of DA uptake (Thompson 1999; Thompson et al. 2000). Moreover, estradiol rapidly enhanced cocaine-stimulated DA increase in dialysate in NAc as in striatum (Hu and Becker 2006). A similar non-genomic effect of estrogen on membrane receptors in NAc is suggested as well (Wong et al. 1996).

A model proposed by Becker hypothesizes that estradiol acts on estrogen receptors located on the membranes of medium spiny striatal neurons to reduce calcium current and result in a decreased GABA release, which then disinhibits DA neuron terminals to enhance stimulated striatal DA release (Becker 1999; Becker and Hu 2008). This hypothesis is further supported by the evidence showing that estradiol treatment rapidly attenuates potassium stimulated striatal GABA release in dialysate (Hu et al. 2006). The

above evidence may explain the effect of estradiol on response to acute treatment of psychostimulants.

Related to the effect of estradiol on behavioral sensitization to cocaine, AMPH-stimulated DA release from striatal tissue is greater in estradiol-treated OVX rats compared to OVX rats after cocaine sensitization (Peris et al. 1991). It is hard to judge, however, if this is due to an interaction of repeated cocaine treatment with long term or acute effects of estradiol or both. A microdialysis study shows that repeated treatments of estradiol produce greater enhancement of AMPH-induced DA release in dorsolateral striatum than a single acute treatment, suggesting estradiol treatment produces both acute and long-term effects in the striatum (Becker and Rudick 1999). Besides enhanced DA transmission, other long term effects of estradiol on DA system, probably through genomic activation, have been reported as well. For example, chronic estradiol treatment increases D2 receptor sensitivity and density in the striatum and NAc (Hruska and Silbergeld 1980; Lammers et al. 1999; Landry et al. 2002; Zhou et al. 2002), increases DA uptake site density in the nigrostriatal dopaminergic system (Morissette and Di Paolo 1993a), increases turnover of DA in the nucleus accumben (Shimizu and Bray 1993), decreases the expression of DAT in striatal astroglia cultures and results in lower clearance of DA from the extracellular space (Karakaya et al. 2007). In cultured striatal neurons from embryonic mouse, chronic estradiol modified the G-protein coupling process to modulate adenylate cyclase activity stimulated by D1 and D2 DA receptor agonist (Maus et al. 1989a; Maus et al. 1989b). Whether or how those changes produced by long term effect of estradiol contribute to specific responses produced by either acute or repeated treatment of psychostimulants, however, still remains open.

For many years, the classical intracellular estrogen receptor (ERα), discovered in the early 1960s, was the only receptor known to mediate the effect of estrogen. Recently, a new β-isoform of estrogen receptor (ERβ) has been identified (Kuiper et al. 1996; Pettersson et al. 1997; Tremblay et al. 1997). None of these receptors, however, has been detected in the nucleus of neurons in adult rat striatum and NAc (Laflamme et al. 1998; Perez et al. 2003; Shughrue et al. 1997; Simerly et al. 1990; Zhang et al. 2002). But ERα and ERβ mRNA expression have been reported in developing and adult mice striatum (Kuppers and Beyer 1999) and in rat substantia nigra (Laflamme et al. 1998; Shughrue et al. 1997). ERβ mRNA and protein have also been found in the VTA (Creutz and Kritzer 2002; Shughrue et al. 1997; Shughrue and Merchenthaler 2001). Investigators are just beginning to differentiate the role of ERα and ERβ in drug-related effects using newly developed ERα- and ERβ-selective agonists. The effect of chronic estradiol treatment to increase D2 receptor density in the striatum and NAc in OVX rats is reported to be through ERβ (Le Saux et al. 2006). Consistent with a critical role of accumbens D2 DA receptors in reinstatement of drug-seeking, ERβ is suggested to mediate estradiol's effect at precipitating relapse (Larson and Carroll 2007). At the same time, ERα has been suggested to mediate estradiol's effect at enhancing psychostimulant-induced locomotor activity (Larson and Carroll 2007). However, those results should be taken very carefully since ERβ-selective agonist DPN (70:1 ERβ: ERα) may stimulate ERα as well, especially after chronic treatment (Meyers et al. 2001).

Even though both ERα and ERβ are traditional intracellular estradiol receptors, some evidence suggests both of them may be present in the cell membrane as well and mediate the rapid responses to estradiol (Milner et al. 2001; Norfleet et al. 1999; Watson

et al. 1999). Distinct from those traditional ERs, a G-protein coupled membrane estrogen receptor (mER) has been identified in the brain and mediates rapid response to estradiol in the hippocampus (Funakoshi et al. 2006; Qiu et al. 2006). A series of very recent studies further identified that, in hippocampal neurons, activation of membrane estrogen receptors, probably through caveolin protein, leads to metabotropic glutamate receptors activation and initiates cell signaling(Boulware et al. 2007; Dewing et al. 2007; Micevych and Mermelstein 2008). It is not known yet whether these receptors are found in the reward system. Nonetheless, compelling evidence showing the rapid effects of estradiol on striatal and accumbens DA neuron and drug-related effects, suggests the existence of membrane-located estrogen receptors in striatum and NAc.

Taken together, estradiol has both acute and long term effects on the ascending DA system, through which it modulates response to psychostimulants. Failure to identify estrogen receptors in NAc and striatum, however, present a big challenge to further clarify the molecular and cellular mechanism involved.

3.3.2 Progesterone and DA

Until now, the effects of progesterone on dopaminergic function as well as psychostimulants-related effects have not been investigated extensively. A series of studies conducted in the Ramirez lab suggest that estradiol and progesterone collaborate to modulate DA neuron. Progesterone treatment either in vivo or in vitro enhances DA release in striatal tissue from extradiol-primed OVX female rats (Dluzen and Ramirez 1984; 1987; 1989a; 1990b; 1991). A microdialysis study has confirmed the idea that progesterone treatment enhances AMPH-stimulated DA in dialysate from dorsolateral striatum in estradiol-pretreated OVX female rats (Becker and Rudick 1999). The effect of

progesterone on DA release from striatum is suggested to be mediated through a surface membrane site on interneurons in the striatum (Dluzen and Ramirez 1989a; b). A membrane-associated protein with high affinity for progesterone has been found in the striatum after estradiol priming (Ke and Ramirez 1990; Ramirez et al. 1996; Tischkau and Ramirez 1993). Estradiol plus progesterone also increases DA levels in the NAc (Russo et al. 2003a). Those effects of progesterone on striatal DA release are not seen without estradiol priming. Thus, some long-term effects of estradiol may be mediated through progesterone.

Without estradiol priming, progesterone alone can influence dopaminergic function as well. Chronic progesterone treatment alone or in combination with estradiol increased DA reuptake sites in striatum and substantia nigra pars compacta to the same extend as estradiol treatment alone (Morissette and Di Paolo 1993a). It also decreases turnover of DA in the NAc (Shimizu and Bray 1993).

Taken together, even though progesterone can work alone to influence DA system, many effects of progesterone on DA system are through an interaction with estradiol.

4. Specific aims

My dissertation work has been trying to further clarify the underlying mechanisms mediating sex differences in response to cocaine. Based on current understanding of neural mechanisms mediating the effects of psychostimulants and sex differences in brain functions, I hypothesize that behavioral sensitization is a process that enhances both behavioral stimulant and reinforcing effect of psychostimulants, probably through enhanced ascending midbrain dopaminergic transmission, in females as in males. In

addition, there are sex differences in cocaine sensitization. Both intrinsic sex differences in the brain, that is independent of gonadal hormones, and estradiol contribute to sex differences in cocaine sensitization. In particular, estradiol may both produce an acute activational effect on sensitizated brain as well as influence neural process associated with sensitization to enhance behavioral sensitization in females. Moreover, the ascending midbrain DA system is sexually dimorphic and is powerfully modulated by estradiol, which is the neural substrate mediating the observed sex differences in behavioral sensitization.

Aim 1(Chapter II): This experiment was designed to further characterize the effect of estradiol on behavioral sensitization in female rats by comparing the effect of estradiol given with repeated cocaine treatments vs. the effect of estradiol on a challenge day to determine: 1) if estradiol enhances behavioral sensitization when it is given with repeated cocaine treatments; 2) if enhanced sensitization produced by estradiol treatment during the time that sensitization is induced by cocaine treatment could be seen when on challenge day, whether or not estradiol is given on the challenge day; 3) if estradiol treatment on challenge day has additional acute effect on behavioral response to cocaine on cocaine-sensitized rats.

Rationale: estradiol has been shown to enhance behavioral sensitization to cocaine in female rats (Chin et al. 2002; Hu and Becker 2003; Sircar and Kim 1999; Yang et al. 2007), but most studies have used implanted estradiol-releasing pellets or have given subcutaneous estradiol injections throughout the whole process of sensitization (Becker et al. 2001; Febo et al. 2003; Peris et al. 1991; Perrotti et al. 2001). It is not clear whether

estradiol modulates the neural process occurring with the development of cocaine sensitization, or just produces an activational effect on naïve brain and sensitized brain, or do both to enhance cocaine sensitization (Figure 1.5)

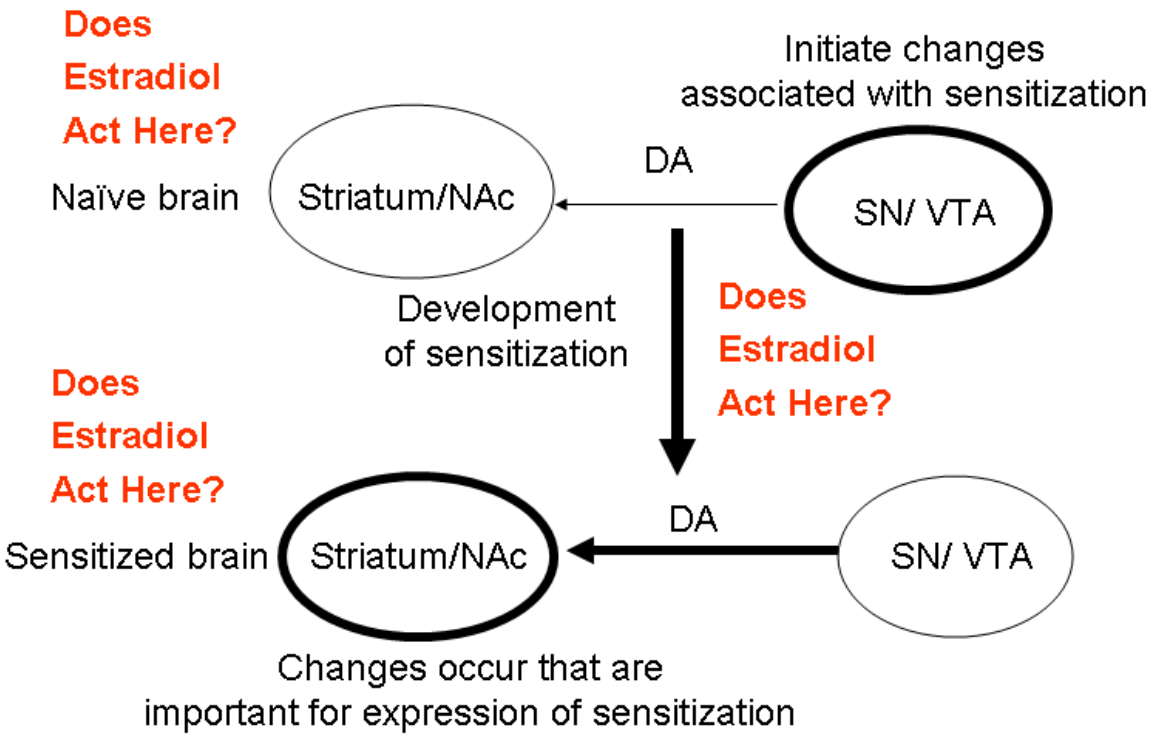


Figure 1.5 Possible mechanisms through which estradiol may work to modulate behavioral sensitizaiton

This study was designed to look at the effect of estradiol on behavioral sensitization when administered during cocaine treatment vs. prior to cocaine injection on the challenge day to provide insight to the above question. I hypothesize that estradiol would facilitate the induction of behavioral sensitization to cocaine; this enhanced behavioral sensitization produced by estradiol treatment with repeated cocaine treatment would be induced by cocaine on challenge day, independently of the presence of estradiol. Estradiol treatment on the challenge day would produce acute enhancing effect on behavioral response to cocaine in cocaine-sensitized rats.

Aim 2 (Chapter III): To determine whether 1) cocaine sensitization promotes the acquisition of cocaine self-administration in female rats; 2) estradiol further promotes the acquisition of cocaine self-administration in cocaine-pretreated rats; 3) there is enhanced DA transmission in striatum and NAc accompanying behavioral changes.

Rationale: there is evidence showing that preexposure to cocaine predisposes the rats to the rewarding effect of cocaine (Childs et al. 2006; Horger et al. 1990). Some studies have shown that prior exposure to psychostimulants enhances cocaine or AMPH self-administration behavior in male rats (Ferrario and Robinson 2007; Piazza et al. 1989; 1990; Suto et al. 2002; Vezina et al. 2002), and this behavioral change is probably mediated through sensitized DA neuron reactivity (Vezina 2004). This hypothesis has not yet been tested in females. This study was designed to investigate the effect of cocaine sensitization on acquisition of cocaine self-administration in females. In addition, estradiol is known to enhance the acquisition of cocaine self-administration in female rats (Hu et al. 2004; Lynch et al. 2001). The effect of estradiol on cocaine self-administration in cocaine-sensitized rats was also examined in this study. AMPH-induced striatal and accumbal DA release were compared between cocaine-experienced rats and cocaine-naïve rats to test if enhanced DA transmission is involved. I hypothesize that prior sensitization would promote the acquisition of cocaine self-administration in female rats, and estradiol would further enhance drug taking behavior in sensitized female rats. Moreover, cocaine exposure would result in enhanced DA transmission in both striatum and NAc.

Aim 3 (Chapter IV): To characterize the extracellular DA in dialysis in striatum of CAST males, OVX females and estradiol-treated OVX females after cocaine sensitization

to determine if: 1) there is a long term change in the nigrostriatal DA system due to repeated cocaine treatment; 2) this change will show a sexually dimorphic pattern or is modulated by estradiol.

Rationale: OVX female rats show enhanced behavioral sensitization relative to CAST male rats, and estradiol treatment further enhances sensitization in OVX female rats (Hu and Becker 2003). Microdialysis studies found that cocaine sensitization is associated with augmented DA overflow in response to psychostimulants in striatum and NAc (Torregrossa and Kalivas 2007). This hypothesis has not yet been tested in vivo in females. Furthermore, striatum is sexually dimorphic and is modulated estradiol (Becker 1999). This experiment was designed to compare the AMPH-induced DA increase in dialysate from striatum after cocaine sensitization among CAST male, OVX female and estradiol-treated OVX female rats to see if sex difference in behavioral sensitization could be accounted for by a difference of sensitivity of DA system developed with repeated cocaine treatment. I hypothesized that there would be augmented AMPH-induced DA release in striatum after cocaine sensitization in both males and females. This changes, however, would show sexually dimorphic patterns and be further modulated by estradiol.

CHAPTER II

EFFECT OF ESTRADIOL ON BEHAVIORAL SENSITIZATION TO COCAINE IN FEMALE RATS

1. Introduction

Behavioral sensitization is defined as the progressive enhancement of drug- induced psychomotor activity after repeated treatment with drugs such as cocaine and amphetamine (AMPH) (Robinson and Becker 1986b; Robinson and Berridge 1993; Stewart and Badiani 1993). Behavioral sensitization is of interest to the study of drug abuse because the same neural substrate that mediates drug addiction may be involved (Robinson and Berridge 1993; 2000a; 2001; Wise and Bozarth 1987).

Patterns of cocaine use have been reported to differ between men and women (Brady and Randall 1999; Griffin et al. 1989). For example, women report a greater level of cocaine cue-induced craving and a greater level of anxiety after cocaine use (Kosten et al. 1996; Robbins et al. 1999). The transition from initial use to addiction is shorter in women (Kosten et al. 1996). Better treatment outcomes are observed in cocaine dependent women compared to cocaine-dependent men in six-month follow-up studies (Kosten 1993; Weiss et al. 1997).

Besides these differences in behavior, cocaine-dependent men and women exhibit different patterns of region-specific blood flow as well as stress-induced brain activity, which suggests that the neural mechanism underlying cocaine addiction may differ

between men and women (Li et al. 2005; Tucker et al. 2004).

Consistent with clinical studies, sex differences in cocaine-related effects have also been reported in rodents, including cocaine-induced locomotor activity, conditioned rewarding effects and cocaine self-administration (Becker and Hu 2008; Becker et al. 2001; Caine et al. 2004; Dalton et al. 1986; Hu et al. 2004; Lynch and Taylor 2005; Russo et al. 2003b; Thompson et al. 1984; Walker et al. 2001). Of particular relevance here is the sex difference in susceptibility to sensitization (Robinson and Becker 1986b). Gonadal hormones, in particular estradiol, have been shown to enhance behavioral sensitization to cocaine in female rats (Chin et al. 2002; Hu and Becker 2003; Sircar and Kim 1999; Yang et al. 2007). It is not clear, however, whether estradiol modulates the neural processes occurring with the development of cocaine sensitization, or just produces an activational effect on sensitized brain, or do both to enhance cocaine sensitization

Behavioral sensitization to cocaine can be measured in two ways: 1) demonstration of the development of an enhanced psychomotor activating effect of cocaine with repeated injection of cocaine by within subject comparison, and/or 2) demonstration of a greater behavioral response to cocaine compared to saline-pretreated rats, on a challenge day that occurs following withdrawal from cocaine. Looking at the behavioral response on the challenge day also allows the investigators to determine the persistence of the sensitized response.

Until now, no research on hormonal influences on cocaine sensitization has tried to differentiate or compare the effect of estradiol given during the development of sensitization vs. the effect of estradiol on the challenge day. For example, many groups

have implanted estradiol-filled silastic capsules throughout the whole experiment (Febo et al. 2003; Peris et al. 1991; Perrotti et al. 2001), while others have looked at the effect of injections of estradiol prior to treatment with cocaine during the development of sensitization (Hu and Becker 2003). In that particular study, 5, 10 or 20 mg/kg cocaine-induced behavioral sensitization was enhanced by estradiol treatment. On the challenge day, this enhanced behavioral response was induced again by 10 mg/kg cocaine, 10 days after withdrawal from drug and hormone.

The present study was designed to determine the effect of estradiol during repeated cocaine treatments vs. the effect of estradiol on behavioral sensitization to cocaine as demonstrated by behavior on the challenge day. These data could provide insight as to whether estradiol is acting to modify the changes in the brain induced by repeated cocaine treatments or is affecting the acute effect of cocaine on behavior, or both. More importantly, it could guide clinical studies and help develop hormone-based treatments of cocaine abuse for women.

2. Materials and Methods

Animals

Female Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 225-250 g at the start of the experiment, were housed 3-4 per cage when they arrived. The room had a 10:14 h dark:light cycle (lights on at 5:30 am and off at 7:30 pm). The animals were housed in a room maintained at a constant temperature of 20-21 °C, with free access to phytoestrogen-free rodent chow (2014 Tekad Global, 14% protein rodent maintenance

diet, Harlan rat chow; Harlan Teklad, Madison, WI) and water available ad libitum. All procedures were performed according to a protocol approved by the University of Michigan Committee for Use and Care of Animals.

Ovariectomy (OVX)

All rats received bilateral ovariectomization surgery (OVX) approximately one week after they arrived. During the surgery, an incision around 1 cm long was made on the back skin along the midline just below the ribs. The muscle immediately under the skin was opened (0.5cm long and 1.5cm lateral to the midline) and the ovary on one side was then externalized with blunt forceps. The tissue between the ovary and uterus was clamped with a hemostat when the ovary was removed. The hemostat remained in place for another 30 seconds until there was no bleeding when the tissue was released and returned to the abdomen. The same procedure was repeated on the other side. The wound was closed with 11 mm wound clips. All animals underwent vaginal lavage testing daily for 10 consecutive days to confirm the cessation of cycling.

Behavior Sensitization

Two weeks after OVX surgeries, the rats were assigned to one of four treatment groups: (1) saline and 0.1 ml peanut oil; (2) 15 mg/kg cocaine and 0.1 ml peanut oil; (3) saline and 5 μg estradiol benzoate (EB) in 0.1 ml peanut oil; (4) 15 mg/kg cocaine and EB.

The testing chamber is a red round chamber (bottom diameter: 41cm; top diameter: 50cms; height: 42 cm) with a cone (bottom diameter: 16cm; height: 42 cm) in the middle, that allowed the rat to move freely around the cone. Animals were injected with EB or oil subcutaneously (s.c) and were placed in the testing chamber. After a 30 min habituation

period, each rat received an intraperitoneally (i. p.) injection of either saline or 15 mg/kg cocaine and was then put back to the testing chamber for one hour. Animals were tested for four days per week with three days off for three consecutive weeks, for a total of 12 testing days (Hu and Becker 2003). No cocaine or saline treatments or hormone were administered on the 3 days off. Intermittent treatment was used because it was better at producing behavioral sensitization than continuous treatment (Robinson and Becker 1986b).

The activity was monitored on day 1(first day of the 1st week), day 8 (first day of the 2nd week), day 15 (first day of the 3rd week) and day 18 (last day). On the above recording days, digital cameras were mounted on the top of each chamber that automatically tracked rats' activity. Behavior was analyzed by the Any-Maze system (Stoelting Inc, Wood Dale, IL 60191). On days when behavior was recorded, animals were allowed to habituate to the apparatus for 30 minutes as usual; they were then given 5 mg/kg cocaine or saline injections (i.p.) and put back in the testing chamber for one hour. Following that, they received 15 mg/kg cocaine or saline injections and were placed in the testing chamber for one hour.

The challenge day was the 14th day after the last treatment. Each of the four treatment groups were further divided into two groups, which either got EB or Oil treatment 30 minutes before they got cocaine. All of the rats received 5 mg/kg cocaine i.p., and their activities in the chamber were tracked for 60 minutes. This experiment was completed in two waves. During the second wave, an additional dose was added with another 60 minutes testing to track rat's locomotor activity induced by 15 mg/kg cocaine injections after the 5mg/kg dose on challenge day. See Table 3.1 for a summary of the

treatment of each group.

Table 2.1 Experimental design and sample size

Initial treatment	Oil				EB			
Drug	Saline		Cocaine		Saline		Cocaine	
Challenge day	Oil	EB	Oil	EB	Oil	EB	Oil	EB
N	8	8	7	7	8	9	7	6

Statistics

Data were subjected to analysis of variance (ANOVA) and Student's t test. Differences were considered significant if $p \leq 0.05$. All data were analyzed using SPSS 11.5 for PC.

3. Results

Development of behavioral sensitization

During the development of behavioral sensitization, there are only four treatment groups: Oil-treated saline (OS) and cocaine (OC) groups as well as EB-treated saline (ES) or cocaine group (EC), Distance traveled in the activity chamber during the first 30 minutes after cocaine (5 mg/kg) or saline injections was used to assess cocaine-induced locomotor activity. The behavioral activation on day 1, 8, 15 and 18 is shown in Figure 2.1. Repeated measures ANOVA analyses found an interaction among time, drug and hormone ($F(3,162) = 3.571$ $p=0.015$). When behavior on each individual day was

compared by two way ANOVA (drug x hormone), cocaine treatment significantly enhanced locomotor activity compared to saline treatment on each testing day (significant effect of drug, $p<0.0001$ for all four days). There was no effect of EB on either cocaine- or saline-induced response on day 1 ($F(1, 54) = 0.05$, $p=0.825$) or day 8 ($F(1, 54) = 0.851$, $p=0.360$). Estradiol treatment, however, interacts with drug treatment on days 15 ($F(1, 54) = 13.641$, $p=0.001$) and 18 ($F(1, 54) = 9.418$, $p=0.003$). Student t-test was then carried out to do individual comparisions. Estradiol not only enhanced the locomotor activity in response to low dose of cocaine on day 15 ($t(26) = 2.66$, $p=0.014$) and day 18 ($t(26) = 2.364$, $p=0.027$), but also decreased the locomotor response in saline rats on day 15 ($t(32) = 3.004$, $p=0.005$) and day 18 ($t(32) = 2.40$, $p=0.023$).

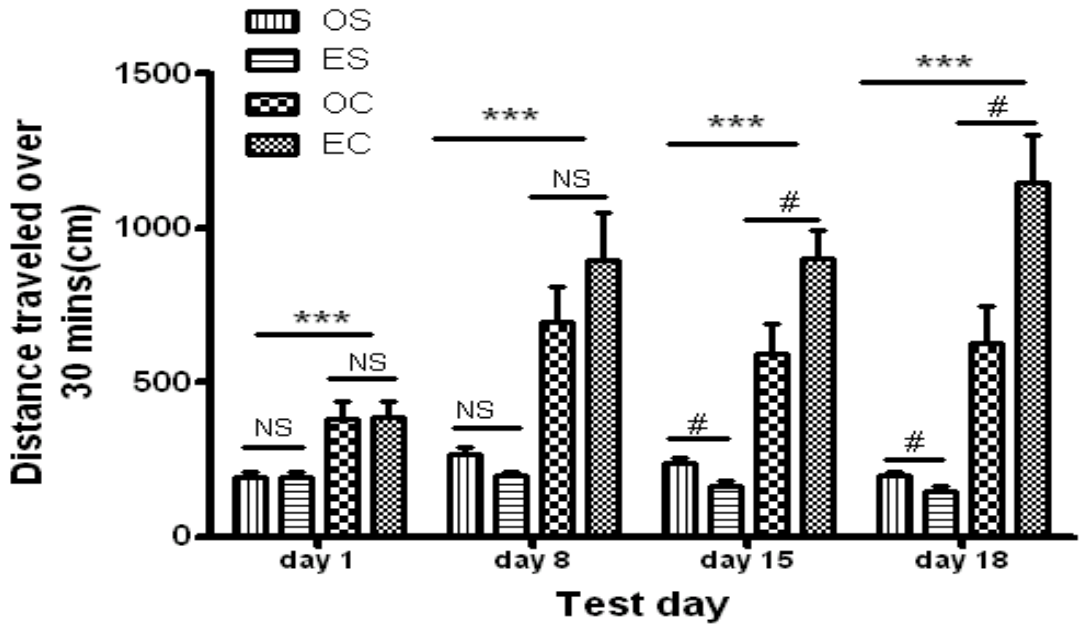


Figure 2.1: **Cocaine- or saline-induced locomotor activity (Mean + SEM) over 30 minutes of each testing of four testing days (day 1, 8, 15 and 18). The *** indicates significant difference in locomotor activity between cocaine-pretreated rats and saline-pretreated rats on that testing day. The # indicates significant difference in locomotor activity between EB-treated cocaine with oil-treated cocaine groups, or between EB-treated saline with oil-treated saline groups. OS: oil treated saline rats (n=17); ES: EB treated saline rats (n=16); OC: oil treated cocaine rats (n=14); EC: EB treated cocaine rats (n=13).**

The difference in the distance traveled by the rats on day 18 minus day 1 was taken as an index of sensitization (Figure 2.2). Both of the two cocaine-pretreated groups were sensitized as the rats' response to cocaine on day 18 minus day 1 were significantly greater than 0 (EC group: t (12) =5.102, p<0.0001; OC group: t (13) =2.151, p=0.05). Neither saline group exhibited sensitization or habituation (ES group: t (15) =-1.523, t=0.149; OS group: t (16) =1.052, p=0.309). Estradiol significantly enhanced behavioral sensitization to cocaine (significant difference between EC and OC group, t=2.74, p=0.011), but had no effect on activity induced by repeated saline treatment (no significant difference between ES and OS group, t=1.87, p=0.071)

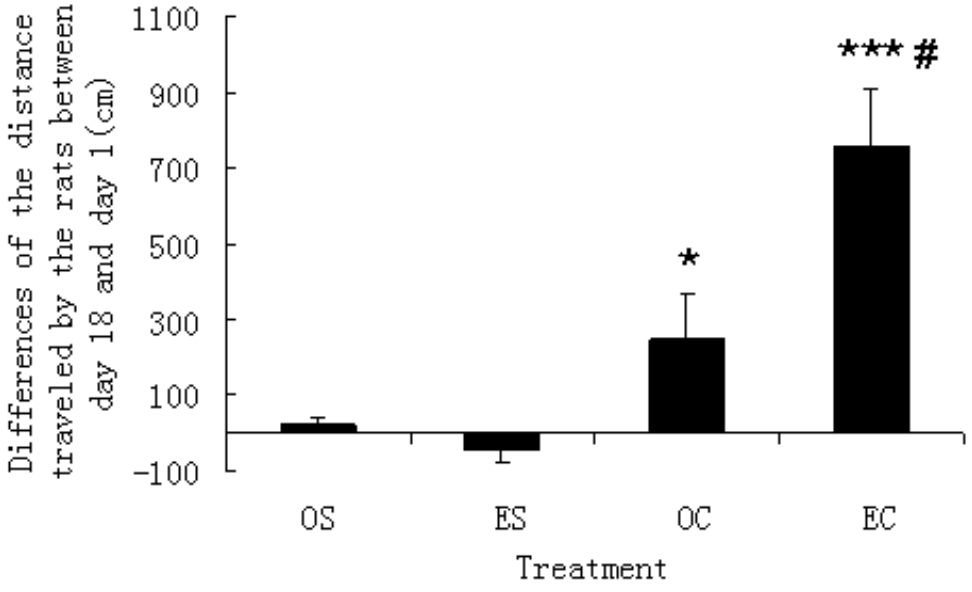


Figure 2.2: The differences of distance (Mean ± SEM) traveled by the rats between day 18 and day 1(distance traveled by the rats on day 18 after cocaine or saline injections minus the distance traveled on day 1 after the same cocaine or saline injections for each groups). The * (p<0.0001) and * (p=0.05) indicate changes in locomotor activities are significantly different from 0. The # indicates significant difference between OC and EC group.**

Locomotor activity induced by 5mg/kg cocaine on challenge day

Cocaine-induced locomotor activity on the challenge day is shown in Figure 2.3 Three-way ANOVA (prior drug treatment X prior hormone treatments X hormone on challenge day) indicates a significant effect of prior drug treatment ($F(1, 53) = 117.409$, $p<0.0001$), suggesting cocaine pretreatment resulted in behavioral sensitization on the challenge day. For all the cocaine groups, two way ANOVA (prior EB treatment given with repeated cocaine treatment x EB treatment on challenge day) indicated a significant interaction ($F(1,23)=6.08$, $p=0.022$). LSD Post Hoc test showed that OE group exhibited more locomotor activity than OO group ($p=0.032$).

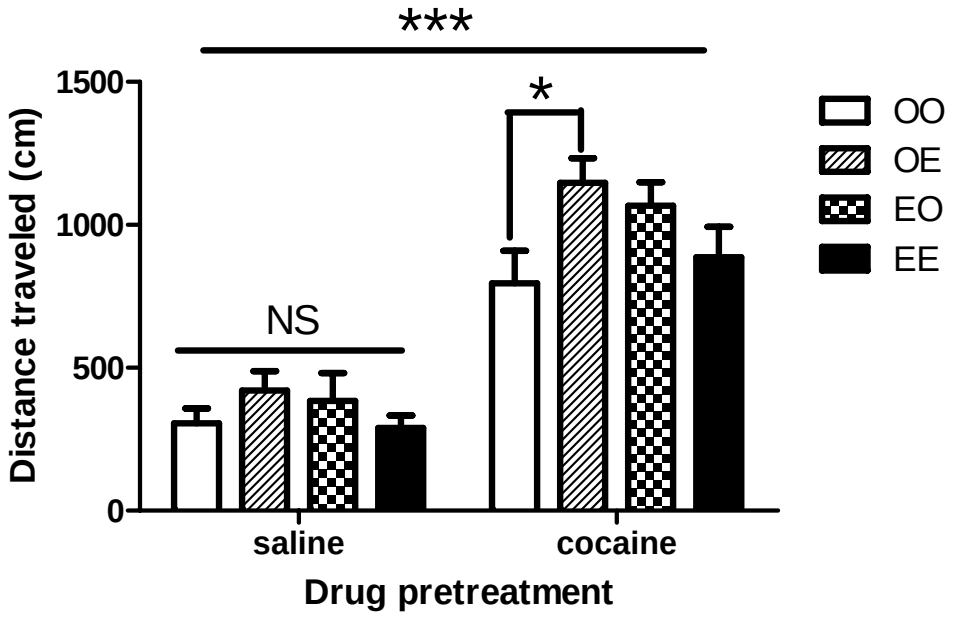


Figure 2.3: Locomotor activity (Mean ± SEM) over 30 minutes after 5mg/kg cocaine injections on challenge day. The * indicates significant difference in locomotor activity between saline-pretreated rats and cocaine-pretreated rats at p<0.0001. The * indicates significant difference between cocaine-pretreated OO and OE group. Saline-pretreated groups: OO (n=6), OE (n=6), EO (n=8); EE (n=9); Cocaine-pretreated groups: OO (n=7), OE (n=7), EO (n=7), EE (n=6).**

Locomotor activity induced by 15mg/kg cocaine on challenge day

Since EB treatment during development of sensitization failed to enhance behavioral activation after 5 mg/kg cocaine injection on challenge day, we hypothesized that a higher dose of cocaine might be needed to see the effect of EB. As illustrated in Figure 2.4 below, 15 mg/kg cocaine caused significant behavioral activation even on the first injection, as illustrated by substantial locomotor activity in saline-pretreated rats. Cocaine pretreatment resulted in behavioral sensitization in response to 15 mg/kg cocaine injection as well, shown by significant effect of drug pretreatment on locomotor activity over one hour $F(1,30)=4.492$, $p=0.042$.

All the rats exhibited a higher rate of locomotor activity immediately after the 15 mg/kg cocaine injection and then slowed, their behavior during the period 15 to 45 min exhibited more between-group variations and therefore were analyzed separately. For the four groups of cocaine-pretreated rats, two way ANOVA (previous EB treatment given with repeated cocaine treatment x EB treatment on challenge day) indicated that previous EB treatment given with repeated cocaine treatment significantly enhanced the cocaine-induced behavioral activation on challenge day ($F (1, 14) =8.436$, $p=0.012$). In addition, the four groups of saline-pretreated rats do not show any difference in cocaine-induced behavioral activation.

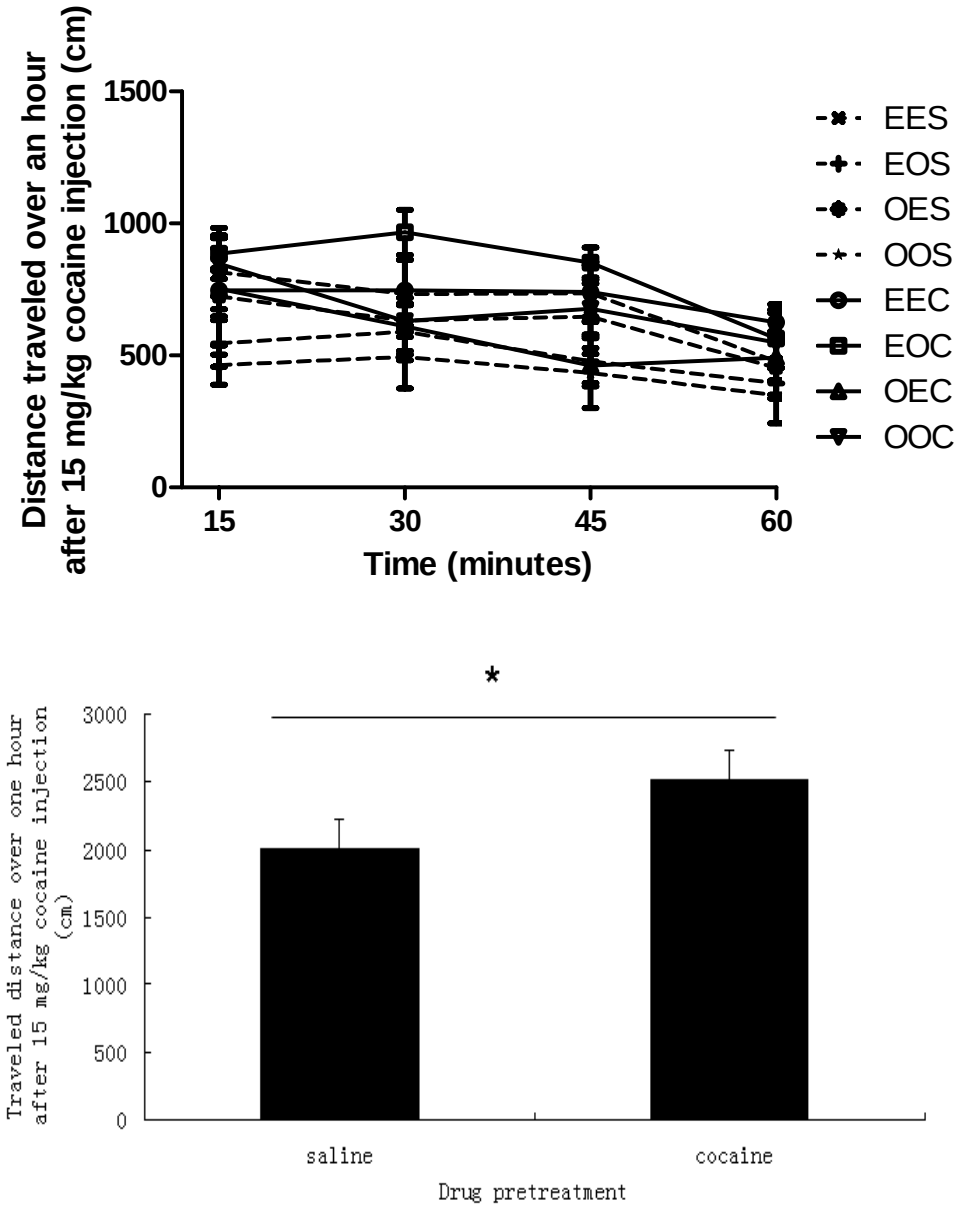


Figure 2.4: Locomotor activity (Mean ± SEM) induced by 15 mg/kg cocaine on challenge day. Distance traveled in 15 min segments over an hour for each group is shown in the top panel. Total activity over an hour was compared between all saline-pretreated rats and all cocaine-pretreated rats in the bottom panel. The * indicates cocaine-pretreated rats exhibited significant greater locomotor activity than saline-pretreated rats. OOS (n=6), OES (n=5), EOS (n=4), EES (n=4), OOC (n=5), OEC (n=5), EOC (n=4), EEC (n=4)

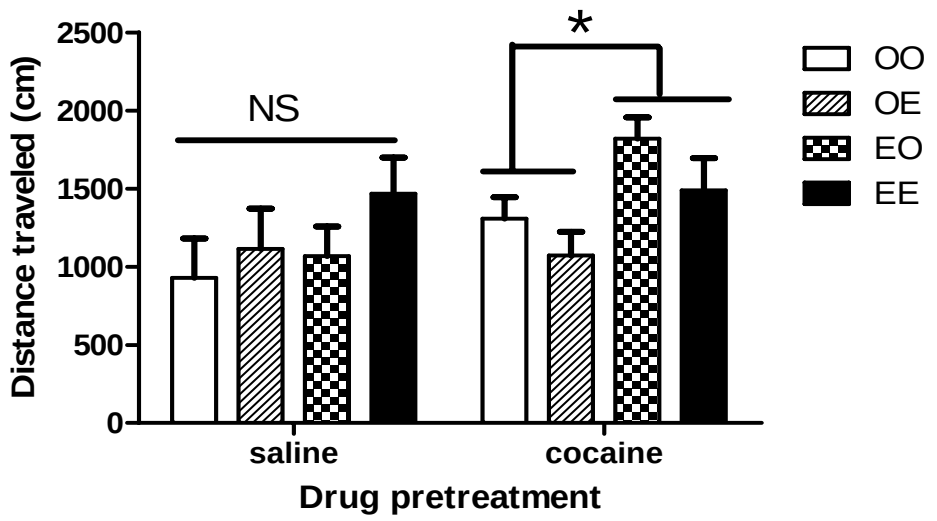


Figure 2.5: Traveled distance (mean ± SEM) over 30 minutes (from 15 to 45 min) after 15 mg/kg cocaine injections on challenge day. The * indicates cocaine-pretreated rats received EB with repeated cocaine treatment exhibited greater behavioral activation on change day than did cocaine-pretreated rats received Oil with repeated cocaine treatment.

4. Discussion

This is the first study investigating the effect of estradiol treatment during sensitization vs. post sensitization on the challenge day. In this experiment, estradiol enhanced behavioral sensitization to cocaine when it was given with repeated cocaine treatments as predicted. More importantly, by comparing behavioral activation post sensitization on challenge day with or without another estradiol treatment, it was found that estradiol acutely enhanced cocaine-induced behavioral activation in cocaine-pretreated rats when it was given on the challenge day, suggesting for the first time that estradiol produce activational effect on sensitized brain to enhance behavioral activation. In addition, the rast that received prior estradiol treatment with repeated cocaine treatments, which exhibited greatest behavioral sensitization, also showed enhanced cocaine-induced behavioral activation post sensitization on challenge day with or without

another estradiol treatment, suggesting estradiol may have also influenced the neural sensitization associated with repeated cocaine treatment.

The present study is consistent with previous reports and supports the idea that estradiol promotes cocaine sensitization in female rats (Becker et al. 2001; Chin et al. 2002; Hu and Becker 2003; Peris et al. 1991; Perrotti et al. 2001; Sell et al. 2002; Sircar and Kim 1999; Zhen et al. 2007). Although this question has been addressed by a few studies, people differed in the ways of quantifying sensitization. In most studies, people compared the absolute amount of behavior induced by cocaine at the end of sensitization to determine the magnitude of sensitization. Sensitization, however, is the change of the behavior with repeated treatment of drugs, rather than the absolute behavior exhibited. If one compares the changes of behavior (day 18 - day 1) for each group, estradiol treatment significantly enhanced this change, demonstrated behavioral sensitization as others have shown.

Only one paper has looked at the persistence of estradiol-produced enhanced sensitization (Hu and Becker 2003). Estradiol treated OVX rats continued to exhibit greater rotational behavioral in response to 10 mg/kg cocaine than OVX rats when they were challenged after 10 days' withdrawal from cocaine and estradiol. Consistent with that observation, when a high dose of cocaine was administered in this study, the rats with previous estradiol treatment and repeated cocaine treatments exhibited more locomotor activity than control rats. Thus, this finding as well as Hu's study both suggests that estradiol, given during cocaine sensitization, may have influenced the neural processes associated with sensitization so that the rat continued to show greater behavioral activation later on even without circulating estradiol (Hu and Becker 2008).

One caution should be kept in mind is that prior EB treatment given with repeated drug treatment didn't significantly interact with repeated drug treatment to influence behavior on challenge day in this study. Thus it is possible that the enhanced behavioral activation on challenge day seen in the rats that received both prior repeated estradiol and cocaine treatment is only due to a long term effect of estradiol. It is known that estradiol could produce profound changes on DA system. For example, OVX increase DA and its metabolites in the striatum and NAc (Bitar et al. 1991, Shimizu, 1993 #689), decrease DAT density in the striatum(Bosse et al. 1997; Le Saux and Di Paolo 2006; Morissette and Di Paolo 1993a) and all those effects could be reversed by estradiol treatment. Moreover, chronic estradiol treatment has also been shown to act on ERβ to increase D2 receptor density in the striatum and NAc in OVX rats (Di Paolo 1994; Lammers et al. 1999; Landry et al. 2002; Le Saux et al. 2006; Zhou et al. 2002). None of the above effects of estradiol, however, is known to persist for two weeks after withdrawal from estradiol. Compared to that, repeated cocaine treatment is known to produce series of long term neuroadaptations in DA system that can persist from weeks to months after withdrawal from cocaine (Thomas et al. 2008). Thus, the enhanced behavioral activation in the rats treated with repeated estradiol and cocaine compared to the rats that received oil and cocaine are more likely due to an interaction of estradiol and cocaine. None the less, more studies are needed to confirm this hypothesis.

Consistent with previous reports from our lab and some other reports, we didn't see an acute effect of estradiol on the locomotor activity after first injection of cocaine in this study (Hu and Becker 2003; Peris et al. 1991; Perrotti et al. 2000; Sircar and Kim 1999; Yang et al. 2007). A role for estradiol, however, has been reported in several other studies

when estradiol benzoate-filled silastic capsules were implanted early before the testing(Perrotti et al. 2001; Quinones-Jenab et al. 2000; Sell et al. 2000). The differential effect may be due to short vs. long exposure to estradiol.

Even though estradiol did not enhance the response to the first cocaine treatment, previously sensitized rats exhibited an enhanced behavioral response when pre-treated with estradiol acutely on challenge day. Thus, when an animal has been sensitized, estradiol exacerbates this effect. The expression of behavioral sensitization is suggested to be related to the enhanced DA transmission in NAc and striatum (Vezina 2004). Moreover, estradiol produces rapid effect on DA transmission in striatum. For example, an acute administration of estradiol to OVX rats induce a rapid increase in AMPH-induced DA release in striatum in vivo (Becker 1990b), induces an increase in striatal DA turnover (Di Paolo et al. 1985) and decrease D2 class DA receptors (Bazzett and Becker 1994). Thus, it is very likely that sensitized DA transmission in striatum after cocaine sensitization made the acute effect of estradiol more pronounced to result in behavioral effect that was not seen before sensitization. More importantly, this effect of estradiol on challenge day is probably simply due to its enhancement of cocaine-induced increases in DA neuron reactivity, which is independent of the neural processes mediating sensitization.

An intriguing thing is no acute effect of estradiol was seen if the rats received prior administration of cocaine as well as estradiol. Note that those animals showed most robust behavioral sensitization during the development of sensitization, it is possible that ceiling effect prevented us from seeing additional acute effect of estradiol. Moreover, if previous long term estradiol treatment has interacted with cocaine and produced some

long term effect on DA system as I pointed out earlier, the way of DA transmission in response to estradiol may have been comprised or changed.

Another observation in this study is estradiol decreased the activity in saline rats at later stage of sensitization. It might be due to anti-depressant and anti-anxiety effect of EB (Walf and Frye 2005a; b; 2006; 2007a; b; Walf et al. 2004). When the rats face novel environment, however, estradiol is also known to enhance spontaneous locomotor activity (Becker et al. 1987; Frye et al. 2000; Morgan and Pfaff 2001; 2002), which may explain why initial response to saline was comparable between estradiol- and oil- treated rats.

In conclusion, this study demonstrated that estradiol treatment enhances behavioral sensitization when given during cocaine treatment as predicted. Importantly, it provides the first evidence suggesting that estradiol can produce activational effect on sensitized brain to enhance behavioral activation in cocaine-sensitized rats. Moreover, prior estradiol treatments, given with repeated cocaine treatment, resulted in greater behavioral activation in response to a high dose of cocaine on challenge day after two weeks withdrawal from cocaine and hormone, suggesting estradiol may have also influenced the neural sensitization occurred with repeated cocaine treatment. More experiments are needed to further clarify each mechanism, which are important for developing appropriate treatments of drug abuse specifically for women.

CHAPTER III

EFFECT OF ESTRADIOL AND COCAINE SNESITIZATION ON COCAINE SELF-ADMINISTRATION

1. Introduction

Cocaine abuse is an important health issue throughout the world. Among all illicit drugs, it has second highest level of dependence among all the illicit drugs in National Survey on Drug Use and Health (SAMHSA 2007). Understanding the underlying mechanisms mediating cocaine use and abuse is important for developing clinical treatment and prevention.

Cocaine self-administration in rodents has been widely used as a preclinical model of cocaine use in humans. Interestingly, in laboratory animals, as seen in humans, there are individual differences in drug taking behavior. Factors such as stress, individual characteristics and prior drug history have been shown to modulate the vulnerability to self-administer cocaine-like psychomotor stimulants (Davis et al. 2008; Fattore et al. 2008; Piazza et al. 1989; 1990).

Of particular interest here is how prior experience with psychomotor stimulants modulates the vulnerability to self-administer them. It has been shown that behavioral sensitization either induced by stress or repeated amphetamine (AMPH) treatment predisposes rats to self-administer low dose of AMPH (Piazza et al. 1989; 1990). Animals that have been previously sensitized to AMPH also acquire cocaine self-administration

faster (Horger et al. 1992; Valadez and Schenk 1994), accelerate escalation of cocaine intake when they are given extended access to cocaine self-administration (Ferrario and Robinson 2007). Similarly, rats that are pre-exposed to cocaine learn self-administration faster and develop a preference to a dose of cocaine that non-exposed rats do not prefer more than saline (Childs et al. 2006; Horger et al. 1990). All of these suggest prior exposure to psychostimulants may have enhanced the reinforcing effect of psychostimulants.

Midbrain dopamine (DA) neuron reactivity, which mediates both stimulant and reinforcing effect of psychostimulants, is reported to be sensitized by repeated exposure to psychomotor stimulant drugs (Torregrossa and Kalivas 2007). Moreover, reactivity of DA neurons is directly related to self-administration of psychomotor stimulant drugs (Vezina 2004). Thus, sensitization to cocaine or AMPH may enhance the reinforcing effect of these drugs through a sensitized dopaminergic system, and therefore promote self-administration (Vezina 2004).

In all the studies discussed above, males were used as subjects. The relationship of sensitization and self-administration has not been examined in females. Clinical studies suggest a gender difference in response to cocaine as cocaine cues induce more cocaine craving in female than male addicts (Robbins et al. 1999). Women also report less pleasure and dysphoria and greater anxiety in response to cocaine (Kosten et al. 1996; Lukas et al. 1996; Singha et al. 2000). Female substance users have greater cocaine use and are more likely to be dependent on cocaine than males (Lejuez et al. 2007). Further more, women cocaine addicts report greater subjective effect of smoked or intravenous cocaine administration in the follicular phase than in the luteal phase, which suggest a

role of ovarian hormones on cocaine-induced response (Evans et al. 2002; Sofuoglu et al. 1999).

In accordance with clinical studies, rodents also show sex differences in response to cocaine. Female rats acquire cocaine self-administration more rapidly and self-administer more cocaine than do male rats (Hu et al. 2004). They are more sensitive to the priming effect of cocaine during reinstatement (Lynch and Carroll 2000). Female rats also show greater behavioral sensitization than male rats (Hu and Becker 2003). Moreover, females' self-administration behavior fluctuates over the estrous cycle (Lynch et al. 2000; Roberts et al. 1989). Further investigation suggests that estradiol enhances the acquisition of cocaine self-administration and cocaine intake in female rats (Hu et al. 2004; Lynch et al. 2001).

Related to above behavioral difference, ascending midbrain DA projections, which are known to mediate psychomotor stimulant and reinforcing effect of cocaine, are also sexually dimorphic and modulated by estradiol (Becker 1999). Only one study has ever looked at the striatal DA reactivity after cocaine sensitization in females, and reported that the DA release from striatum in vitro was enhanced after cocaine pretreatment (Peris et al. 1991). Nobody has ever tested if cocaine pretreatment also results in enhanced DA dopamine (DA) release in vitro from nucleus accumbens (NAc) in females.

This study was designed to investigate the effects of cocaine sensitization on cocaine self-administration in female rats. Moreover, from the previous sensitization experiment (chapter II), we saw that acute estradiol enhanced behavioral activation in cocaine-sensitized rats. Thus we also investigate whether estradiol further enhance cocaine self-administration in cocaine-sensitized rats. Lastly, we test the hypothesis that cocaine

experience results in enhanced release of DA from both the nucleus accumbens and dorsal striatum in an in vitro superfusion experiment.

2. Materials and Methods

Animals

Female Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 225-250 g at the start of the experiment, were housed 3-4 per cage when they arrived. The room has a 10:14 h regular dark:light cycle (lights on at 5:30 am and go at 7:30 pm). The animals were housed in a room maintained at a constant temperature of 20-21 °C, with free access to phytoestrogen-free rodent chow (2014 Tekad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI) and water available ad libitum. All procedures were performed according to a protocol approved by the University of Michigan Committee for Use and Care of Animals.

Ovariectomy

All rats were bilaterally ovariectomized (OVX) approximately one week after they arrived. During the surgery, an incision around 1 cm long was made on the back skin along the midline just below the ribs. The muscle immediately under the skin was opened (0.5 cm long and 1.5 cm lateral to the midline) and the ovary on one side was then externalized with blunt forcepts. The tissue between the ovary and uterus was clamped with a hemostat when the ovary was removed. The hemostat was remained in place for another 30 seconds until there was no bleeding when the tissue was released and returned to the abdomen. The same procedure was repeated on the other side. The wound was

closed with 11 mm wound clips. All animals underwent vaginal lavage testing daily for 10 consecutive days to confirm the cessation of cycling.

8 rats were randomly selected as cocaine naïve control group and kept in the home cages until they were sacrificed for the superfusion experiment. All other animals were assigned to one of the four treatment groups: (1) rats received saline treatment during sensitization and oil treatment during self-administration (SO, n=12); (2) rats received saline treatment during sensitization and EB (5µg estradiol benzoate dissolved in 0.1 ml peanut oil) treatment during self-administration (SE, n=14); (3) rats received cocaine treatment during sensitization and oil treatment during self-administration (CO, n=12); (4) rats received cocaine treatment during sensitization and EB treatment during self-administration (CE, n=14).

Cocaine Sensitization

The testing chamber is a round chamber (bottom diameter: 41cm; top diameter: 50cms; height: 42 cm) with a cone (bottom diameter: 16cm; height: 42 cm) in the middle, which allowed the rat to move freely around the cone. Two weeks after OVX surgeries, animals received an injection of 0.1 ml oil subcutaneously and placed in the testing chamber. After a 30 min habituation period, each rat received an intraperitoneally (i.p) injection of either saline or 15 mg/kg cocaine (according to their treatment group) and was then put back into the testing chamber for one hour. Animals were tested for four days per week, followed by three days off, for three consecutive weeks, for a total of 12 testing days (Hu and Becker 2003). No cocaine or saline were administered on the 3 days off. Intermittent treatment was used here because it was better at producing behavioral sensitization than continuous treatment (Robinson and Becker 1986b).

The activity was monitored on day 1 (first day of the 1st week), day 8 (first day of the 2nd week), day 15 (first day of the 3rd week) and day 18 (last day). On the above recording days, digital cameras were mounted on the top of each chamber to automatically tracked rats' activity. Behavior was analyzed by the Any-Maze system (Stoelting, Inc. Wood Dale, IL). On days when behavior was recorded, animals were allowed to habituate to the apparatus for 30 minutes as usual; they were then given 5mg/kg cocaine or saline injections (i.p.) and put back in the testing chamber for one hour. Following that, they received 15 mg/kg cocaine or saline injections and were placed in the testing chamber for one hour. On those four days, cameras were mounted on the top of each bucket to videotape and monitor the activity of the rats throughout the testing. Animals' behavior was quantified by the Any-Maze system.

Catheter implantation

10 days after sensitization training, rats were prepared with indwelling intravenous jugular catheters connected to a backport. Catheters were constructed by gluing Silastic tubing (0.51mm ID, 0.94 mm OD; Dow Corning, Midland, MI) to an external guide cannula. The cannulae were then glued to polypropylene mesh with cranioplastic cement.

Rats were anesthetized with a combination of ketamine (45 mg/kg, i.p.) and medetomidine (0.3 mg/kg, i.p.). The free end of the Silastic tubing was inserted into the right jugular vein and secured with 4.0 silk sutures around the venous tissue. The catheters exited dorsally on the animal's back. Dummy stylets were inserted into the catheters when rats were not connected to infusion pumps. Catheters were flushed daily with 0.1 ml heparinized saline (30 U/ml, in 0.9% sterile saline buffered at pH 7.4) and a

0.1 ml gentamicin solution (0.8 mg/ml) to prevent occlusions and to circumbent microbial buildup in the catheter.

For each self-administration session, catheters were flushed with 0.1 ml of saline before sessions began and with 0.1 ml gentamicin after session ended.

Cocaine self-administration

Self-administration apparatus is a standard operant chamber (25 x 27 x 30 cm) that was placed in a sound-attenuating cabinet (Med Associates, Inc., Georgia, VT). There were a red house light, a tone generating apparatus, and two nose-poke holes in each chamber. Rats were connected to an infusion syringe and tethered via a steel cable to a swivel. The swivel was mounted on a counterbalanced arm so that the animal can move freely in the test cage. The infusion pumps were mounted on the outside doors of the cabinets. At the start of the experiment, the red house lights were illuminated and remained on for the whole session so that the rat's behavior could be monitored through the observer's camera on the cabinet door. Nose poke on the active hole resulted in an intravenous injection of 50 µl of cocaine-HCl in saline delivered over 2.8 s. This process was accompanied by a compound stimulus consisting of a white stimulus light and a tone (85 dB). There was a 5 s time-out period after each infusion, during which time further nose pokes had no programmed consequences. But nose pokes were still recorded. Nose pokes in the inactive hole were also recorded, but had no programmed consequences.

17 days after the last treatment with cocaine or saline (around five days after recovery from catheter surgery), rats began cocaine self-administration. Rats received either EB or Oil (s.c), according to their treatment group, 30 min before self-administration session started. Rats were allowed to nose poke to obtain an i.v. infusion

of cocaine on an FR1 schedule of reinforcement. The self-administration test occurred five days per week with two days off for four weeks. No cocaine or saline treatment or hormone was administered during the two days off. The doses of cocaine that was self-administered during each week were 0.1, 0.1, 0.15, 0.4 mg/kg/infusion. The test session last 120 min every testing day during the first three weeks and 60 min during the last week. We reduced the session time in the last week to prevent overdose. Rats didn't receive any training for nose poking and they were not primed with cocaine.

Superfusion

Due to a limit in the number of chambers that could be tested with the superfusion apparatus (Brandel Inc., Gaithersburg, Md., USA) each day, only brains from CE group and the 8 cocaine naïve rats that had been kept in the home cage since OVX surgeries, were used for superfusion experiment.

After self-administration testing ends, animals in CE group were given 15 mg/kg cocaine treatment (i.p.) every day for another four days to reduce variability of cocaine experience during self-administration. Ten days after the last cocaine treatment, they got one i.p. injection of low dose cocaine (5 mg/kg) and locomotor activity was monitored. Brains were obtained for superfusion experiment three to four days after the cocaine challenge.

Superfusion experiments were performed using an automated superfusion system (Brandel Inc., Md., USA). The procedure used was as described previously (Xiao et al. 2003). On the day of superfusion, a Ringer's solution was oxygenated by bubbling with 95%O_2/5%CO_2 for at least 30 min and the pH was adjusted to 7.4 in the morning. Animals were decapitated, and the brain was removed and placed in ice-cold Ringer's

solution within 5 mins. The dorsal striatum and ventral striatum (including NAc and medial olfactory tubercle) was dissected from each rat separately. Each brain region was cut into approximately 0.5 to 1 mm^3 fragments and placed into different superfusion chambers filled with Ringer's solution. The brain fragments were allowed to equilibrate for 60 mins before sample collection while Ringer's solution flowed at 100 μl/min. Following the stabilization period, two 5-min baseline samples were collected. Then a 10 μM d-AMPH was delivered to all the chambers for 2.5 min followed by continuously delivery of the Ringer's again. Effluent samples were collected every 5 minutes throughout the whole experiment. The solutions were warmed to 37°C via water bath prior to reaching the chambers and the chambers were warmed in a water bath at 37°C. Each collection vial contained 25 μl of 0.05 N $HClO_4$ with dihydroxybenzylamine as an internal standard to control for any variation in volume and loss of DA due to oxidation. Tissue in each chamber was removed and weighed immediately following the superfusion. The amount of DA in each sample was measured by the HPLC-ED (ESA Biosciences, Inc. Chelmsford, MA, US).

Statistics

Data were subjected to analysis of variance (ANOVA) and Student's t test. Differences were considered significant if $p \leq 0.05$. All data were analyzed using SPSS 11.5 for PC.

3. Results

Induction of cocaine sensitization

The difference in distance traveled over 60 minutes on day 1 vs. day 18 was

calculated to assess sensitization (Figure 3.1). As expected, cocaine treatment produced behavioral sensitization (t (25) =2.173, p=0.039); and saline treatment resulted in behavioral habituation (t (23) =-4.964, p<0.0001).

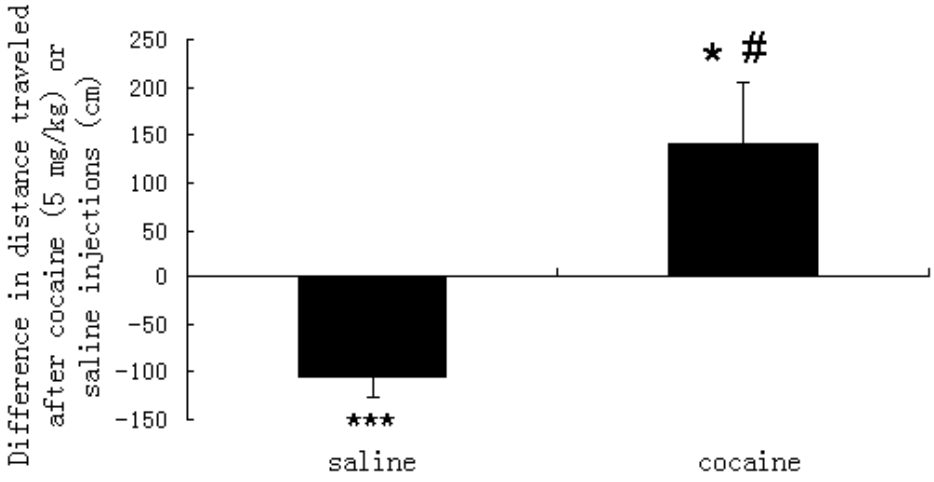


Figure 3.1: Difference in distance traveled (Mean ± SEM) after cocaine (5mg) or saline injections (day 18 - day 1). The * and * indicate significant difference from 0. The # indicates cocaine group was significantly different from saline group.**

Acquisition of cocaine self-administration

Acquisition of cocaine self-administration was defined as when nose poking in the active hole was more than 20 per two hour session or more than 10 per one hour session for three consecutive days and nose poking in the active hole was more than twice that in the inactive hole. This criterion was chosen based on the average number of nose pokes in the inactive hole during two hours session for the first week, which was not greater than 10. This criterion has been used in other self-administration studies (Hu et al. 2004; Jackson et al. 2006). Rats that acquired the cocaine self-administration according to this criterion showed a stable increase in cocaine intake over the remaining experiment.

Fig 3.2 shows the percentage of animals in each group that acquired self-

administration throughout the experiment. By the end of the first week, half of the rats from CE group had already acquired self-administration, whereas only 30% of rats from other groups had done so. By the end of the second week, 80% of rats from CE group and more than 60% from CO group had acquired self-administration whereas only 40% of saline-pretreated animals had done so. On week 3 when the dose increased to 0.15 mg/kg/inf, all the rats in SE group acquired self-administration, whereas a few rats from SO group did so. Some SO animals only acquired self-administration when the dose was increased to 0.4 mg/kg/inf during week 4. Chi square analyses showed the acquisition curves were different among the four groups, $X^2(3) = 4.432$, $p=0.0397$. Individual comparisons between each pair of curves indicated that CE group have different acquisition curves compared to SO group, $X^2(1) = 4.586$, $p=0.032$

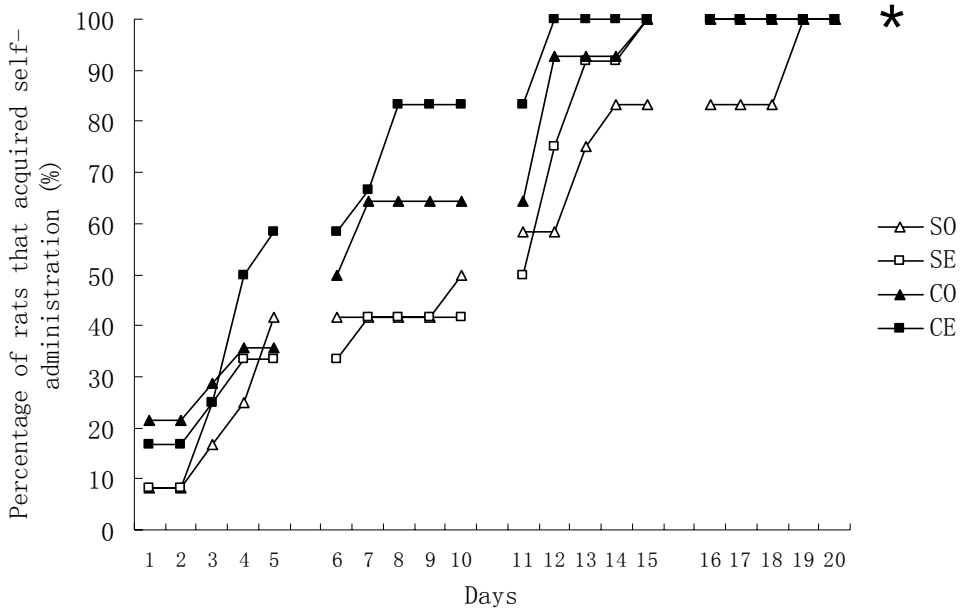


Figure 3.2: The self-administration acquisition curves of each group. The * indicates EC group acquired self-administration faster than OS group. CE (n=12): cocaine-pretreated rats received EB during self-administration session; CO (n=14): cocaine-pretreated rats received O during self-administration session; SE (n=12): saline-pretreated rats received EB during self-administration session; SO (n=12): saline-pretreated rats received O during self-administration session.

Figure 3.3 shows the average number of days to acquire self-administration for each group. Two-way ANOVA analyses (drug X hormone) indicated a significant effect of prior cocaine treatment ($F(1, 46) = 4.926$, $p=0.03$).

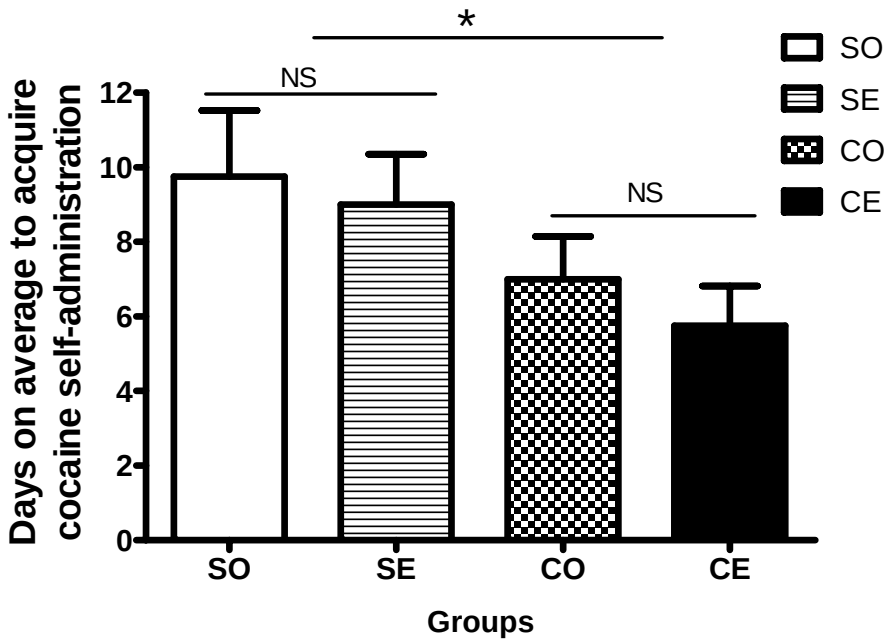


Figure 3.3: The average days (Mean ± SEM) to acquire cocaine self-administration for each group. The * indicates prior cocaine treatment significantly accelerated the acquisition of cocaine self-administration regardless of hormone treatment.

The total number of cocaine infusion of each week

When the total number of cocaine infusions per week (Figure 3.4) was examined by two-way ANOVA (drug X hormone), prior cocaine treatment greatly enhanced cocaine taking behavior during week 2 ($F(1, 46)=8.405$, $p=0.006$), week 3 ($F(1, 46)=9.672$, $p=0.003$) and week 4($F(1, 46)=7.040$, $p=0.011$). Post hoc comparison showed that CE group always took more cocaine than SO group ($p<0.05$).

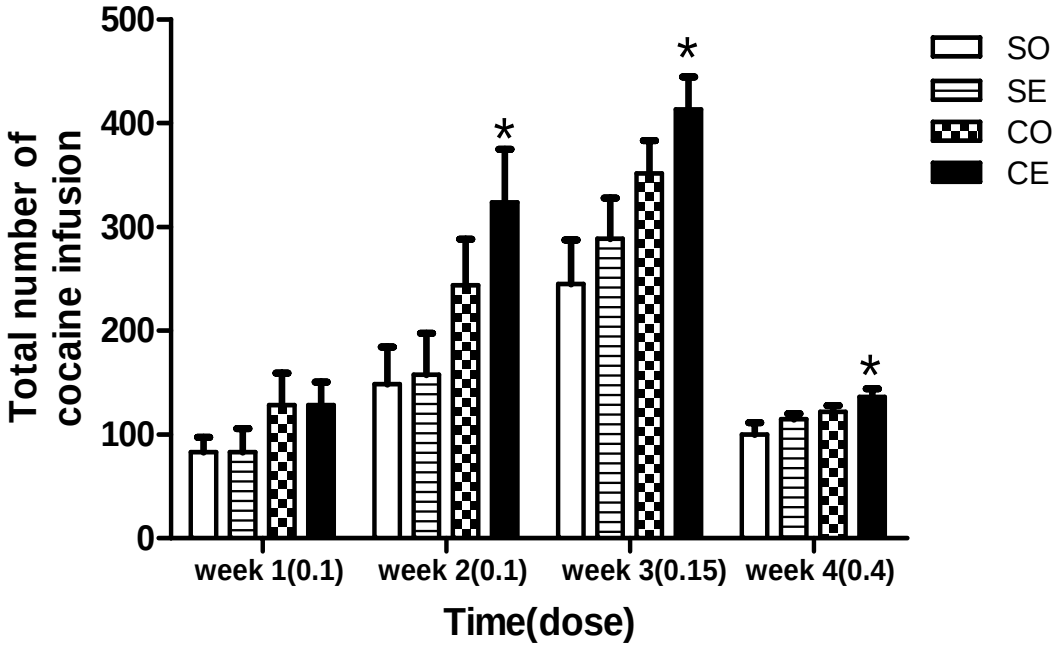


Figure 3.4: Total number of cocaine infusions (Mean ± SEM) per week for each group of rats. Cocaine sensitization greatly enhanced cocaine self-administration throughout the whole experiment. The * indicates that CE group always took more cocaine then SO group.

For the last 6 self-administration sessions (Figure 3.5), two-way ANOVA indicated there was both a main effect of drug pretreatment ($F(1, 46) = 9.171$, $p = 0.004$) and a main effect of hormone ($F(1,46) = 3.898$, $p = 0.05$).

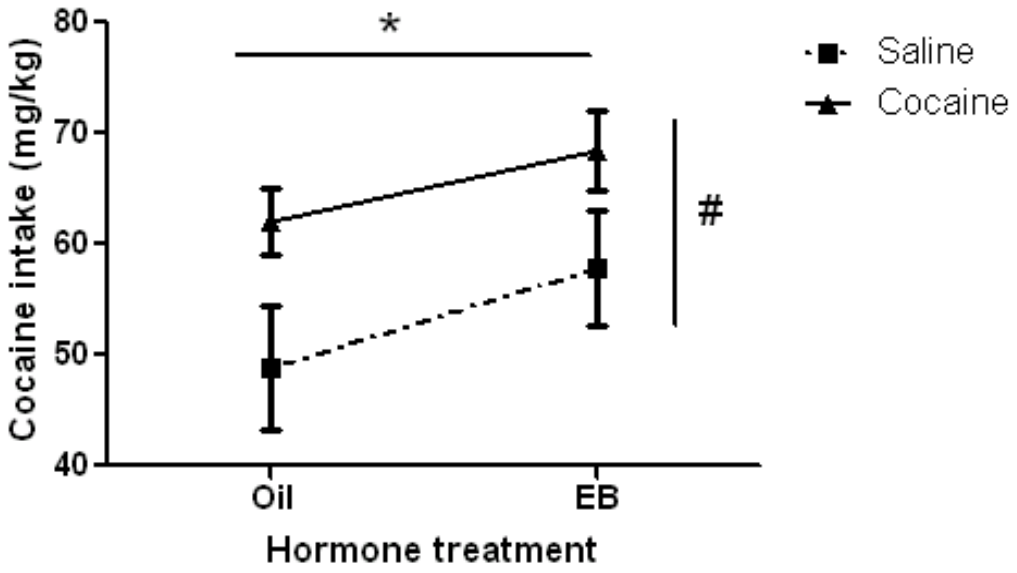


Figure 3.5: Total cocaine intake over the last 6 sessions for each group (Mean ± SEM). The * indicated a significant effect of hormone treatment. The # indicated a significant effect of drug pretreatment.

Total inactive nose pokes of each week

To make sure that the treatment did not affect general activity, nose pokes in the inactive hole were analyzed (Figure 3.6). There was no significant difference in inactive nose pokes among the four groups of rats during the first three weeks. EB treatment, however, significantly enhance inactive poking in the last week ($F(1, 46) = 6.8$, $p = 0.012$), when the high dose of cocaine was self-administered by the rats.

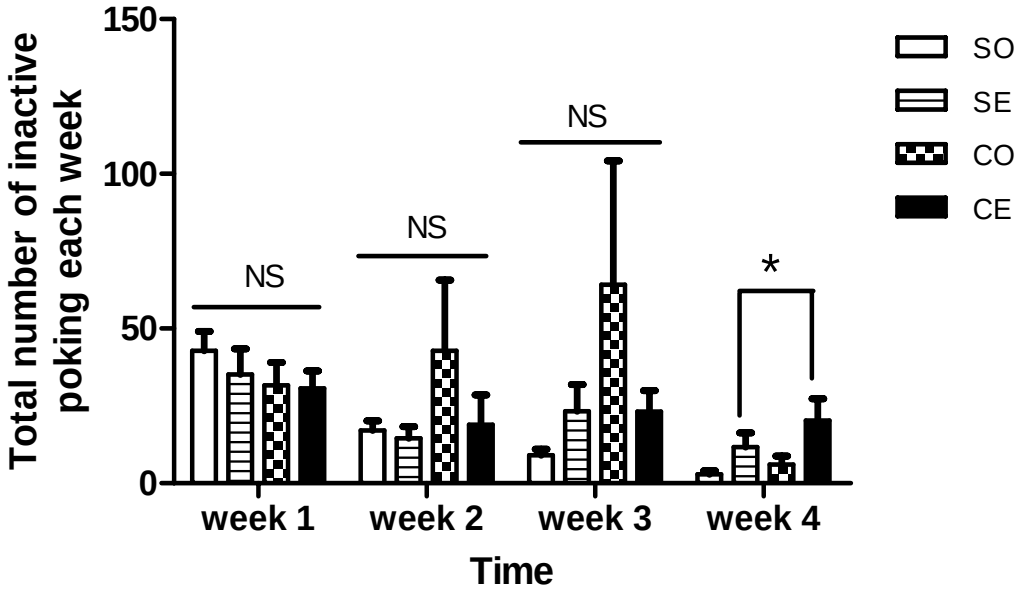


Figure 3.6: Total inactive poking of each week for each group of rats (Mean ± SEM). The * indicates EB treatment immediately before cocaine self-administration resulted in more total inactive poking in week 4 when the dose was 0.4 mg/kg/inf.

Cocaine-induced locomotor activity on challenge day

The CE group was compared with cocaine naïve rats for analysis of AMPH-induced DA (DA) release using in vitro superfusion. EC rats were injected with 5 mg/kg cocaine 3 to 4 days prior to the superfusion experiment, to determine if they were still sensitized to cocaine. Locomotor activity induced by 5 mg/kg cocaine upon first injection and challenge injection of cocaine in EC group is illustrated in Figure 3.7. Behavioral sensitization to cocaine was seen 10 days after cocaine self-administration (3 to 4 days before the superfusion experiment) ($t=2.174$, $p=0.047$).

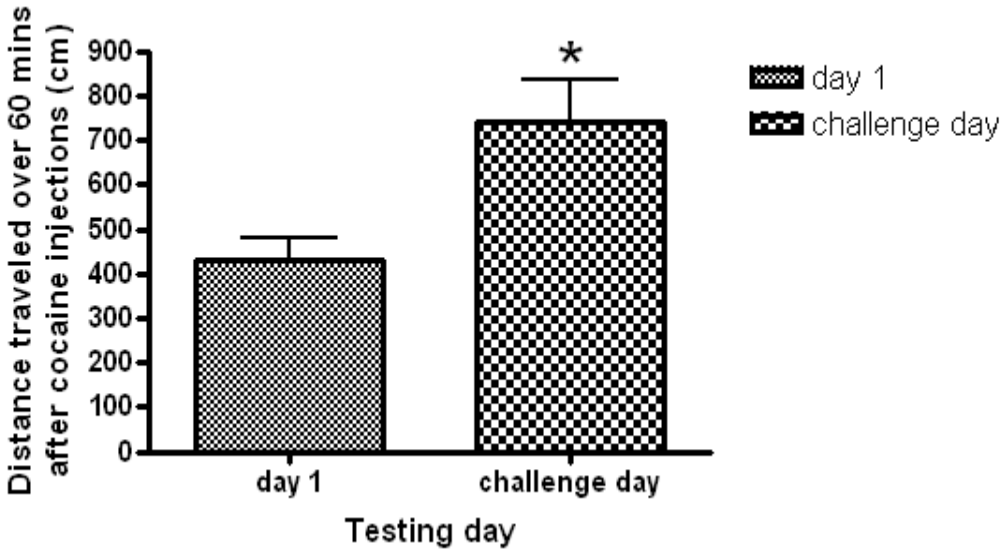


Figure 3.7: Distance traveled (Mean ± SEM) by the cocaine rats over 60 mins after 5 mg/kg cocaine injections on day 1 and challenge day. The * indicated that cocaine rats exhibited greater locomotor activity on challenge day than on day 1.

AMPH-induced DA efflux in the dorsal striatum

The DA efflux (pg/mg) induced by AMPH from the dorsal and ventral striatum tissue is shown in Figure 3.8 (the average of two samples collected immediately before AMPH application is used as basal DA for each rat). AMPH significantly induced DA efflux at 5, 10 and 15 mins in both dorsal and ventral striatum from cocaine rats and cocaine-naïve rats (all bars are significantly different from 0 at p=0.05). Furthermore, ANOVA analyses indicated cocaine experience interacted with the pattern of DA release in both dorsal striatum ($F(1,11)=7.077$, $p=0.022$) and ventral striatum ($F(1,12)=5.480$, $p=0.037$). Student' t-tests were used to compare DA efflux at each time point between groups. There was greater striatal DA release 10 mins post-AMPH in dorsal striatal tissue ($p=0.05$) and 5 mins post-AMPH in ventral striatal tissue ($p=0.035$) from rats that had previously received cocaine compared to cocaine naïve rats.

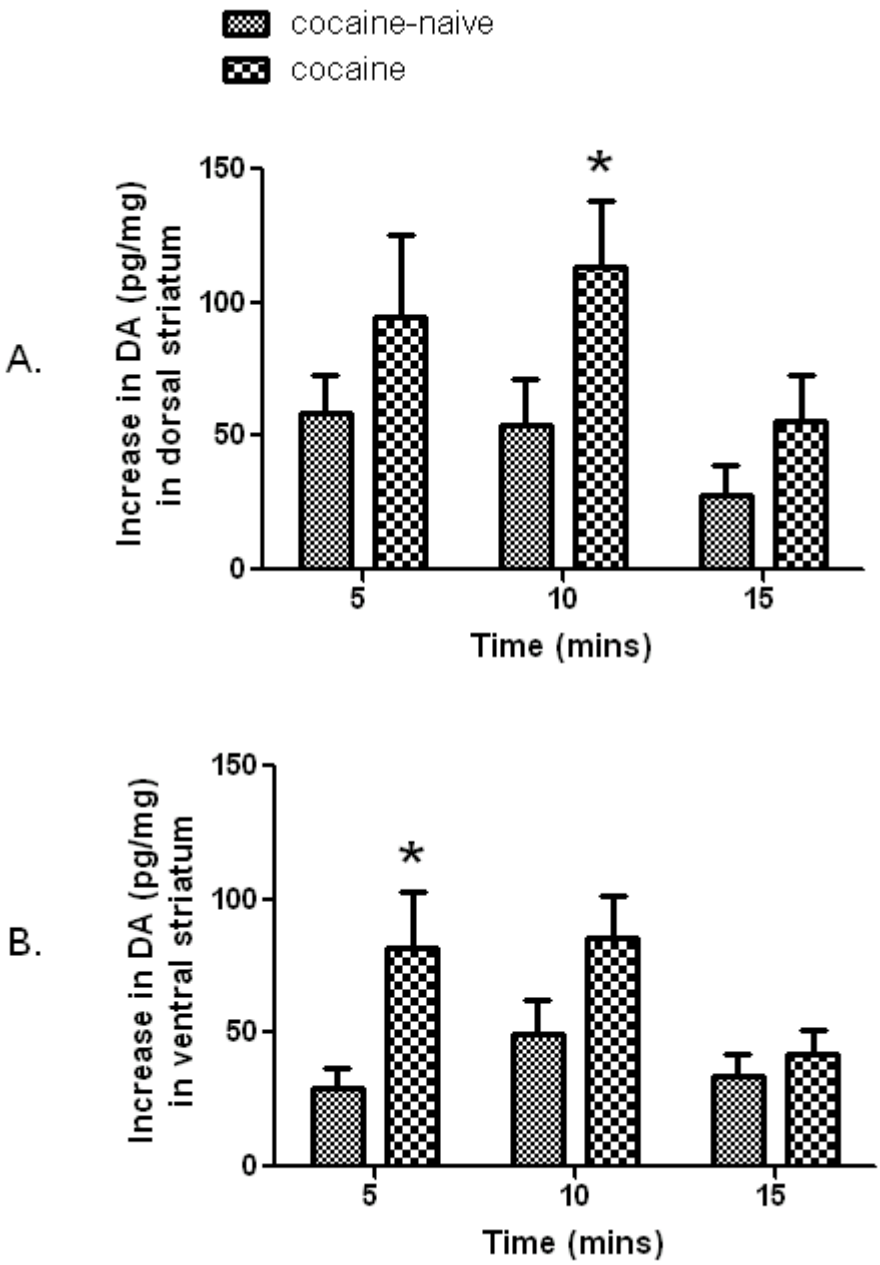


Figure 3.8: Increase in DA (Mean ± SEM) after AMPH application to the dorsal (A) and ventral (B) striatum . AMPH induced significant DA efflux (significant different from 0) in both dorsal and ventral striatum from cocaine-naïve rats and cocaine rats. The * indicated greater DA increase in striatal tissue from cocaine rats than did cocaine-naïve rats.

4. Discussion

In the present study, we show that prior exposure to cocaine significantly promoted the acquisition of cocaine self-administration and cocaine intake in female rats. While there were some effects of estradiol to enhance cocaine intake, the effect of prior cocaine sensitization was much more powerful than the hormone effect. Importantly, we confirmed that sensitization of AMPH-induced DA release in vitro was seen in dorsal and ventral striatum from animals after acquisition of cocaine-taking behavior.

The present finding is consistent with previous reports found that preexposure to psychomotor stimulants enhanced self-administration of cocaine or AMPH in male rats (Ferrario and Robinson 2007; Horger et al. 1990; Piazza et al. 1989; Pierre and Vezina 1997; Schenk and Partridge 2000; Suto et al. 2002; Vezina 2004; Vezina et al. 2002). In male rats, it has been suggested that both cocaine sensitization and enhanced cocaine self-administration is a consequence of similar neuroadaptations (Vezina 2004). Either behavior can be initiated or promoted by AMPH pretreatment in VTA (Hooks et al. 1992; Kalivas and Weber 1988; Suto et al. 2002; Suto et al. 2003; Vezina et al. 2002) and are both related to an enhancement of DA transmission in DA terminal area (Akimoto et al. 1989; Cadoni et al. 2000; Hooks et al. 1994; Kalivas and Duffy 1993; Paulson and Robinson 1995; Pierce and Kalivas 1995; Wolf et al. 1993).

To be specific, in vitro studies have shown that AMPH application to striatal and accumbal slices from rats pretreated with AMPH stimulate greater DA release than did controls (Castaneda et al. 1988; Kantor et al. 1999; Kolta et al. 1989; Robinson and Becker 1982). Enhanced DA transmission in striatum and NAc after AMPH sensitization has been demonstrated in vivo as well (Cadoni et al. 2000; Paulson and Robinson 1995;

Wolf et al. 1993). Similar to AMPH, prior cocaine treatment enhances AMPH-stimulated DA release in striatal tissue (Peris et al. 1990; Peris et al. 1991; Peris and Zahniser 1989). An enhancement of psychostimulant-induced increase in DA transmission in NAc as a result of cocaine sensitization, however, has only been demonstrated by in vivo micordialysis studies (Cadoni et al. 2000; Kalivas and Duffy 1993; Paulson and Robinson 1995; Pierce and Kalivas 1995). This study not only confirmed there is an enhanced DA efflux in striatum in female rats after prior cocaine experience, but also demonstrated that cocaine experience leads to similar enhancement in AMPH-induced DA release in NAc in vitro. Taken together, it suggests that the same neural adaptations exist in females as well and may account for observed behavior in this experiment.

Even though NAc and striatum share very similar neural characteristics, they are mediating different motor components (locomotion vs. stereotypy) activated by psychostimulants (Amalric and Koob 1993; Castall et al. 1977; Costall et al. 1977). Moreover, NAc, rather than striatum, is found to be the key neural substrate mediating the reinforcing effect of psychostimulants. Compared to that, a role for dorsal striatum in reinstatement of self-administration was suggested recently (Fuchs et al. 2006; See et al. 2007; Vanderschuren et al. 2005). In particular, extracellular DA was increased in the dorsal striatum rather than the NAc during responding for cocaine-associated cues after chronic cocaine self-administration in animals or in cocaine-dependent subjects (Ito et al. 2000; Ito et al. 2002; Volkow et al. 2006; Wong et al. 2006). Thus, even though reactivity of both NAc and dorsal striatum DA neuron is enhanced by cocaine experience, they may serve different functions.

An effect of estradiol at promoting the acquisition of cocaine self-administration and

cocaine intake has been previously established with doses between 0.3 to 0.5 mg/kg (Hu and Becker 2008; Hu et al. 2004; Jackson et al. 2006). No effect of estradiol on the acquisition of cocaine self-administration was seen in this study. It suggests prior cocaine experience is much more powerful than estradiol at modulating the acquisition of cocaine self-administration.

Consistent with previous findings, estradiol enhanced cocaine intake during the last six test sessions with cocaine doses from 0.15 to 0.4 mg/kg. There was also an effect of estradiol on nose pokes in the inactive hole during the last week of self-administration with 0.4 mg/kg cocaine. Enhanced nose pokes in estradiol-treated rats may be due to enhanced motivation to take drugs or enhanced locomotor activation or both. One thing that should be kept in mind is that the animals were mostly at the acquisition stages of cocaine self-administration. It is known that sensitization powerfully modulates the acquisition of drug self-administration at sub-threshold doses but has no effect on acquisition of self-administration or drug intake at high doses (Lorrain et al. 2000). It is possible that an effect of estradiol may have emerged as playing a more important role on intake after acquisition, if self-administration had been extended for a longer time.

Even though drug-pretreated and drug-naïve animals are not distinguishable when they self-administer high dose of drug with little work demand, they do differ in the motivation to take high dose of drugs when the work demands are increased in progressive ratio schedule. Animals that have been sensitized work harder to obtain drugs than do drug naïve rats (Lorrain et al. 2000; Mendrek et al. 1998). Estradiol is also known to enhance females' motivation to take cocaine as exhibited by higher breaking point on a progressive ratio schedule (Becker and Hu 2008). Thus, it remains to be determined

whether estradiol and cocaine sensitization interact to influence female rats' motivation to work for high dose of cocaine on a progressive ratio schedule.

In conclusion, the above study strongly suggests that cocaine sensitization made female rats more vulnerable to the effect of cocaine as it promoted the acquisition of cocaine self-administration and cocaine intake, as has been seen in male rats. Furthermore, prior cocaine experience is much more powerful than estradiol at enhancing at acquisition of cocaine self-administration. Associated with cocaine experience, there is enhanced AMPH-induced DA release in both ventral and dorsal striatum in vitro. This study adds important evidence to the current literature suggesting similar mechanisms and relationship between cocaine sensitization and cocaine self-administration exists in females as well. Understanding the mechanism underlying these behavioral changes is important for us to understand cocaine abuse in women.

CHAPTER IV

PRIOR COCAINE TREATMENT PRODUCES CROSS-SENSITIZATION TO AMPHETAMINE IN SEXUALLY DIMORPHIC PATTERN

1. Introduction

Psychomotor stimulant drugs such as amphetamine (AMPH) and cocaine are known to produce acute psychomotor activating effect by activating nigrostriatal and mesolimbic dopamine (DA) neurotransmission (Hurd and Ungerstedt 1989). When these drugs are repeatedly administered, the ability to produce a psychomotor activating effect is usually enhanced so that a later challenge administration of those drugs could result in a greater psychomotor activating effect, evidenced by a greater psychomotor stimulants-induced locomotor activity or stereotype compared to the behavioral activation after first injection (Robinson and Becker 1986a; Robinson and Berridge 1993). Accompanying this behavioral sensitization, an increase of DA overflow in the nucleus accumbens (NAc) and striatum has been observed in sensitized animals both in vitro and in vivo (Kalivas and Stewart 1991; Kantor et al. 1999; Pierce and Kalivas 1997; Robinson et al. 1982; Robinson and Berridge 1993; Vanderschuren et al. 1999a) and is suggested as an important mechanism underlying addiction.

The above notion is largely based on experimental evidence conducted on male rodents. Cocaine and AMPH sensitization, however, is also modulated by sex and ovarian

hormones (Chin et al. 2002; Hu and Becker 2003; Robinson and Becker 1986b; Sircar and Kim 1999; Yang et al. 2007). Specifically, OVX female rats show greater behavioral sensitization to cocaine than CAST male rats, and estradiol further enhance behavioral sensitization in OVX female rats (Hu and Becker 2003).

Related to sex differences in psychostimulant-related responses, sex differences in mesostriatal dopamine (DA) function have been reported (Becker 1999). Both basal DA levels in vivo and AMPH-stimulated DA release from striatal tissue are higher in CAST males compared to OVX females (Becker and Ramirez 1981b; Castner et al. 1993). Moreover, basal striatal DA levels fluctuats over the estrous cycle and acute estradiol enhances AMPH-induced DA release in OVX female rats (Becker and Rudick 1999; Castner et al. 1993; Xiao and Becker 1994). These findings suggest that presynaptic DA function in striatum is sexually dimorphic and modulated by estradiol treatment.

In terms of sensitization-related neuroadaptations, as in male rats, prior AMPH injection or cocaine treatment enhances AMPH-stimulated striatal DA release in females in vitro (Peris et al. 1991; Robinson et al. 1982). But no direct comparison between males and females has yet been made. Furthermore, in female rats, enhanced AMPH-induced DA release from striatal tissue after sensitization is more pronounced in cocaine-sensitized rats chronically treated with estradiol compared to OVX rats (Peris et al. 1991). Until now, no study has examined the effect of cocaine sensitization on reactivity of striatal DA neuron in vivo in female rats. In addition, previous work has shown long term (12 days) changes in behavioral activation when animals were treated with estradiol during cocaine sensitization (Hu and Becker 2003). It is not known if it is related to a long term effect of estradiol on neuroadaptations in DA system.

This study was designed to compare the AMPH-induced increase of DA in dialysate in the striatum after cocaine sensitization among CAST male, OVX female and OVX female rats treated with estradiol, to test if there are long term changes in the nigrostriatal DA system due to cocaine sensitization in both male and female rats. We also investigated whether this change is sexually dimorphic or modulated by estradiol. It was hypothesized that there will be enhanced AMPH-induced DA release in striatum after cocaine sensitization and this neuroadaptation would differ between male and female rats, and be enhanced in OVX rats that received estradiol during sensitization.

2. Materials and Methods

Animals

Male and female adult Sprague-Dawley rats (Harlan, Indianapolis, IN), weighting 225-250 g at the start of the experiment, were housed 3-4 per cage when they arrived. The room has a 10:14 h regular dark: light cycle (lights on at 5:30 am and off at 7:30 pm). The animals were housed in a room maintained at a constant temperature of 20-21 °C, with free access to phytoestrogen-free rodent chow (2014 Tekad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI) and water available ad libitum. All procedures were performed according to a protocol approved by the University of Michigan Committee for Use and Care of Animals.

Gonadectomy

One week after the rats arrived, they all went through gonadectomy surgery under

isoflurane anesthesia. All female rats received bilateral ovariectomization (OVX) surgery. During the surgery, an incision around 1cm long was made on the back skin along the midline just below the ribs. The muscle immediately under the skin was opened (0.5cm long and 1.5cm lateral to the midline) and the ovary on one side was then externalized with blunt forcepts. The tissue between the ovary and uterus was clamped with a hemostat when the ovary was removed. The hemostat remained in place for another 30 seconds until there was no bleeding, at which time the tissue was released and returned to the abdomen. The same procedure was repeated on the other side. The wound was closed with 11mm wound clips. All male rats received castration (CAST) surgery. The scrotal sac is opened and the testis is externalized. The blood supply to the testis is closed with suture material, and the testis is removed. The wound is then closed with wound clips. All female animals underwent vaginal lavage testing daily for 10 consecutive days to confirm the cessation of cycling.

Cocaine Sensitization

Two weeks after gonadectomy surgeries, the rats were assigned to one of six groups: (1) castrated male rats treated with saline and peanut oil (n=4); (2) castrated male rats treated with cocaine and oil (n=4); (3) OVX female rats treated with saline and oil (n=6); (4) OVX female rats treated with cocaine and oil (n=5);(5) OVX female rats treated with saline and 5 μg estradiol benzoate (EB) dissolved in peanut oil (n=7); (6) OVX female rats treated with cocaine and EB (n=8).

The testing chamber is a square plastic cage (8 inch x 17 inch x 7 inch). Animals were injected with either EB or 0.1 ml peanut oil subcutaneously (s.c.), according to their

group treatment, and were placed in the testing chamber. After a 30 min habituation period, each rats received an intraperitoneally (i.p.) injection of either saline or 20 mg/kg cocaine and was then put back to the testing chamber for one hour. Animals were tested for five days per week with two days off for three consecutive weeks, for a total of 15 testing days. No cocaine or saline treatments or hormone was administered on the 2 days off. Intermittent treatment was used here because it was better at producing behavioral sensitization than continuous treatment (Post 1980; Robinson and Becker 1986b). On the first and last test day, the testing was videotaped for behavioral analysis. Behaviors were scored by observers blind to the experiment. The numbers of headbobs, forelimb movements, rearings, and quadrant crossings were counted for 10 seconds every five minutes.

Cannula implantation

Immediately after the last cocaine or saline treatment and testing, rats were anesthetized with a combination of ketamine (45mg/kg, i.p.) and medetomidine (0.3mg/kg, i.p.). A guide cannulus was implanted through the skull and aimed at the dorsolateral striatum (from Bregma in mm: anterior, 0.2; lateral, 3.2; ventral, 1) using stereotaxic procedures. Dental acrylic and jeweler's screws were used to hold the cannulus on each skull.

Microdialysis testing

Microdialysis testings were conducted 9 to 14 days after the last injection of cocaine or saline. Microdialysis probes (CMA/11, CMA/Microdialysis AB, Chelmsford, MA,

USA) were tested for recovery in vitro at 37°C prior to the experiment, as described by Becker (Becker and Rudick 1999). On the day prior to dialysis, microdialysis probes (4 mm dialysis membrane) were inserted into the dorsolateral striatum under methoxyflurane anesthesia. A Ringer's solution (145 mM $NaCl_2$, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.25 mM ascorbic acid; pH = 7.3) was perfused through the probe overnight at a rate of 0.3 µl/min. On the day of testing, the Ringer's was pumped through the probes at the flow rate of 1.5µl/min using a Harvard Apparatus pump (Holliston, MA). Samples were collected at 10-minute intervals. Three baseline samples were collected before the administration of 0.1 ml oil vehicle (s. c.) followed by an additional three samples. Then a challenge dose of AMPH (i.p.) was administered (2.5 mg/kg for male rats and 2.0 mg/kg for female rats). Different doses of AMPH were used to overcome gender differences in AMPH metabolism. Dialysate samples continued to be collected up to 1.5 hour. The concentration of DA in dialysate was determined using high performance liquid chromatography and electrochemical detection as described previously (Becker and Rudick 1999). The rats' behavior was videotaped, and the numbers of headbobs, forelimb movements, rearings, and quadrant crossings were counted for 10 seconds every five minutes.

At the end of microdialysis testing, animals were anaesthetized with sodium pentobarbital and perfused with 0.9% saline followed by 4% paraformaldehyde. The brains were removed and the brain sections were stained with cresyl violet. The placement of the probes was examined under the microscopy and any animals with probes outside of the dorsolateral striatum were excluded from subsequent analyses.

Statistics

Data were subjected to analysis of variance (ANOVA) and Student's t test. Differences were considered significant if $p \leq 0.05$. All data were analyzed using SPSS 11.5 for PC.

3. Results

Cocaine-induced locomotor and stereotyped behavior during sensitization

For saline-pretreated rats, saline-induced headbobs, forelimb movements, rearing, and quadrant crossing upon either the 1st or 15th injection (last) did not differ among CAST males, OVX females, OVXE females. Saline-induced behavior upon 1st and 15th injection of saline were compared and no group show behavioral habituation or sensitization (data not shown).

For cocaine-pretreated rats, one-way ANOVA test indicated that CAST male, OVX female, OVXE female did not show any differences in headbobs ($F(2,12)=1.304$, $p=0.307$), forelimb movements ($F(2,12)=0.626$, $p=0.551$), rearings ($F(2,12)=3.075$, $p=0.084$), or quadrant crossings ($F(2,12)=0.951$, $p=0.132$), upon the 1st injection of cocaine (Figure 4.1-A).

Cocaine-induced behavior upon the l5th injection of cocaine minus the 1st injection of cocaine was calculated to assess sensitization or habituation due to repeated cocaine injections (Figure 4.1-B). One sample t-test showed that only the OVXE female group exhibited sensitized stereotypy as total number of headbobs and forelimbs movements were significantly different from 0 ($t(6)=2.640$, $p=0.039$). OVX females and CAST

males did not show sensitization or habituation. When CAST males, OVX females and OVXE females were compared by one-way ANOVA, there was a significant effect of sex (OVX females and OVXE females didn't differ in any behavior) as male and females differed significantly in sensitized headbobs ($F(1,13)=8.638$, $p=0.012$) and forelimb movements ($F(1, 13)= 4.588$, $p=0.05$). LSD post hoc analysis indicates significant difference between CAST male and OVXE female (headbobs $p=0.010$, forelimb movements $p=0.035$).

AMPH-induced behavior on challenge day (day of microdialysis)

Total number of headbobs, forelimb movement, rearing and quadrant over 30 mins after AMPH injections (2.5 mg/kg for male and 2.0 mg/kg for female) for saline- or cocaine-pretreated CAST males, OVX females, OVXE females on challenge day, during microdialysis, are shown in Figure 4.2.

One-way ANOVA indicated that saline-pretreated CAST males, OVX females and OVXE females did not differ in their behavioral response to acute AMPH injection on the challenge day. The cocaine-pretreated three groups, however, exhibited different amount of headbobs ($F (2, 14) = 4.917$, $p=0.024$) and rearings ($F(2,14)=6.197$, $p=0.012$) in response to AMPH. LSD post hoc analysis indicated that OVXE female rats exhibited fewer headbobs than both CAST males ($p=0.05$) and OVX females ($p=0.012$), but more rearing than CAST males ($p=0.007$).

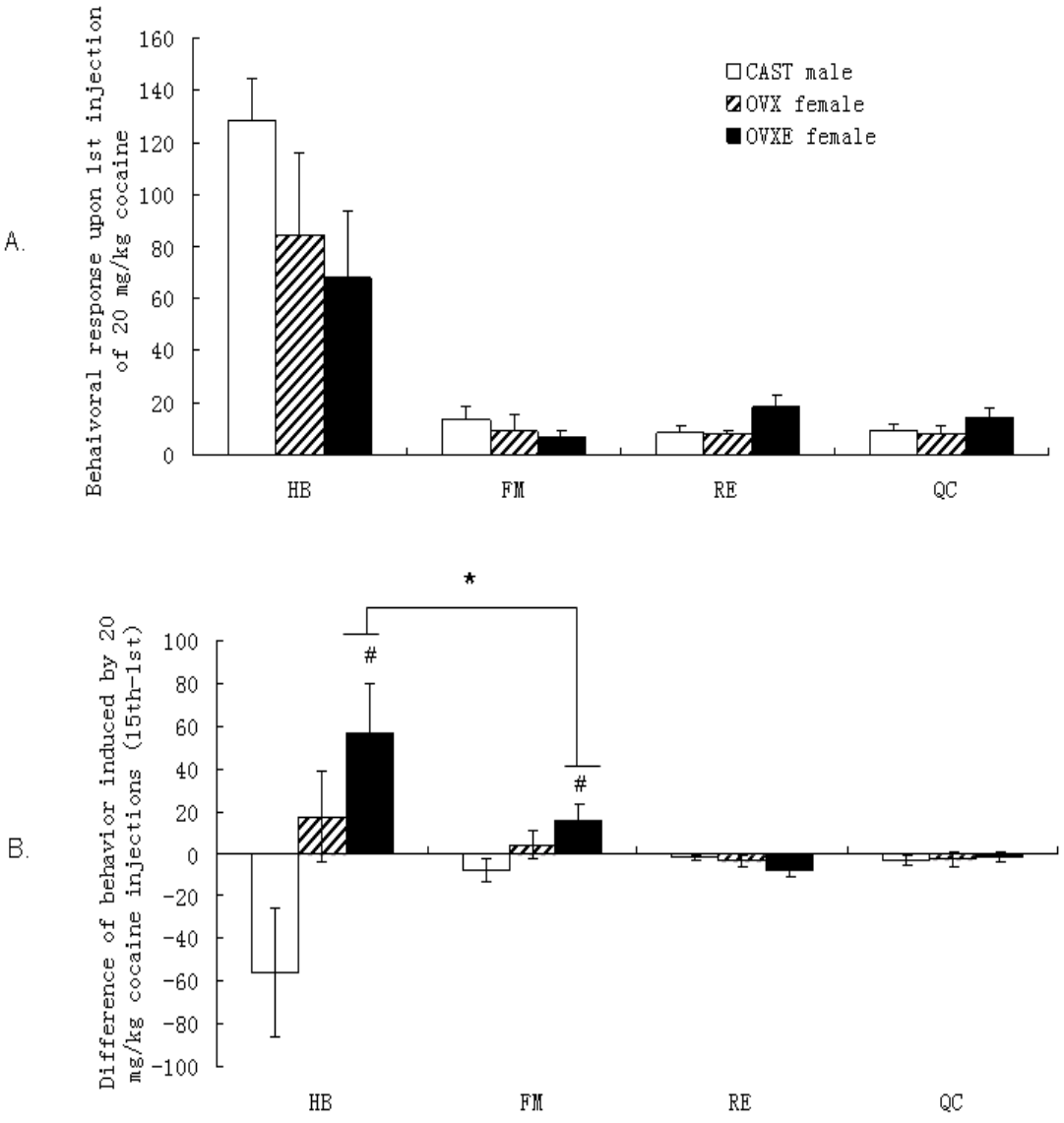
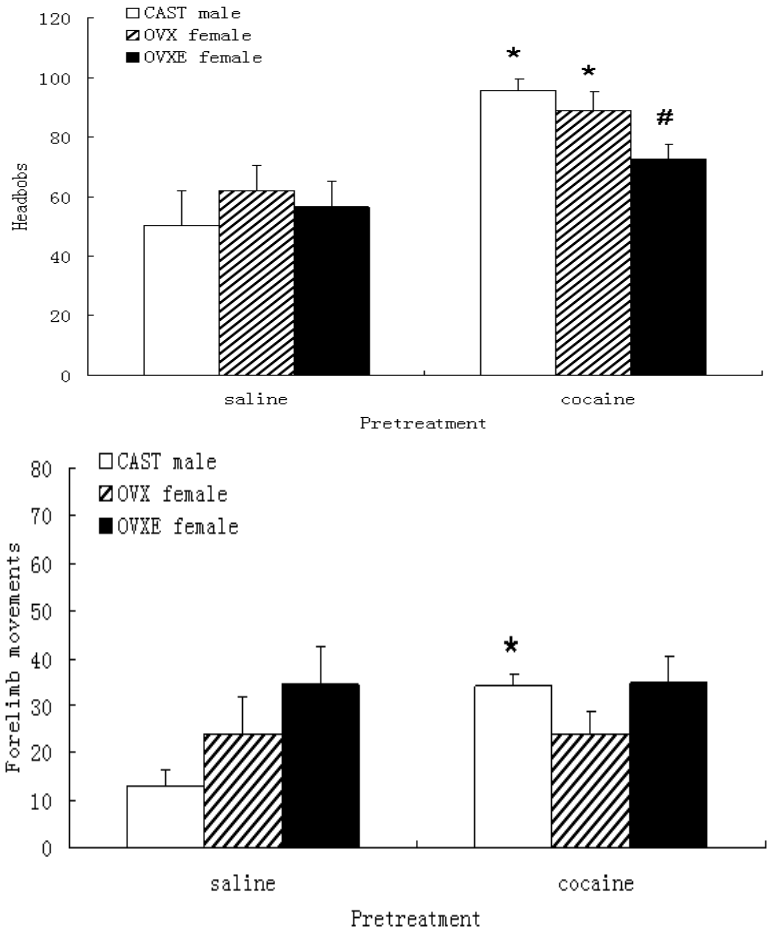


Figure 4.1: Behavior (Mean ± SEM) induced by 20 mg/kg cocaine with repeated treatment of cocaine. A. CAST male, OVX female, OVXE female did not differ on cocaine-induced headbobs, forelimb movements, rearings and quadrant crossings upon the 1st injection of cocaine. B. Cocaine-induced behavior upon the l5th injection of cocaine minus the 1st injection of cocaine was calculated to assess sensitization or habituation due to repeated cocaine injections. The * indicates changes of stereotypy (total of headbobs and forelimb movements) in OVXE group was significantly greater than 0. The # indicates significant difference between OVXE females and CAST males. HB: headbobs; FM: forelimb movements; RE: rearing; QC: quadrant crossing.

When the behavior of cocaine and saline treated groups are compared by Students' t

tests, the cocaine-pretreated CAST male rats exhibited greater stereotypy as indicated by more headbobs (p=0.011) and forelimb movements (p=0.003) in response to AMPH compared to saline-pretreated rats. Cocaine-pretreated OVX female rats also showed greater stereotypy than did controls as indicated by more headbobs (p=0.038) in response to AMPH. In addition, they exhibited less rearings (p=0.037) compared to saline-pretreated rats. Interestingly, cocaine-pretreated OVXE rats were not different from saline-pretreated rats on challenge day.



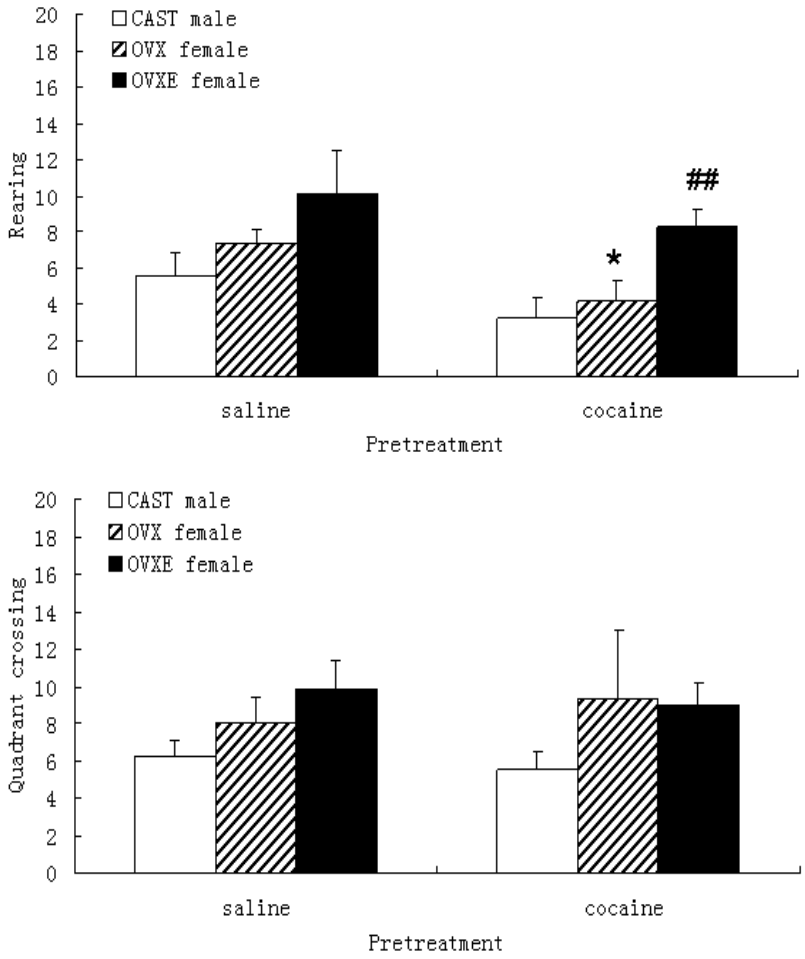


Figure 4.2: Behavior induced by AMPH injections on challenge day. The * indicated significant difference compared to paired saline rats. The # indicated different amount of headbobs compared to both CAST males and OVX females. The ## indicated significant different amount of rearings compared to CAST males.

AMPH-induced changes of extracellular DA in dorsal striatum

Two-way ANOVA indicated that CAST male, OVX female and OVXE female had different basal extracellular DA levels ($F(2, 28) = 6.73$, $p=0.004$) as CAST male rats had higher basal DA than both OVX females ($p=0.027$) and OVXE females ($p=0.001$) (Table 4.1).

Table 4.2: Basal extracellular DA in the dorsal striatum (pg/μl)

	CAST males	OVX females	OVXE female
Saline-pretreated	3.2 (0.453) *	2.09 (0.37)	1.40 (0.343)
Cocaine-pretreated	3.03 (0.453) *	2.17 (0.406)	1.91 (0.321)

The data was expressed as Mean (SEM). * indicated different basal DA between Males and Females.

There was no effect of cocaine pretreatment on basal DA, thus DA in striatum is expressed as the percent of baseline for each rat. AMPH-induced DA in dialysate from cocaine- or saline-pretreated CAST males, OVX female and OVXE females are shown in Figure 4.3. Repeated ANOVA was used to compare the six samples after AMPH injections between paired cocaine- and saline-pretreated groups. It indicated that cocaine-pretreated CAST males had significantly greater DA increase in dialysate than did saline-pretreated controls ($F(1, 6) = 7.082$, $p = 0.037$). Cocaine-pretreated OVX females and OVXE females, however, did not show enhanced DA in dialysate compare to equivalent saline groups. (OVX females: $F(1, 9) = 1.507$, $p = 0.251$. OVXE females: $F(1, 13) = 0.384$, $p = 0.546$).

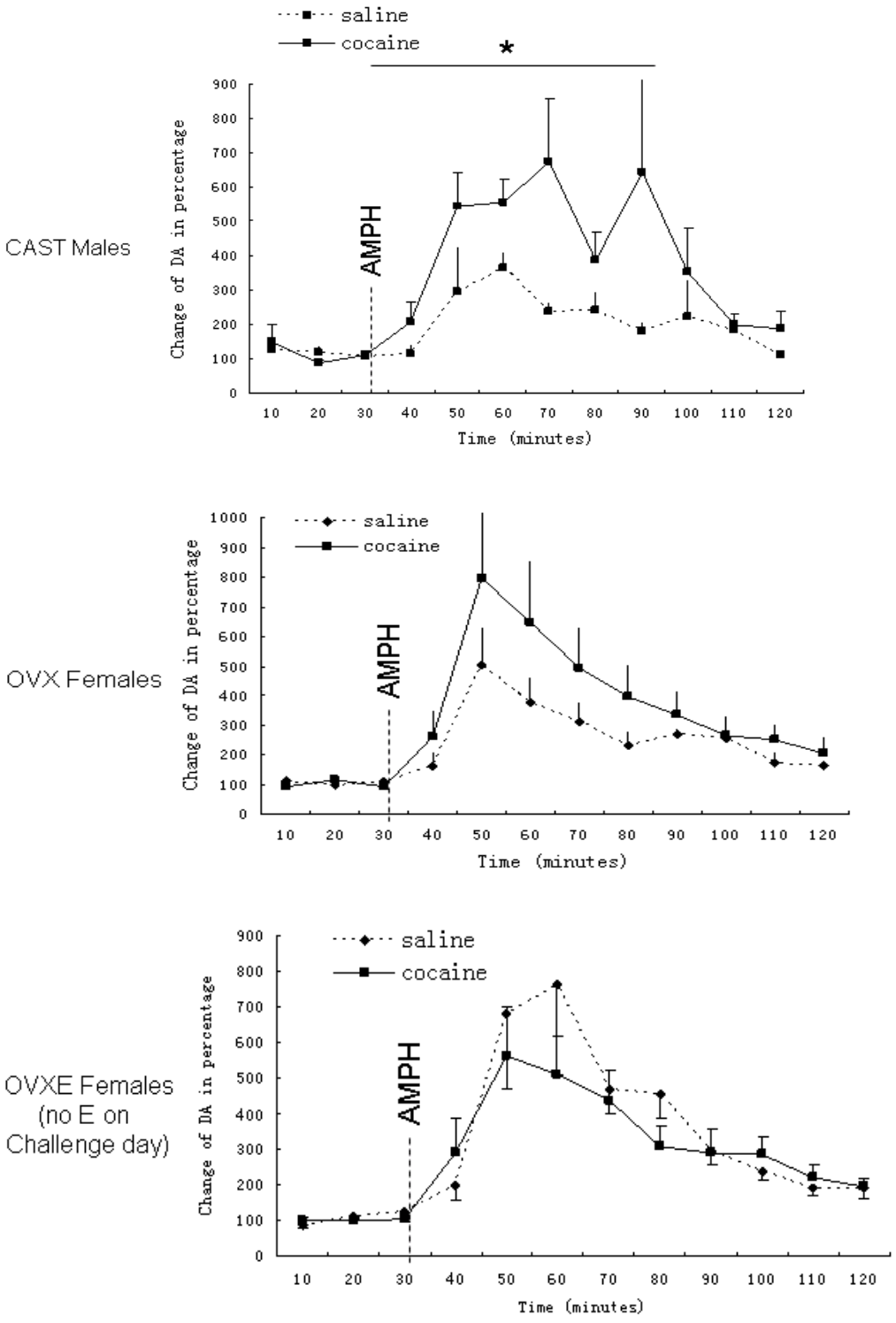


Figure 4.3: AMPH-induced DA in dialysate from cocaine- and saline- pretreated CAST males, OVX females and OVXE females. The * indicated significant difference between cocaine- and saline- pretreated CAST males.

Relation of AMPH-induced DA peak with behavior

For male rats, the AMPH-induced DA peak correlated with the number of AMPH-induced forelimb movements as shown in Figure 4.4. This relationship was not seen in female rats.

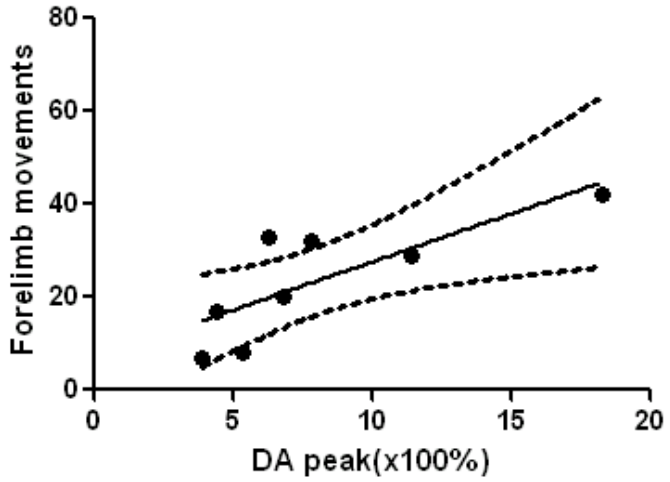


Figure 4.4: For male rats (including cocaine- and saline-pretreated animals), Pearson correlation test indicates that AMPH-induced DA peak significantly correlated with AMPH-induced forelimb movements on challenge day r^2 (8) =0.613, p=0.021

4. Discussion

In this experiment, repeated cocaine treatment resulted in sexually dimorphic patterns of both the behavioral response and the AMPH-induced increase in DA in dialysate from striatum. Specifically, estradiol-treated OVX female rats exhibited greater behavioral sensitization to cocaine during the sensitization period, than did CAST male rats as was predicted. On challenge day, however, repeated cocaine resulted in an enhanced behavioral response to AMPH, and enhanced AMPH-induced DA in dialysate only in CAST male rats. In females, repeated cocaine resulted in very limited behavioral

cross-sensitization to AMPH, and no enhanced AMPH-induced DA in dialysate.

It has been known that OVX female rats show greater behavioral sensitization compared to CAST male rats and this sensitization is further enhanced by estradiol treatment in female rats (Becker et al. 2001; Hu and Becker 2003). We saw a similar pattern in this study. Female rats, especially EB-treated female rats, showed greater sensitization of stereotypy (headbobs and forelimb movements) compared to CAST male rats. More specifically, the estradiol-treated OVX group is the only group that exhibited sensitized stereotypy in this study, when the behavioral sensitization was assessed by difference between behavior induced by the 1^{st} and the 15^{th} cocaine injections. CAST male rats even show a trend of decreased stereotypy, though not at a significant level. Rats exhibit big individual differences in susceptibility to cocaine sensitization (Flagel et al. 2008). Moreover, the sample size is relatively small in this study. It is possible that we had a population of rats that had greater resistence to sensitization so that sensitization was not detected as a whole. Moreover, cocaine-induced behavior was videotaped and was counted by an observer in this study. It is also possible that the headbobs and forelimb movements counting were not the most sensitive measurement to detect changes of stereotypy at the dose of 20 mg/kg cocaine. Nonetheless, females exhibited greater behavioral sensitization to cocaine than males in this study as predicted.

Surprisingly, we found the opposite pattern on AMPH-induced behavioral responses on challenge day. Cocaine-pretreated CAST males showed cross sensitization to AMPH as they exhibited twice the number of headbobs and three times the number of forelimb movements exhibited by saline-pretreated rats. Compared to male rats, OVX female rats showed very limited cross sensitization to AMPH as they only exhibited 30% more

headbobs compared to saline-paired group. Lastly, OVXE rats did not show any behavioral cross sensitization to AMPH even though they showed the most robust sensitization to cocaine during repeated cocaine treatment.

Cross-sensitization between AMPH and cocaine has been demonstrated in several paradigms. Repeated systemic AMPH administrations are known to produce sensitized locomotor activation to cocaine (Bonate et al. 1997; Schenk et al. 1991; Shuster et al. 1977), and a sensitized reinforcing effect to cocaine as assessed by cocaine self-administration (Horger et al. 1992; Valadez and Schenk 1994). Direct AMPH exposure in VTA also enhances cocaine self-administration under progressive ratio procedures (Suto et al. 2002; Suto et al. 2003). The above studies, however, have all focused on the ability of repeated AMPH to produce cross-sensitization with the effects of cocaine. There are very few studies addressing the ability of repeated cocaine to produce cross-sensitization with the effects of AMPH. A very early study has demonstrated that cocaine does not necessarily produce cross- sensitization to AMPH when vice versa is true (Shuster et al. 1977). Similarly, cross-sensitization to AMPH produced by morphine did not guarantee that AMPH could produce cross-sensitization to morphine either (Vanderschuren et al. 1999c). Thus, we need to be very careful when we consider the ability of cocaine to produce cross-sensitization to AMPH.

Our observation is consistent with a previous study showing that cocaine-pretreated male rats show cross-sensitization to AMPH (Pierce and Kalivas 1995). Cocaine exposure has also been demonstrated to produce sensitized rewarding effect of AMPH (Liu et al. 2007). Even though cross-sensitization has not been examined in females in vivo, in vitro superfusion experiments suggest cocaine sensitization results in enhanced

striatal DA neuron reactivity to AMPH in both males and females (Peris et al. 1990; Peris et al. 1991). Thus, it is not likely that female rats did not develop cross sensitization to AMPH at all.

It is known that drug administration environment powerfully modulates both the development and expression of behavioral sensitization (Badiani and Robinson 2004; Robinson et al. 1998). When a drug is repeatedly administered to rats in one environment, robust sensitized response is produced when the drug is subsequently administered in the same drug-paired environment. But sensitization may not be expressed if the drug is administered in a non-paired environment (Anagnostaras and Robinson 1996; Mattson et al. 2008). Cross-sensitization between AMPH and cocaine has been shown to be context-dependent as well (Bonate et al. 1997). In this study, all animals were challenged by AMPH in microdialysis chamber, which was a novel environment for them. The observation that female rats, but not male rats, failed to express cross-sensitization to AMPH in novel environment may suggest greater vulnerability to the influence of context in females. But van Haaren's observation, that intact female rats develop sensitization to AMPH in home cages while males fail to do so, suggest that females may be more insensitive to the environment (van Haaren and Meyer 1991). This difference between males and females in van Harren's study, however, may also due to different metabolism rate of AMPH as the effect of context on sensitization also depends on drug doses and time of context exposure (Todtenkopf and Carlezon 2006). I am not aware of any other studies looking at the effect of context on the expression of cocaine sensitization in female rats. Thus this hypothesis still remains open for further investigation. Nonetheless, if the above hypothesis is correct, it may contribute to clinical

observation that better treatment outcome is observed in cocaine dependent women compared to cocaine-dependent men (Kosten 1993; Weiss et al. 1997).

Another possibility is the sex differences in the stress system. In males a novel environment activates the stress axis and enhances sensitization and drug taking (Piazza and Le Moal 1998; Sorg and Kalivas 1991). The effect of stress on drug related response is suggested to be mediated by circulating glucocorticoids. Evidence suggests that an optimal level of glucocorticoids is necessary for favoring drug taking. If the levels of circulating glucocorticoids exceed this optimal range, it may decrease drug taking (Goeders and Guerin 1996; Kabbaj et al. 2001). It is known that there are greater stress responses, that include greater hypothalamic-pituitary-adrenal (HPA) axis activation and higher glucocorticoids level following stress, in females than males (Gozen et al. 2007; Mitsushima et al. 2003; Wilson and Biscardi 1994). It is possible that greater stress response induced by the novel environment in females has already exceeded the optimal stress ranges, thus compromised or inhibited behavioral response to drug. This would explain why the female rats sensitized in the home cage and then didn't show sensitization when challenged in a novel testing chamber.

Consistent with previous reports (Becker and Ramirez 1981b; Castner et al. 1993; Xiao and Becker 1994), nigrostriatal DA system is sexually dimorphic as males had higher extracellular DA than females. Similar to different behavioral response to AMPH, we saw different neurochemical response to AMPH as well. In male rats, cocaine-pretreated rats show enhanced AMPH-induced release of DA in striatum. And more importantly, the DA peak in male rats is correlated with forelimb movements in male rats, which is consistent with the notion that stereotypy is mediated by the dorsal striatum

(Castall et al. 1977). Similar relationship of AMPH-induced stereotypy and DA release in striatum has been reported before (Lienau and Kuschinsky 1997). It supports the idea that behavioral sensitization is associated with sensitization of presynaptic DA transmission (Kalivas and Stewart 1991). However, cocaine-pretreated female rats fail to show sensitized DA activity in response to AMPH, which is parallel with very limited expression of behavioral sensitization. Thus, it is very likely that diminished DA response is due to the same reason I mentioned above causing diminished behavioral response. Neural mechanisms mediating the effect of context or stress may directly or indirectly influence DA neuron reactivity. (Gozen et al. 2007; Klebaur et al. 2002; Mattson et al. 2008; Uslaner et al. 2001a; Uslaner et al. 2001b).

Another thing that should be kept in mind is the relationship between DA transmission and behavioral sensitization to cocaine has always been inconsistent (Heidbreder et al. 1996; Hooks et al. 1994; Kalivas and Duffy 1990; 1993; Meil et al. 1995; Segal and Kuczenski 1992b). Studies reporting a sensitization of DA transmission to cocaine utilized an interval of more than 14 days (Hooks et al. 1994; Kalivas and Duffy 1993) while those who failed to observe it utilized an interval of 10 days or less. It was hypothesized that enhanced DA release in NAc and striatum is developed after long withdrawal from chronic psychostimulants administration (Kalivas and Duffy 1993). Peris's studies suggest the more treatments were given, the longer withdrawal it requires to develop DA sensitization (Peris et al. 1990; Peris and Zahniser 1987; 1989). It is possible that females need shorter or longer withdrawal to develop DA sensitization compared to males. Further experiment with different withdrawal in females is needed to test this hypothesis.

DA sensitization associated with cocaine treatment has also been shown to be dose- and subregion-dependent (Cadoni and Di Chiara 1999). When cocaine pretreatment results in behavioral sensitization to both low and high dose of cocaine challenge, sensitized DA transmission is only observed to the lower dose of cocaine challenge in the NAc core while a reduction of DA response was observed after the higher dose in the shell region. Clinical studies also found that AMPH-induced DA release was decreased in striatum in cocaine-dependent subjects as assessed by PET scan (Martinez et al. 2007). It suggests that the reactivity of striatal DA neuron may exhibit a reversed U shape in response to psychostimulants or could be impaired by high dose of psychostimulants. Thus, it is possible that the regimen of high dose of cocaine treatments and high dose of AMPH challenge was too aggressive for females that it leads to the ceiling effect or attenuated DA response.

Taken together, the current study suggests that males and females exhibit sexually dimorphic patterns of behavioral sensitization to repeated treatments of cocaine and in response to AMPH challenge. Females, though exhibit greater behavioral sensitization to cocaine than do males, but do not show cross-sensitization between cocaine and AMPH to the same extent as do males. This sex difference in sensitization may be related to sexually dimorphic pattern of DA neuron activity in striatum. Understanding the underlying neural mechanisms associated with long term use of psychostimulants in both males and females is critical to understand sex difference in cocaine abuse and develop gender-specific strategy to prevent cocaine addiction.

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTION

1. Summary of findings and working hypothesis

The patterns of cocaine use have been found to differ between men and women (Wetherington 2007). Both clinical and preclinical studies suggest sex differences in response to cocaine are mediated by both intrinsic sex differences and ovarian hormones. This dissertation was designed to further characterize the underlying mechanisms mediating sex differences in responses to cocaine, in particular the response to repeated cocaine treatment. The questions that have been addressed in the preceding chapters are: 1) how does estradiol treatment affect behavioral sensitization to cocaine? Does estradiol produce an acute activating effect on sensitized neural circuits? Does estradiol influence the neural processes involved in sensitization to repeated cocaine treatment? Or both? 2) How do previous repeated cocaine treatments and estradiol influence acquisition of cocaine self-administration? Is this cocaine experience associated with enhanced dopamine (DA) transmission in nucleus accumbens (NAc) and striatum in vitro in females? 3) Are sex differences in cocaine sensitization associated with a corresponding sexually dimorphic pattern of DA sensitization in the striatum in vivo?

The experiments described in Chapter II was the first experiment trying to understand how estradiol influence cocaine sensitization, either through activational

effect on sensitized brain or influence neural processes associated with sensitization or both. For the first time, it was found that acute estradiol on challenge day enhanced behavioral activation in cocaine-sensitized rats. This result suggests that estradiol produces an independent acute activating effect on sensitized neural circuits to affect behavioral sensitization. Estradiol, given with repeated cocaine treatment, enhanced cocaine-induced behavioral activation post sensitization on challenge day after withdrawal from cocaine and estradiol, suggesting estradiol may also directly influence neural sensitization occurring with repeated cocaine treatment.

Sensitization is thought to be a process associated with an enhancement of the reinforcing effect of drugs. This hypothesis has been proposed largely based on preclinical studies on males. Experiment in Chapter III, for the first time, found that prior cocaine exposure, which was much more powerful than estradiol, promoted the acquisition of cocaine self-administration and cocaine taking in females, suggesting the same hypothesis is applicable to females as well. Moreover, it was the first experiment showing that chronic cocaine experience was associated with enhanced DA transmission in NAc as well as in striatum in vitro.

Even though DA system was found to be sensitized in females as in males after cocaine exposure in vitro, this hypothesis has not been tested in vivo in females yet. Chapter IV directly compared male and females in one experiment to test the hypothesis that sex differences in cocaine sensitization are associated with sexual dimorphic pattern in DA sensitization with repeated cocaine treatments. It was found that males and females did exhibit sexually dimorphic patterns of behavioral sensitization to repeated treatments of cocaine and in response to AMPH challenge. Females, though exhibited greater

behavioral sensitization to cocaine than did males, but did not show cross-sensitization to AMPH on challenge day while undergoing microdialysis, while males did. More importantly, the DA system in response to AMPH challenge in vivo after cocaine sensitization was also sexually dimorphic, which is consistent with the hypothesis that sex differences in cocaine sensitization are associated with a sexually dimorphic pattern of DA sensitization.

2. Mechanisms mediating sex differences in the effects of cocaine

Based on current knowledge, the following model is presented to explain some mechanisms mediating sex differences in cocaine abuse. First of all, cocaine produces psychomotor stimulating and reinforcing effects through its activation of the ascending midbrain DA system, in particular by enhancing extracellular DA levels in NAc and striatum. Repeated DA activation associated with repeated cocaine treatment first initiates some transient neuroadaptations in cell body regions (VTA and SNc), such as subsensitivity of autoreceptors that may contribute to an enhanced basal activity of DA neurons and transient increase in basal DA transmission in DA terminal regions. These transient responses might be important for initiating more persistent changes in DA terminal areas (NAc, striatum and prefrontal cortex), including enhanced psychostimulants-induced DA release, D1 receptors supersensitivity and upregulated D2-high receptors. The enhanced DA transmission in terminal area may be responsible for the expression of behavioral sensitization and enhanced reinforcing effect of

psychostimulants resulted from repeated cocaine exposure (Figure 5.1).

Related to sex differences in the acute response to cocaine, the ascending midbrain DA system, in particular the striatum and NAc, is sexually dimorphic. The sex differences in acute response to cocaine are probably mediated directly by striatum and NAc. In particular, independent of gonadal hormones, castrated (CAST) males have higher basal DA level in striatum than ovariectomized (OVX) females (Castner et al. 1993). If DA levels in striatum and NAc critically determine psychomotor stimulant and reinforcing effect of cocaine, the sex differences in basal DA tone of the striatum and NAc may have determined a different amount of DA that is required to induce behavioral activation or reinforcing effect in males vs. females (more DA may be required for males). Thus, with same amount of cocaine stimulation, females are more likely to show greater behavioral activation and feel greater subjective reinforcing effect of cocaine than are males. In addition, one acute effect of estradiol in the striatum and NAc is to directly enhance psychostimulants-induced DA release. This acute effect probably happens through an activation on estrogen receptors located on the extracellular membrane of medium spiny striatal neurons to reduce calcium current and results in a decreased GABA release, which then disinhibits DA neuron terminals to enhance stimulated striatal DA release (Becker 1999; Becker and Hu 2008). Thus, DA mediated postsynaptic transmission are further enhanced in estradiol-treated females (Figure 5.2).

The reason that an acute effect of estradiol on the acute behavioral response to cocaine is not usually seen is this enhanced DA output is not strong enough to result in behavioral effect. After repeated cocaine treatment, psychostimulants-induced DA transmission in striatum and NAc is enhanced. Thus, estradiol directly works on

sensitized DA terminals in striatum and NAc to further enhance cocaine-stimulated DA increase and results in enhance behavioral output after cocaine sensitization. This is probably one of the mechanisms contributing to the enhancing effect of estradiol on cocaine sensitization.

In addition to the acute effect of estradiol, chronic estradiol treatment also results in neural functional changes on striatum and NAc that require longer time to occur. Chronic estradiol treatment results in increased DA D2 receptor sensitivity and density (Hruska and Silbergeld 1980; Lammers et al. 1999; Landry et al. 2002; Zhou et al. 2002), increased DA uptake site density (Morissette and Di Paolo 1993a), increased turnover of DA in NAc (Shimizu and Bray 1993), decreased expression of DAT in striatal astroglia cultures (Karakaya et al. 2007), and modified G-protein coupling process stimulated by D1 and D2 DA receptor agonist (Maus et al. 1989a; Maus et al. 1989b). Thus, as a result of progressively prolonged estradiol exposure with repeated estradiol treatment, estradiol may also enhance behavioral effects of cocaine at the later stage of sensitization through some of the above mechanisms. This may also contribute to enhanced behavioral sensitization in estradiol-treated females.

Until now, there is no direct evidence showing that the effect of estradiol on the straitum or NAc (either acute or long term effect I mentioned above) would interact with the neural adaptitive changes produced by repeated cocaine treatment. Even though estradiol didn't significantly interact with cocaine treatment to influence cocaine-induced behavior on challenge day, estradiol treatment did enhance cocaine-induced behavioral activation only in cocaine-pretreated rats (Chapter II). Similar observation has been reported by another study (Hu and Becker 2003). Moreover, cocaine-pretreated OVX

females exhibited some sensitized stereotypy (headbobs) in response to AMPH challenge while OVX females that received estradiol treatment with repeated cocaine treatment did not exhibit any cross-sensitization to AMPH at all on challenge day (no estradiol treatment on challenge day, Chapter IV). Taken together, those behavior data suggest that estradiol may also interact with repeated cocaine treatment to influences the neural adaptations associated with cocaine sensitization.

Additionally, a very recent electrophysiological study demonstrated that both basal and cocaine-induced activity of VTA DA neurons fluctuates significantly during the estrous cycle, and was also modulated by systemic estradiol treatment (Zhang et al. 2008). This fluctuation of VTA neuron activity is in agreement with the fluctuation of DA concentration in medial prefrontal cortex measured by microdialysis during the estrous cycle (Dazzi et al. 2007). Even though ERβ is present in VTA (Creutz and Kritzer 2002; Shughrue et al. 1997; Shughrue and Merchenthaler 2001), there is no evidence showing a direct effect of estradiol on VTA. Thus, this regulation of VTA DA activity by estradiol could be mediated by a direct effect of estradiol on VTA or an indirect influence of estradiol on striatum and NAc, or both. Nonetheless, the regulation of VTA DA neuron by estradiol, and a critical role of VTA in initiating sensitization, present a possibility that estradiol may influence some neural events occurred with cocaine sensitization.

Taken together, repeated cocaine treatment produces persistently enhanced DA transmission in NAc and striatum to enhance the behavioral and reinforcing effects of cocaine. Estradiol, to a lesser extent, produces an independent action on DA transmission in striatum and nucleus accumben to enhance behavioral and reinforcing effects of cocaine. These two processes, though occur independently, but work together and result

in greater behavioral sensitization in estradiol-treated female rats than in OVX female rats. Further experiments are needed to verify if estradiol influence the neural process occurred with cocaine sensitization (Figure 5.1).

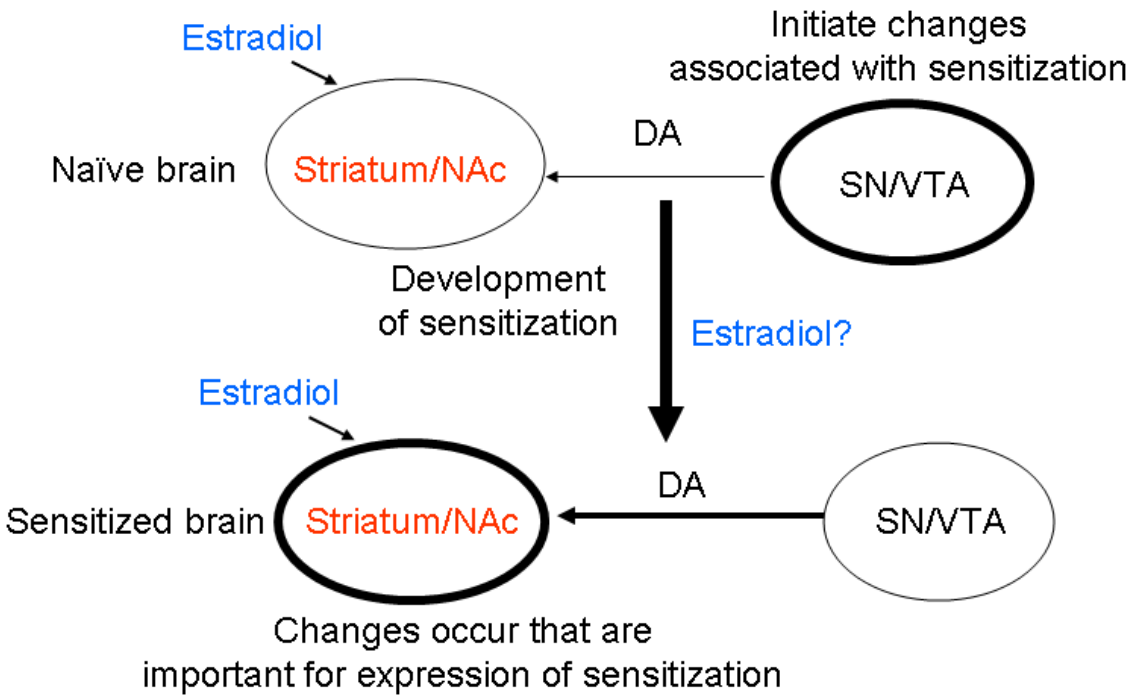


Figure 5. 1: A model representing mechanism mediating sex differences in the effects of cocaine.

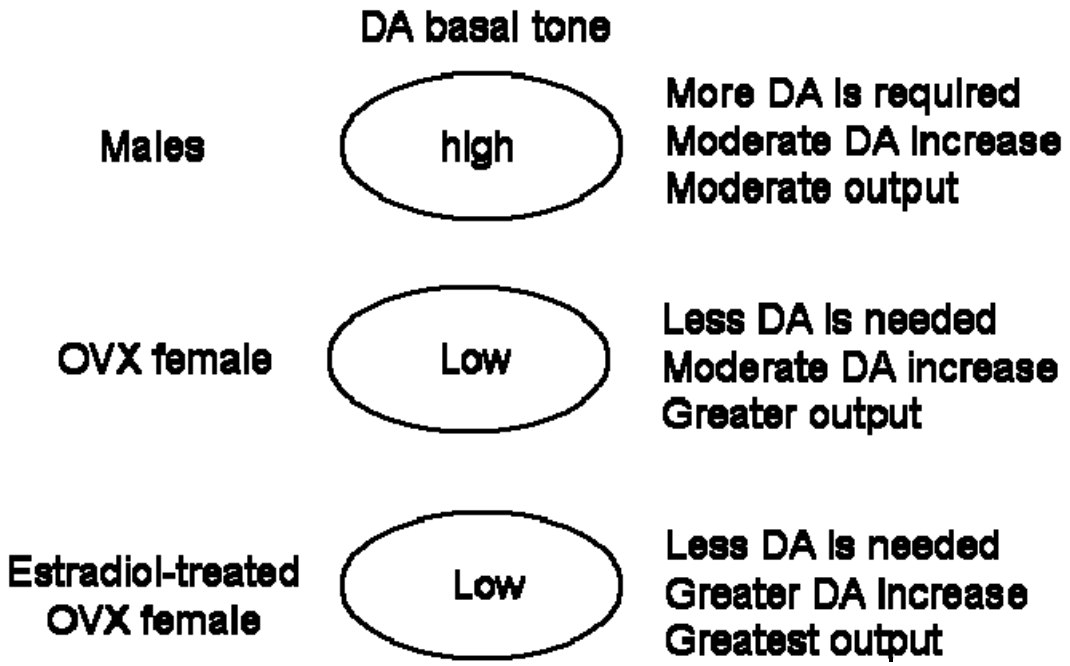


Figure 5.2 Sex differences in basal and cocaine stimulated-DA increase in DA terminal areas (NAc and striatum) determine the sex differences in psychostimulant and reinforcing effects of acute cocaine administration.

Even though a large body of preclinical and clinical studies suggests that dopaminergic neurotransmission is critical in mediating the reinforcing effects of cocaine in humans. The intriguing thing is treatment based on antidopaminergic mechanism fails to show an effect at treating cocaine dependence (Sherer et al. 1989). Thus the role of DA in cocaine abuse needs to be carefully examined. The hallmark of cocaine addiction, distinguished from casual cocaine use, is compulsive drug abuse despite negative consequence, and high rates of relapse during periods of abstinence (Dackis and O'Brien 2001; Mendelson and Mello 1996). Recently, a critical role of glutamate projections from medial prefrontal cortex (mPFC) to VTA and NAc in cocaine sensitization has been recognized (Kalivas 2004; Kalivas et al. 2003; Wolf 1998) and is found to mediate

relapse to cocaine-seeking behavior (Kalivas and McFarland 2003; Kalivas et al. 2003). Pathology of glutamate transmission from prefrontal cortex to NAc after sensitization has also been hypothesized to account for impulsive drug abuse in addiction (Kalivas et al. 2005).

Therefore, DA may be mediating the reinforcing effect of cocaine and be important for maintaining initial use of cocaine. With repeated cocaine treatment, DA transmission in DA terminal areas is enhanced, which is responsible for behavioral sensitization and enhanced reinforcing effect of psychostimulants. More importantly, the persistent DA release might be important for initiating a series of neural events to progressively recruit glutamatergic system and associated limbic brain regions and the prefrontal cortex, so that gluatamate neurons start to show dramatic response to cocaine and related stimuli, which contribute to compulsive drug abuse in addiction (Kalivas et al. 2005).

Thus, if the acending midbrain mesolimbic DA system, which mediate the primary behavioral and subjective effect of cocaine, is sexually dimorphic and is modulated by estradiol in females, it is very likely that there are sex differences in DA-initiated neural events with repeated cocaine use, that lead to a sex difference in glutamate transmission in NAc, cortical area and other related limbic structure. This may contribute to a sex difference in the transition from casual cocaine use to compulsive cocaine abuse as well as sex differences in relapse. Until now, the knowledge of the role of glutamate on cocaine sensitization in females is very poor. Thus, looking at the interaction of the DA and glutamate systems in the process of cocaine sensitization in females and understanding its role for compulsive cocaine taking and relapse is an important step for us to understand sex differences in cocaine abuse.

APPENDIX

Effect of progesterone on behavioral sensitization to cocaine in female rats

1. Introduction

Cocaine is one of the most widely abused drugs in western countries. Cocaine abuse, particularly by women, has increased in the last decade. According to the 1998 National Household Survey on Drug Abuse, currently among the 1.8 million Americans who use cocaine, approximately 36% are female (NHSDA, 1998). Thus, cocaine dependence in women is a growing public health concern. A great portion of women who try cocaine develop lifetime dependence upon cocaine (Kandel et al. 1995) Clinical studies suggest that women show a different pattern of cocaine abuse than do men. Women begin using cocaine and enter treatment at earlier ages than men (Griffin et al. 1989). After being addicted, cocaine cues induce more drug craving in female than male addicts (Robbins et al. 1999). In addition, women report less pleasure and dysphoria, and greater anxiety in response to cocaine than men (Kosten et al. 1996; Lukas et al. 1996; Singha et al. 2000). Collectively, these data suggest that women may be more vulnerable to the addictive effect of cocaine than men.

Repeated exposure to psychomotor stimulants, such as cocaine, results in enhanced behavioral responses to subsequent drug treatment in humans and laboratory animals. This is called behavioral sensitization (Robinson and Berridge 2003). It is suggested that

sensitization-related neuroadaptations play an important role in the processes of drug addiction (Robinson and Berridge 1993; 2000b; Wyvell and Berridge 2001). Thus, behavioral sensitization is often used to explore the mechanism underlying drug addiction in animal models.

Preclinical studies on rodents indicate that there are differences between the biological sexes. Female rats displayed greater cocaine induced stereotypy and locomotor activity than males (Festa et al. 2004; Sell et al. 2000). They acquired self-administration of cocaine at a faster rate than male rats, and a greater percentage of females acquired cocaine self-administration (Lynch and Carroll 1999). Moreover, female rats showed more robust sensitization to cocaine than males (Cailhol and Mormede 1999). The fact that brain concentrations of cocaine after systemic administration are comparable for male and female rats promotes us to explore mechanism other than cocaine pharmacokinetics (Bowman et al. 1999).

Some evidence suggests that intrinsic sex differences in brain organization during development may partially account for sex differences in responses to psychomotor stimulants. Ovariectomized (OVX) female rats showed greater behavioral sensitization to cocaine and self-administer more cocaine at a faster rate than castrated (CAST) male rats (Hu and Becker 2003; Hu et al. 2004).

Besides the genetic sex difference, circulating gonadal hormones are also thought to play a role in modulating vulnerability to drug use in females since the response to cocaine differs across the estrous cycle in females (Becker 1990a; b; 1999; 2000; Kippin et al. 2005; Lynch et al. 2000). For example, hyperactivity induced by cocaine was greatest in female rats during proestrus and estrus (Sell et al. 2000). Female rats

responding for cocaine under a progressive ratio schedule also reach a higher breaking point during estrus than during other phases of the estrous cycle (Roberts et al. 1989).

Further studies identified estradiol as a key factor modulating cocaine-induced response. It is known to facilitate behavioral responses, including cocaine-induced locomotor and stereotyped activity, self-administration of cocaine as well as cocaine-induced behavioral sensitization, to either acute or repeated injection of cocaine in females (Hu and Becker 2003; Lynch et al. 2001; Sell et al. 2000).

Progesterone is another major hormone, besides estradiol, fluctuating over the menstrual cycle in women. Clinical reports showed that women have a greater subjective response to both cocaine (Evans et al. 2002; Sofuoglu et al. 1999) and AMPH (Justice and de Wit 1999; White et al. 2002) in the follicular phase of the menstrual cycle, when estradiol is predominant, as compared to the luteal phase, when both estradiol and progesterone are elevated. Since the luteal phase is characterized by higher progesterone levels than the follicular phase is, progesterone is implicated as an agent that may attenuate the cocaine response in women. These results were consistent with preclinical studies that examined progesterone and cocaine interactions. Two recent studies indicate that progesterone treatment blocked the conditioned place preference for cocaine in mice (Russo et al. 2003b) and rats (Russo et al. 2003a). Administration of estradiol and progesterone suppressed cocaine-induced locomotion when compared to estradiol treated animals (Quinones-Jenab et al. 2000). A recent study conducted by (Jackson et al. 2006) also demonstrated that concurrent administration of progesterone with estradiol ameliorates the enhancement effect of estradiol in cocaine self-administration. Contrary to that, acute administration of progesterone was shown to enhance cocaine-induced

locomotor activity in estradiol primed OVX rats (Perrotti et al. 2001). At the same time, OVX rats implanted with silastic capsules containing both estradiol and progesterone showed greater magnitude of cocaine conditioned place preference (Russo et al. 2003a). It is possible that progesterone plays a role in the facilitation or inhibition of estradiol's effect on cocaine-induced behavior. Due to these conflicting data, the effect of progesterone still remains to be determined. Thus, the goal of present study is to determine the effect of progesterone on behavioral sensitization to cocaine in female rats and seeks to determine how progesterone and estradiol interact to modulate the behavioral response to cocaine.

2. Materials and Methods

Animals

Female Sprague Dawley rats (Harlan Inc, Indianapolis, IN), weighing 225-250 gm at the start of the experiment, were housed two to three per cage under a 14/10 hr light/dark cycle. Animals were housed in a room maintained at a constant temperature of 20-21℃ with phytoestrogen-free rodent chow (2014 Teklad Global Harlan rat chow; Harlan Teklad, Madison, WI) and water available *ad libitum*. All procedures were performed according to a protocol approved by the University of Michigan Committee for Use and Care of Animals.

Surgery

Approximately one week after arrival, animals received 6-hydroxyDA (6-OHDA) lesions of the nigrostriatal DA pathway. Animals were given pargyline (35 mg/kg, i.p.) to

inhibit 6-OHDA metabolism, desipramine (15 mg/kg, i.p) to protect noradrenergic cells at least half hour before 6-OHDA infusion. They were anesthetized with ketamine (40 mg/kg) and domitor (medetomidine hydrochloride, 0.3 mg/kg). Lesion coordinates measured from bregma, skull flat, were as follows: posterior, 5.0 mm; lateral, 2.0 mm; and ventral, 7.7 mm. A solution of 8.26 μg of 6-OHDA hydrobromide (2 μg/μl in 0.1 mg/ml L-ascorbic acid in 0.9% sterile saline) was infused into the substantia nigra through a 29 gauge cannula at a rate of 0.5 μl/min for 8.25 min (for a total infusion of 4.13 μl). The infusion cannula was left in place for 2 min before being slowly raised to allow the infusion to disperse.

Two weeks later, all rats underwent a test with apormophine because apomorphine-induced rotation can be used to predict lesions of the striatum over 90% (Hudson et al. 1993). Each rat was placed into the rotometer for habituation and rotational behavior was recorded from the beginning of habituation. After 30 min, each rat was injected intraperitoneally with apomorphine (25 mg/kg) and then tested for 1 hr. Satisfactory performance were defined as having over 100 full turn per hour and >95% of them were the turn to the side contralateral to the lesion side.

Only the rats that passed the apormorphine test underwent the following surgery. The rats underwent gonadectomy or sham surgery under isoflurane anesthesia. Ovariectomy is conducted using a dorsal approach. The skin is opened with an incision ~ 1 cm long along the midline just below the ribs, and a small incision (~ 0.5 cm) is made through the muscle ~ 1.5-2 cm lateral to the midline. The ovary is externalized with blunt forceps, and the tissue between the ovary and uterus is clamped with a hemostat. The ovary is removed, and the hemostat remains in place until there is no

bleeding when it is released. The uterus with associated tissue is returned to the abdomen. The procedure is repeated on the other side, and wound closure is via 11 mm wound clips. After 4 d of recovery, all female rats underwent vaginal lavage testing daily for 10 consecutive days to confirm cessation of cycling.

Rotational behavior

Subjects were assigned to one of five groups: (1) Ovariectomized (OVX) females treated with 0.2 ml of peanut oil vehicle; (2) OVX females treated with 5μg of 17β-estradiol in 0.1ml of peanut oil (OVX+E) and 0.1ml of peanut oil; (3) OVX females treated with 125 μg of progesterone in 0.1 ml of peanut oil (OVX + P) and 0.1 ml of peanut oil; (4) OVX females concurrently treated with 5μg of 17β-estradiol in 0.1ml of peanut oil and 125 μg of progesterone in 0.1 ml of peanut oil (CPE) (5) OVX rats sequentially treated with 5 μg of 17β-estradiol in 0.1 ml of peanut oil and 125 μg of progesterone in 0.1 ml of peanut oil (estradiol for 3 days, progesterone on the 4^{th} day, oil on the 5^{th} day). Each rat was placed into the rotometer for habituation for half an hour and then was injected subcutaneously with either hormone or vehicle. Rotational behavior was recorded from the beginning of habituation. 30 min after hormone/vehicle injection, each rat was injected intraperitoneally with either saline or 10mg/kg cocaine and then tested for additional 1 hr. The computer program recorded 360° rotation in 5 min intervals during the whole 120 min period. Numbers of animals in each group are included in the figure legends. Rats were tested for 5 consecutive days followed by 2 d off each week for 3 consecutive weeks. On each test day, animals received hormone or vehicle, and on the 2 d off, no hormone or cocaine was administered.

Striatal DA determination

Around two weeks after testing, all rats were decapitated. Their brains were removed rapidly, and the striatum on each side was dissected. The two striatal samples were each weighed and then homogenized in 400µl of a solution containing an internal standard (dihydroxybenzylamine), 2 mM EDTA, and 0.1 M sodium metabisulfate dissolved in 0.05 N perchloric acid. These samples were then centrifuged at 3500×g, filtered through 0.2 µm filters, and transferred to autosampler vials for DA analysis using HPLC with electrochemical detection (Coulochem II, ESA, Waltham, MA), as described previously (Becker and Freed 1988). Percentage DA depletions were calculated by subtracting the concentration of DA per milligram of wet tissue weight on the lesioned side from the value on the intact side. This result is divided by the value on the intact side, providing a percentage depletion of DA. Rats whose percentage depletion was <95% and rats who turned in the wrong direction or who did not show a clear bias during testing were eliminated from the study.

Statistical analysis

The total number of net rotations was calculated as the sum of the number of contraversive rotations during the 60 min testing period minus the number of ipsiversive rotations for each rat. Comparisons were made by ANOVA and subsequent post hoc comparisons with Fisher's PLSD test, with significance set at $p \leq 0.05$ for individual comparisons. All data were analyzed using the computer program Statview 4.5 (Systat, Evanston, IL) for Macintosh (Apple Computers, Cupertino, CA) and SPSS 11.5 (SPSS, Chicago, IL) for Windows (Microsoft Corporation, Redmond, WA).

3. Results

Acute response to cocaine

Acute effects of the first treatment with cocaine or saline on rotational behavior are shown in Figure A.1. There was no significant effect of hormone on the first day of testing either among saline groups ($F_{(4, 56)}$=1.29, p=0.285) or cocaine groups ($F_{(4, 65)}$=1.215, p=0.313).

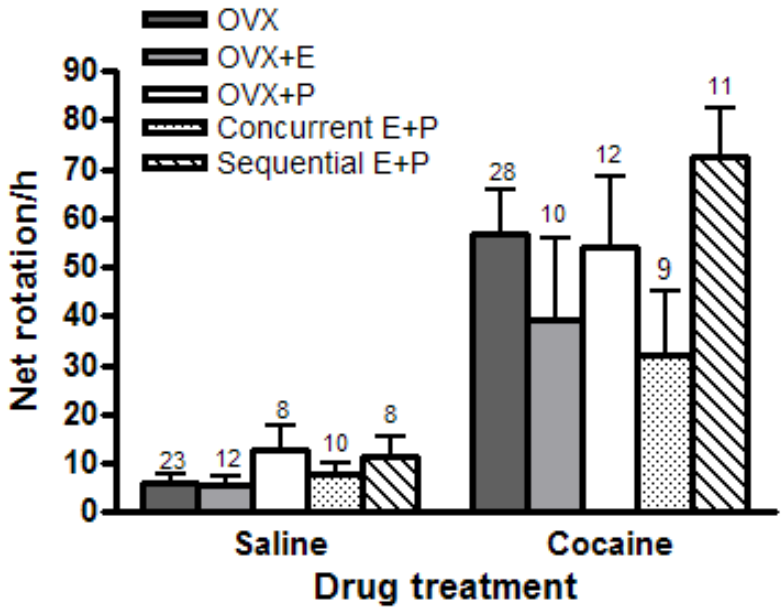


Figure A.1: Rotational behavior induced by first injection of cocaine or saline. There was no significant effect of hormone on the first day of testing either among saline groups or cocaine groups.

Response to repeated treatment of cocaine

In order to test the effect of sensitization, I chose day 1 as the start of sensitization. Compare to that, the mean of day 13, 14 and 15 may be more appropriate to represent the

end of sensitization because OVX+E+P group got different treatment on the last three days (E on 13^{th}, P on 14^{th}, O on 15th).

All rats that received repeated injection of cocaine exhibited sensitization of rotational behavior, as defined by a progressive enhancement of rotational behavior with repeated drug treatment. For all cocaine groups, there was a significant interaction between hormone and testing days ($F_{(4,65)}$= 3.136, p=0.020), main effect of testing day ($F_{(1,65)}$=223.85, p<0.0001), main effect of hormone ($F_{(4, 65)}$=3.366 p=0.014).

Figure A.2 shows the mean $\pm$ SEM number of net rotations per hour for each cocaine group at both the start and end of testing. Post hoc comparison showed that in the end of sensitization, OVX+E group rotated significantly more than OVX group (p=0.014), OVX+P group (p=0.017) and concurrent E+P group (p=0.006). Besides that, sequential E+P group also rotated significantly more than OVX group (p=0.031), OVX+P group (p=0.034) and concurrent E+P group (p=0.013), but not more than OVX+E group (p=0.072). There is no difference among OVX, OVX+P, concurrent E+P groups in the last three days. As we have already shown before, there is no difference among the five groups at the beginning of sensitization. Thus, sensitization is enhanced in OVX+E and sequential OVX+E+P groups compared to all the other three groups.

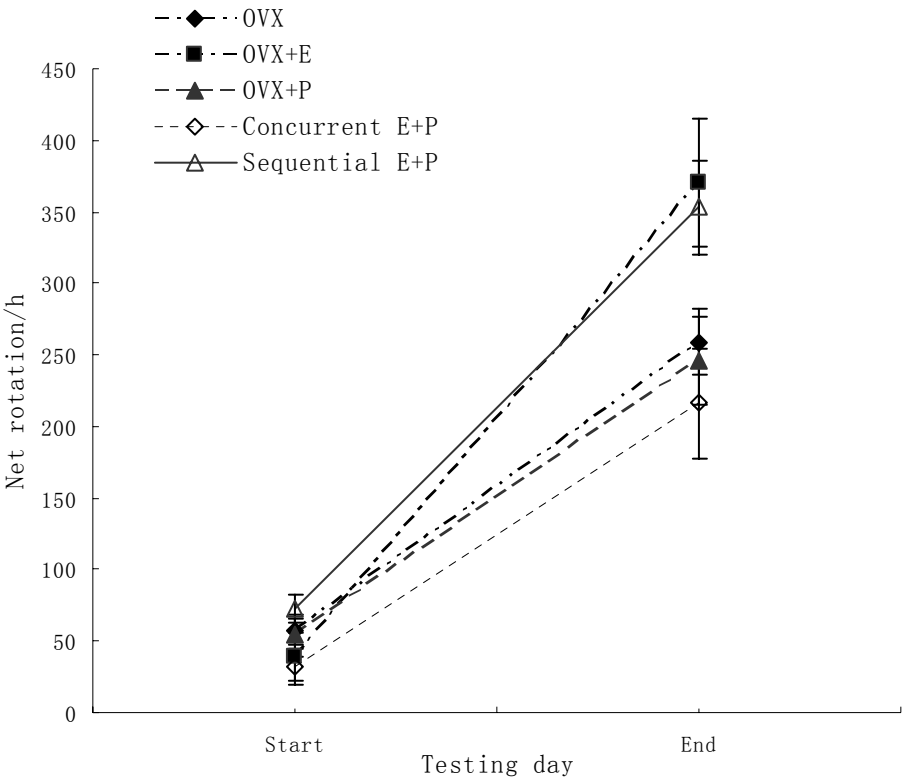


Figure A.2: Cocaine-induced rotational behavior at the start (1st day) and the end (mean of last three days) of sensitization. Sensitization was enhanced in OVX+E (n=10) and sequential E+P (n=11) groups compared to OVX (n=28), OVX+P (12), concurrent E+P (n=9) groups. There is no difference between OVX+E and sequential E+P groups, or among OVX, OVX+P and concurrent E+P groups.

Response to repeated treatment of saline

For all saline groups, there was no interaction between hormone and testing day ($F_{(4, 56)}$=1.243, p=0.303), no main effect of hormone ($F_{(4, 56)}$ = 0.299, p=0.877), but main effect of testing day ($F_{(1, 56)}$=49.210, p<0.0001), as shown in Figure A.3. Thus saline groups, to a very limited extend, also showed behavioral sensitization.

I divided the rats into cocaine and saline groups in order to compare their performance on challenge day. However, data showed that the saline groups also got sensitized on challenge day (data not shown here). Thus I will only focus on the process

of sensitization.

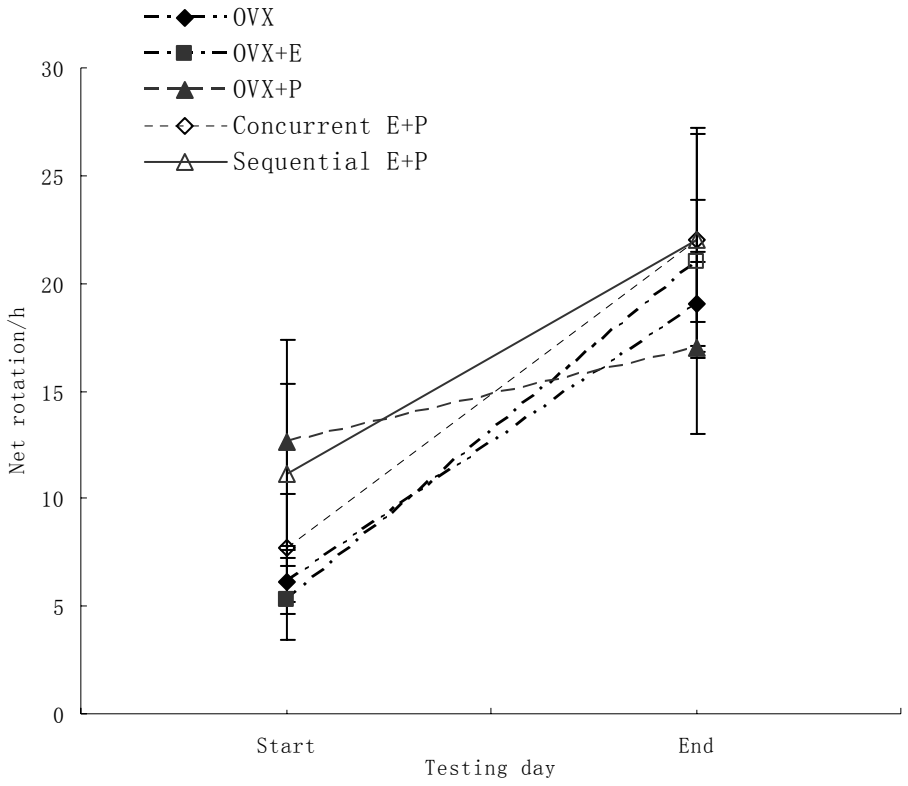


Figure A.3: Saline-induced rotational behavior at the start (1st day) and the end (mean of last three days) of sensitization. There was no interaction between hormone and testing day, no main effect of hormone, but main effect of testing day. OVX (n=23), OVX+E (n=12), OVX+P (n=8), concurrent E+P (n=10), sequential E+P (n=8).

4. Discussion

In the present study, we replicated earlier finding that in the OVX rats, estradiol enhances the behavioral sensitization to cocaine (Hu and Becker 2003). More importantly, we now first report that (1) concurrent administration of progesterone with estradiol ameliorates this effect of estradiol, (2) sequential administration of estradiol and

progesterone also enhances cocaine induced behavioral sensitization as estradiol, and (3) progesterone alone does not have any effect on behavioral sensitization to cocaine in female rats. Thus, progesterone can either facilitate or impede the effect of estradiol on behavioral sensitization to cocaine based on how they are administered.

The enhancement effect of estradiol on cocaine sensitization has been reported by Hu and Becker (Hu and Becker 2003). It is demonstrated again in this study. Actually, estradiol has long been known to facilitate the psychomotor activating and rewarding effect of psychomotor stimulant drugs (Becker 1990b; Becker et al. 1982; Carroll et al. 2004; Hu and Becker 2003; Hu et al. 2004; Jackson et al. 2006; Lynch et al. 2000; Lynch et al. 2001). This effect of estradiol may be mediated by its actions on DA (DA) neurotransmission in striatum (Becker 1999). Female rats during estrus have a significantly greater basal and AMPH-induced increase in extracellular DA in striatum and estradiol enhances the AMPH-induced striatal DA release both in vitro and in vivo (Becker 1990a; b; Becker and Cha 1989; Xiao and Becker 1994). In addition, acute or chronic estradiol treatment increases DA turnover (Di Paolo et al. 1985), modulates DA receptor density and affinity as well as uptake sites (Di Paolo et al. 1988; Levesque and Di Paolo 1988; 1989; Morissette and Di Paolo 1993a; b).

Consistent with past studies, our results show that s.c. estrogen administration, however, does not affect behavioral response to acute cocaine administration (Hu and Becker 2003; Quinones-Jenab et al. 2000; Sircar and Kim 1999). It is possible that estrogen's effects on cocaine-induced activity varies with the route of exposure because it was shown that estrogen, if administered via SILASTIC capsules, potentiates cocaine-induced behavioral effects (Perrotti et al. 2001; Sell et al. 2000).

Progesterone alone was found to have no effect on behavioral response either to acute or repeated cocaine treatment in this study. It is in agreement with most studies showing that progesterone has no effect on cocaine-induced locomotive acitivity (Perrotti et al. 2001; Sell et al. 2000; Sircar and Kim 1999), especially at the dose we were using (Niyomchai et al. 2005). However, administration of 50 and 500 μg of progesterone 24 h before cocaine administration inhibited rearing responses (Niyomchai et al. 2005). In addition, Russo et al. (Russo et al. 2003a) showed that progesterone replacement via SILASTIC capsules attenuated cocaine conditioned place preference (CPP). Thus, the effect of progesterone is also dose and route dependent. Different mechanisms might be involved and have yet to be determined.

Another important finding here is that progesterone counteracted the effect of estradiol on behavioral sensitization. This is not surprising because Jackson (Jackson et al. 2006) has demonstrated that progesterone counteracted the effect of estradiol on cocaine self-administration. Jackson proposed three mechanisms to explain the inhibitory effect of progesterone on cocaine self-administration. Progesterone could counteract the effect of estradiol by either dimishing some subjective effect of cocaine, or by influencing DA system, which is assumed to mediate the reinforcing and incentive motivational effects of cocaine (Robinson and Berridge 1993; 2000b). Besides that, a third, but not exclusively, hypothesis considers the effect of progesterone on changes in DA neurotransmission associated with the development of behavioral sensitization. This study does support the idea that progesterone may antagonize the facilitory effect of estradiol on self-administration by antagonizing psychomotor sensitization. A study conducted by Peris and her colleges (Peris et al. 1991) have similar finding. OVX rats that received chronic

estradiol and progesterone administration had less cocaine-induced stereotyped and locomotor behaviors and lower AMPH-stimulated DA release from striatal slices in vitro than OVX rats that received estradiol alone. However, there are also some studies which showed that concurrent treatment of estrogen and progesterone results in greater cocaine-induced behavior. The OVX rats implanted with silastic capsules containing estrogen plus progesterone showed greater magnitude of cocaine conditioned place preference and increased DA levels in the NAc (Russo et al. 2003a). Sell and her colleagues also found that hormone replacement with E+P in OVX rats resulted in greater cocaine-evoked hyperactivity than OVX animals (Sell et al. 2000). But the procedures used in those studies, which implant rats with silastic capsules that continuingly release estrogen and progesterone, are different from what we used here. Because of the different procedure, the hormone doses that have been released would probably different from the dose of injection in our study.

It is also possible that the continuous release of estrogen primed the rats before progesterone exerts its effect. The hypothesis that estrogen primes progesterone can be supported by our observation that when progesterone were given after treatment of estradiol for three days, cocaine induced rotational behavior were greatly enhanced compared to the OVX, the OVX+P, the concurrent E+P groups. This result indicates that progesterone modulates the effect of estradiol in different ways depending on how they are administered. It is in consistent with a previous report, which demonstrated that estrogen primed OVX rats with acute administration of progesterone had higher counts of locomotor activity in response to chronic cocaine than did vehicle control or progesterone-treated OVX rats (Perrotti et al. 2001). They also reported that cocaine

induced ambulatory and rearing activity peaked only when progesterone was administered 24 hr after estrogen and, more importantly, this effect does not correlate with the temporal interaction between estrogen and progesterone in the regulation of cocaine metabolism and HPA activation (Perrotti et al. 2003). Thus, a more proper explanation is progesterone interacts with estradiol to modulate nigrastriatal DA function.

Dluzen and his colleagues (Dluzen and Ramirez 1984) found that direct infusion of progesterone into superfusion chambers containing corpus striatum tissue fragments of estrogen primed ovariectomized female rats augmented spontaneous and AMPH-stimulated DA release in vitro. This effect can be also demonstrated in castrated male rats pretreated with estrogen (Dluzen and Ramirez 1990a). Becker (Becker and Rudick 1999) also reported that acute progesterone injection after three days of EB treatment resulted in a significant enhancement both on AMPH induced stereotyped behaviors and striatal DA release during microdialysis. The effect of progesterone was very rapid and the significant increases in DA release occurred at 30 min post-progesterone (Dluzen and Ramirez 1990b). In addition, a membrane associated protein, which showed highly affinity for progesterone, was also found in striatum after estrogen priming (Ke and Ramirez 1990; Tischkau and Ramirez 1993). Until now, there is no evidence showing that the effect of progesterone on striatal DA release can be observed without estrogen priming. That may explain why we did not observe the increased behavioral sensitization when estrogen and progesterone were given concurrently.

Finally, how estradiol and progesterone work together to modulate DA function is not clear at this moment. The effect of progesterone upon DA release is thought to happen through interneurons within the striatum rather than direct interaction with

DArgic nerve terminals (Dluzen and Ramirez 1989a). Medium spiny GABAergic neurons in the striatum could be one target that influenced directly by estradiol and progesterone, which then modulate DA system because estradiol was shown to rapidly inhibit KCl stimulated GABA outflow in striatum from a microdialysis study (Hu et al. 2006).

One interesting finding is that saline groups also got sensitized; although to a very limited extend to saline. They had similar response to cocaine on challenge day as cocaine groups. One possible reason might be the stress brought by handling and injection. Stress has long shown to influence psychomotor DA system (Piazza et al. 1990). Cross sensitization of stress and cocaine has also been described a lot (Kikusui et al. 2005; Prasad et al. 1995; Prasad et al. 1998; Sorg 1992). In this study, each rats got three injections per day in the rotometer, which could cause a lot of stress.

Inherent sex differences in brain organization during development and the sex difference in the effect of circulating hormones both contribute to the gender differences in cocaine abuse that is observed. In regarding the effect of hormones, we should keep in mind that different hormone may not separately involved. The temporal/bimodal interaction between estradiol and progesterone in modulating behavioral response to cocaine is more critical in understanding characteristic pattern of cocaine abuse in women.

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