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BEHAVIORAL AND NEUROANATOMICAL SUBSTRATES CONTRIBUTING

TO MOTIVATION IN THE POSTPARTUM FEMALE RAT

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ABSTRACT OF THE DISSERTATION

Behavioral and neuroanatomical substrates contributing to motivation in the postpartum female rat

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The experiments described in this dissertation characterize the unique motivational state of the postpartum female rat. To effectively protect and care for offspring (pups), postpartum females must be strongly motivated to seek out and interact with pups. A combination of place preference studies, behavioral observations, and neurobiological interventions were used to explore females' motivational state across the postpartum period.

To challenge postpartum females' maternal motivation, Chapter 1 presented females with a choice between chambers paired with pups and highly salient cocaine. While most late postpartum females preferred cocaine, many early postpartum females retained striking preference for the pup-paired chamber. To explore whether cocaine's incentive value changed across the postpartum period, Chapter 2 examined females' preference for cocaine- versus saline-paired chambers. Across a broad range of drug administration parameters, postpartum females consistently expressed similar, strong preference for the cocaine-paired chamber. Surprisingly, cocaine preference was stronger in postpartum females than virgin females or males. Females' locomotor response to pup,

cocaine, and saline stimuli predicted their preference for those stimuli. Chapter 3 revealed that the length of pup exposure and nature of female-pup interactions can even affect the motivational state of females that have not given birth. Virgin females were exposed to young pups for various lengths of time and then tested for pup-paired chamber preference. Striking pup-paired chamber preference emerged even in virgin females only briefly exposed to pups, matching the preference expressed by strongly motivated postpartum females. Experiments in Chapter 4 revealed that the ventral tegmental area (VTA), a brain region critical to motivated behavior, drives the incentive value of pup but not cocaine stimuli. Postpartum and virgin females were tested for their preference for pup- or cocaine-paired chambers, respectively, after transient VTA inactivation. Pup preference was abolished by VTA inactivation and restored after recovery. Cocaine preference remained intact despite VTA function.

Maternal motivation is resilient to challenge during early postpartum and is at least partially driven by exposure to pups. As the choice of other salient stimuli (e.g., cocaine) during postpartum may jeopardize maternal motivation, females' motivation to interact proactively with pups is critical to the offsprings' survival and viability.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Chapter 3

Chapter 4

GENERAL INTRODUCTION

Cocaine use and abuse in women

The prevalence of cocaine use, dependency and abuse in the United States is a serious socioeconomic problem. In 2000, over three million people in the United States reported occasional cocaine use and an estimated 2.7 million people reported chronic cocaine use (ONDCP Drug Policy Information Clearinghouse Fact Sheet for Cocaine, 2003). Cocaine use amongst women is particularly concerning: one in ten women report using cocaine at least once in their lives (Substance Abuse and Mental Health Services Administration, 2005) and both human and animal models of drug use indicate that females are highly vulnerable to drug dependence and addiction. Compared to males, females respond more positively to mild drug doses, rapidly increase drug usage, and are more prone to relapse after abstinence and/or treatment (Becker and Hu, 2008). Therefore, even casual use amongst women can rapidly escalate into chronic and problematic drug-taking behavior.

The issue of cocaine use amongst women of childbearing age is particularly critical, as cocaine use can have devastating implications for parenting behavior and child development. Nearly 4% of pregnant women reported using an illicit drug such as cocaine or crack within the past month (Substance Abuse and Mental Health Services Administration, 2005) and over 15% of women report cocaine use during pregnancy in some urban populations (Frank et al., 1988). Cocaine use frequently co-occurs with single parenthood and use of nicotine, alcohol, and other illicit substances (Richardson, 1998).

As cocaine and its metabolites readily cross the placenta (Woods, 1998), pregnant women who use cocaine risk exposing their fetus to cocaine and subsequent cocaine-associated pathophysiologies, even after a sole exposure to the drug (Plessinger and Woods, 1993). Cocaine alters transplacental oxygen available to the fetus (Plessinger and Woods, 1993; Woods, 1998), reduces the length of gestation and increases the rate of preterm birth (Chasnoff et al. 1987; Richardson, 1998), and has been negatively correlated with newborn birth weight and head circumference (Chiriboga, 1998). *In utero* cocaine exposure also negatively affects the development of important neurotransmitter systems for dopamine (Rønnekleiv et al., 1998) and serotonin (Whitaker-Azmitia, 1998). Cocaine-exposed children also exhibit more emotional and behavioral problems, including increased levels of anxiety, depression, and withdrawal behavior (Hawley et al., 1995) and irritability (Chiriboga, 1998). Such temperamental and behavioral problems may persist well into toddler years (Richardson, 1998), representing continued effect of gestational cocaine exposure across the vital period of early-childhood development.

Perhaps most critically, cocaine severely limits a mother's ability to effectively care for her offspring. Cocaine-addicted mothers are substantially more likely to emotionally and physically neglect or abuse their children, consider abandoning their children, and provide less stable, nurturing home environments for their children (Wasserman and Leventhal, 1993; Hawley et al., 1995). Cocaine-addicted mothers are also more psychologically and emotionally detached from their children (Hawley et al., 1995), report high levels of frustration and anger while parenting (Coyer, 2003), and exhibit more psychological distress during the postpartum period (Singer et al., 1997).

These factors indicate a troubling lack of maternal motivation, which may significantly impact a child's socioemotional and physical development.

Together, the combined effect of ineffective and incomplete caregiving and maternal neglect in cocaine-abusing mothers, paired with the adverse developmental and neurobehavioral outcomes associated with *in utero* cocaine exposure, constitutes a serious societal problem. The adverse consequences of cocaine use in pregnant women and young mothers may severely affect the likelihood that the child will assume a positive and productive role in society.

The postpartum rat as an informative model

Cocaine use and abuse in women, particularly those who are recently mothers, is a complex area of research confounded by many intervening factors such as polydrug and alcohol use, environmental influences and sociocultural pressures. While there are a number of complex cognitive contributions that participate in maternal behavior and motivation in the human parent, such contributions cannot be modeled in laboratory animals. However, the female rat has been successfully utilized as a more simplistic, preclinical model to better understand the fundamental biological substrates supporting maternal neglect and negative developmental outcomes associated with cocaine use during the postpartum period. Cocaine's motivational, behavioral, and pharmacokinetic properties have also been extensively studied in the rat model. As important neurobiological and endocrinological principles identified in rat accord closely with clinical studies with humans, research using rat as subjects can contribute meaningfully

to our understanding of the specific underpinnings of drug use and maternal motivation in humans.

Reinforcement, reward, and motivation

To help characterize the quality of stimuli encountered by an animal in its environment, the concepts of *reinforcement*, *reward*, and *motivation* must be clearly defined. A *reinforcing* stimulus is defined as any stimulus that increases the frequency of a target behavior. Importantly, a reinforcing stimulus can be presented (positive reinforcement) or removed (negative reinforcement) to increase the frequency of the target behavior. For instance, cocaine addicts will self-administer cocaine because of the feelings of well-being and euphoria that it provides (positive reinforcement) and/or because it ameliorates the negative affect associated with drug withdrawal (negative reinforcement); in each case, cocaine-taking behavior will increase. As aversive stimuli can also be reinforcing (by increasing the frequency of avoidance or fearful behaviors), it is important to emphasize the hedonic neutrality of the term *reinforcement*.

Reinforcing stimuli can, however, be rewarding. A *rewarding* stimulus is stimulus perceived as intrinsically or subjectively positive (Everitt and Robbins, 2005) or that possesses positive affective, pleasureful, or hedonic value (i.e., "liking") (White, 1989; Berridge and Robinson, 1998; Salamone and Correa, 2002; Everitt and Robbins, 2005; Berridge, 2007; Berridge et al., 2009). As cocaine commonly induces feelings of well-being or euphoria when consumed (Gawin, 1991), cocaine is typically considered a rewarding pharmacological stimulus. Outside of human self-report, however, the range of species-specific behaviors that accurately measures an animal's subjective response to

a rewarding stimulus is limited at best. Objective measures of pleasure, such as rhythmic tongue protrusions in response to a sweet taste, are considered to represent "unconscious liking reactions" to that taste (Berridge and Robinson, 1998; Berridge, 2007) but are used infrequently and inconsistently in the literature. While indicative of hedonic pleasure, reward value does not adequately characterize how a subject produces motivated behavior directed toward attaining a stimulus.

Any stimulus, regardless of its reward value, is attributed with a certain incentive value. Incentive value is considered to represent an animal's core *motivation* to pursue, seek out, attain, or gain proximity to a particular stimulus (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Salamone and Correa, 2002; Salamone 1996; Salamone et al., 2007; Berridge, 2007; Berridge et al., 2009). Motivation also can be interpreted as "wanting" or desiring a target stimulus or goal (Berridge and Robinson, 1998; Berridge, 2007; Berridge et al., 2009). If a rewarding stimulus is attributed with strong positive incentive value, the presentation of that stimulus will initiate highly motivated behavior and effortful decision-making directed toward attaining that stimulus. An aversive stimulus also has incentive value that evokes strongly motivated behavior directed towards avoiding that stimulus. In either case, stronger motivation requires greater energy to initiate the desired stimulus-directed behavior, so long as it remains within a subject's behavioral capacity (Brehm and Self, 1989). Importantly, motivation is strongly influenced by an animal's current physiological state, attention and arousal, environment and context, and ability to produce goal-directed behavior. Finally, motivation can be easily quantified using a number of well-established behavioral paradigms, which are widely considered to reflect the incentive value attributed to a stimulus (detailed later).

The dissociation between motivation and reward

The direction of a stimulus' reward and incentive value often coincide during the initial exposures to a stimulus. In other words, a highly rewarding stimulus typically will evoke strongly motivated behavior toward that stimulus, whereas a highly aversive stimulus will evoke strongly motivated behavior away from that stimulus. A neutral stimulus that lacks strong rewarding or aversive properties will not elicit strong motivation. Thus, measuring the direction of motivated behavior can often provide a vague and indirect indicator of reward value exclusively during early stimulus exposures.

Following persistent exposure to a stimulus, however, the incentive value and reward value of that stimulus can be dissociated (Berridge et al., 2009). In the case of abused drugs, such as cocaine, repeated drug use over time produces tolerance to the drug's reward value (i.e., the same drug stimulus is "liked" less after repeated exposures) but greatly intensifies the incentive value of the drug (i.e., the drug is "wanted" or craved/desired more). This dissociation is most evident in drug addicts, who display pathological motivation to seek and administer drugs but report diminished reward value from drug consumption (Koob and Kreek, 2007). Limiting the number of stimulus exposures effectively minimizes the confounded interpretations arising from potential reward/incentive dissociations that often emerge after chronic stimulus exposure.

Recently, elegant neuroanatomical studies have identified discrete regions of the nucleus accumbens (NAc) that respond exclusively to reward but are not involved in motivated behaviors (Peciña and Berridge, 2005; Peciña, 2008; Berridge et al., 2009).

These findings provide some of the first evidence that the neural circuits participating in reward and motivation are at least partially dissociable.

Motivated behavior can be appetitive or consummatory

Motivation is a complex concept that encompasses a variety of behaviors. One of the most notable distinctions in motivated behavior is whether the behavior is directed at a stimulus that is present or that is absent. To consider this distinction, it is useful to categorize motivated behaviors as an appetitive or consummatory response (Sherrington, 1906; Ikemoto and Panksepp, 1999). Appetitive behaviors represent preparatory, anticipatory or stimulus-seeking states, and typically precede any direct interactions with the target stimulus itself. Thus, appetitive behaviors are performed in an effort to approach or gain access to the stimulus. Once the stimulus has been approached or accessed, the animal can perform a number of responses direct at the stimulus itself, collectively referred to as consummatory behaviors. These behaviors may include interacting with, consuming, withdrawing from, or attacking the stimulus. Once an animal has performed a consummatory response toward the stimulus, the appetitive value of that stimulus changes and the probability that the animal will perform future consummatory responses is altered. For instance, a hungry animal may forage for food (appetitive behavior) until finding and consuming it (consummatory behavior). The presence of food evokes a consummatory response (i.e., eating); eating produces satiety that consequently reduces the animal's motivation to continue seeking out food for consumption.

Importantly, both appetitive and consummatory behaviors incorporate aspects of motivated behavior. For example, a maternal female rat is strongly motivated to actively seek out her offspring in their absence (appetitive behavior) (Lee et al., 2000; Mattson et al., 2001; Pereira et al., 2008). Once her offspring are retrieved, she will perform motivated caregiving behaviors directed at her offspring (consummatory behavior) (Numan and Insel, 2003; Numan, 2007; Pereira et al., 2008). Components of both of these behaviors – seeking and caregiving – reflect the offspring's high incentive value to the maternal female. However, as performing consummatory behavior toward a target stimulus can reduce its appetitive value in subsequent interactions, examining motivated behavior during the initial, appetitive phase of this behavioral sequence provides the clearest representation of the incentive value of this stimulus.

Behavioral measures of motivation

While consummatory responses to a stimulus typically consist of observable behaviors or physiological responses that can be recorded and quantified, motivated appetitive (stimulus-seeking) behaviors are typically assessed using one of two paradigms.

In an operant paradigm, subjects are trained to bar-press for delivery of a stimulus. The total number, rate, and persistence of bar-pressing behavior are typically considered to reflect a subject's motivation to attain the stimulus. However, these measures require that some bar-presses result in stimulus delivery to reinforce continued bar-pressing behavior; stimulus delivery elicits a consummatory response from the subject that affects the subsequent appetitive value of that stimulus in future

presentations. To eliminate this confound of stimulus presentation, the "breakpoint," or maximum number of bar-presses that the subject is willing to make antecedent to the first stimulus delivery, can be used to quantify motivated behavior. Importantly, breakpoint measures a stimulus' incentive value in the absence of intervening consummatory behaviors.

In a conditioned place preference (CPP) paradigm, motivation is also measured in the absence of the stimulus by teaching subjects to associate the stimulus with a neutral chamber containing unique contextual cues (Tzschentke, 1998, 2007). After this unconditioned (US) stimulus is repeatedly presented in the context of the cue-decorated chamber (*conditioning phase*), the cue-decorated chamber acquires the value of the unconditioned stimulus and becomes a conditioned stimulus (CS). The incentive value attributed to the unconditioned stimulus (US) can be assessed by measuring the amount of time spent in the stimulus-associated chamber (CS), in the absence with the unconditioned stimulus (*post-conditioning test session*). The more time spent in one stimulus-associated chamber reflects a greater positive incentive value attributed to the stimulus associated with that (preferred) chamber. As behavior during the CPP test session reflects a subject's motivation to seek out conditioned cues associated with a stimulus in the absence of the stimulus itself, CPP testing represents the initial appetitive phase of a motivated behavior sequence. As this phase provides the clearest representation of incentive value, my dissertation work primarily utilizes CPP methodology.

The CPP method has been used to measure the incentive value of a variety of stimuli, including natural stimuli, such as pups, food, and copulatory opportunities

(Fleming et al., 1994; Mattson et al., 2001, 2003; Maes and Vossen, 1993; Mehrara and Baum, 1990; Oldenburger et al., 1992); as well as pharmacological stimuli, including psychostimulants (e.g., cocaine, amphetamine), opiates, ethanol, and nicotine (Bardo et al., 1995; Tzschentke, 1998, 2007). As subjects can be conditioned and tested for CPP within a relatively brief period of time (Bardo et al., 1995, 1999), CPP offers the unique advantage of assessing motivation in a stimulus-free state within a biologically finite period, such as postpartum.

Maternal behavior and motivation

The postpartum period is one of the most complex and dynamic phases in the natural lifecycle of the female. Unique neurobiological, hormonal and physiological changes occur during gestation, parturition, and lactation to help prepare the female rat to protect and care for her offspring (pups). During this period, the female rat is strongly motivated to seek out and interact with her pups and will vigorously perform a variety of appetitive and consummatory behaviors directed toward pups and pup-related stimuli. Appetitive behaviors correspond to maternal motivation; consummatory behaviors correspond roughly to the expression of maternal behavior.

Maternal caregiving behaviors are essential to the survival of all mammals (Rosenblatt and Lehrman, 1963; Numan and Insel, 2003; Lonstein and Morrell, 2006; Numan et al., 2006). Immediately after parturition and throughout lactation, the postpartum female rat performs a unique array of species-specific behaviors to care for and protect her offspring. She will construct a compact maternal nest, retrieve stray pups to her nest, hover and crouch over her pups to nurse them and keep them warm, perform

anogenital licking and grooming of pups to facilitate pups' waste expulsion, and attack intruder animals in defense of her pups (Rosenblatt and Lehrman, 1963; Numan and Insel, 2003; Lonstein and Morrell, 2006; Numan et al., 2006). Hormones present during late gestation and parturition are necessary for the onset but not the maintenance of maternal behavior in the postpartum female; once established, maternal behavior is maintained by interaction with pups (Rosenblatt and Lehrman 1963; Numan and Insel, 2003). In fact, virgin female rats will not behave maternally toward pups and will treat them as aversive stimuli (Fleming et al., 1979; Fleming and Luebke, 1981; Numan et al., 2006) unless exposed repeatedly to pups over a series of days (Rosenblatt, 1967; Fleming and Rosenblatt, 1974) or stimulated with exogenous hormones (Siegel and Rosenblatt, 1975; Siegel et al., 1978; Mayer and Rosenblatt, 1980; Fahrbach et al., 1984; Mayer et al., 1990; Fleming et al., 1994).

While the expression of maternal behavior has been comprehensively studied, females' motivation to seek out and interact with pups in their absence (i.e., maternal motivation) is less understood. The appetitive value of pups has been compellingly demonstrated using operant and conditioned place preference paradigms. In the absence of pups, postpartum females will bar-press vigorously for access to pups, retrieving hundreds of pups over a period of hours (Wilsoncroft, 1969; Hauser and Gandelman, 1985; Lee et al., 2000). Postpartum females will also express robust conditioned preference for a pup-paired chamber (pup CPP; Fleming et al., 1994; Mattson et al., 2001) over chambers paired with other rewarding stimuli such as food (Fleming et al., 1994) and even cocaine (Mattson et al., 2001). Females will voluntarily spend a great deal of time caring for their pups (Grota and Ader, 1969, 1974; Pereira et al., 2008), will

readily retrieve pups from anxiety-provoking areas (Bridges et al., 1972; Stern and Mackinnon, 1976; Pereira et al., 2005), and will defend their pups at great risk to their safety (Lonstein and Gammie, 2002).

Previous work has revealed a striking dichotomy in maternal motivation between parturient females in the early postpartum period compared to those in the late postpartum period. While almost all early postpartum females strongly prefer a pup-paired chamber over a chamber paired with moderately rewarding cocaine, most late postpartum females prefer the cocaine-paired chamber and spend little time in the chamber paired with pups (Mattson et al., 2001). This motivational shift, proposed to occur around postpartum day 10 (Mattson et al., 2003), accords closely with behavioral data suggesting that voluntarily time that a female spends with pups declines dramatically as the postpartum period progresses (Grota and Ader, 1969, 1974; Reisbick et al., 1975; Pereira et al., 2008). In the case of the pup-cocaine dual-choice CPP paradigm (Mattson et al., 2001), it is possible that early postpartum is a period of extremely strong maternal motivation (i.e., robust preference for the pup-paired chamber) and/or a period of blunted responsivity to the reward value of cocaine (i.e., low preference for the cocaine-paired chamber). These possibilities have not been systematically explored.

It is likely that postpartum females' attribution of incentive value to pups is partially mediated by the unique neuroendocrine state of the postpartum period. Early postpartum is characterized by low levels of estrogen, increasing levels of progesterone, and very high levels of prolactin (Grota and Eik-Nes, 1967; Smith and Neill, 1977; Taya and Greenwald, 1982). As the postpartum period progresses, estrogen rises and

progesterone and prolactin gradually return to normal levels of a cycling female (Grota and Eik-Nes, 1967; Smith and Neill, 1977; Taya and Greenwald, 1982). These changes could underlie the distinctive preference patterns expressed during early and late postpartum. Past work also revealed that ovariectomized virgin females will only prefer a pup-paired chamber if exposed to pups and administered a hormonal regimen mimicking parturition and early postpartum (Fleming et al., 1994). However, the relative contributions of hormones and pup exposure in maternal motivation have not been elucidated.

Cocaine and maternal responsivity

Cocaine has been used to disrupt both maternal behavior and maternal motivation in rodents. Maternal caregiving behavior was impaired dramatically after a systemic injection of cocaine (Zimmerberg and Gray, 1992; Kinsley et al., 1994; Johns et al., 1994) and recovered completely once cocaine reached undetectable levels in the bloodstream (Vernotica et al., 1996). Microinfusions of cocaine into discrete brain regions involved in maternal responsivity, such as the medial preoptic area (MPOA), also transiently abolished maternal caregiving behavior (Vernotica et al., 1999). When presented with their pups in a cocaine self-administration chamber, postpartum female rats spent substantially less time tending to their pups when they are self-administering cocaine compared to sucrose (Hecht et al., 1999). During late postpartum, females preferred to spend time in a chamber associated with moderately rewarding cocaine than one associated with their pups (Mattson et al., 2001). The incentive value of cocaine across postpartum has not been characterized, and it is possible that, by manipulating cocaine administration parameters, such as administration route and length of exposure,

differences will emerge in females' motivation to seek out cocaine in early versus late postpartum.

Cocaine's mechanisms of action

Cocaine ($C_{17}H_{21}NO_4$) is an alkaloid substance obtained from leaves of the coca plant that acts as a local anesthetic, psychomotor stimulant, and euphoria-inducing agent (Gawin, 1991; Nestler et al., 2001; Koob and LeMoal, 2006). Cocaine binds selectively to plasma membrane transporters for dopamine (DAT), serotonin (SERT), and norepinephrine (NET) (Uhl et al., 2002). These transporters, located on the axon terminals of associated monoaminergic neurons, bind and transport synaptically released neurotransmitter back into the pre-synaptic cell for re-release, storage, or degradation (Nestler et al., 2001). When bound and competitively inhibited by cocaine, however, transporters are unable to clear excess extracellular neurotransmitter from the synaptic cleft, so neurotransmitter continues to bind post-synaptic receptors and stimulate the post-synaptic cell.

Each membrane transporter participates in distinct aspects of cocaine's mechanisms of action. Cocaine-induced hyperlocomotion has been attributed exclusively to DAT function (Giros et al., 1996), as cocaine-induced locomotion is not present in DAT knockout mice but does emerge normally in SERT and NET knockout mice (Uhl et al., 2002). However, both DAT and SERT knockout mice will exhibit preference for a cocaine-associated chamber in CPP paradigms (Sora et al., 1998; Medvedev et al., 2005), revealing a functional distinction between the role of DAT in determining appetitive (reward) and locomotor-activating properties of cocaine. As cocaine-associated chamber

preference is completely abolished in DAT/SERT double-knockout mice (Sora et al., 2001), other compensatory mechanisms must contributing to cocaine's reward value in the absence of either DAT or SERT. In contrast, NET function may actually mediate cocaine's aversive properties (Uhl et al., 2002).

Acute and chronic exposures to cocaine are associated with a variety of morphological and electrophysiological changes. While chronic cocaine can induce substantial neuroplasticity in the brain, particularly within the dopaminergic receptor system (Alburges et al., 1996; Nestler et al., 2001; Koob and LeMoal, 2006), chronic drug exposure paradigms are outside the immediate goals of this dissertation research and will not be discussed further here. Acute cocaine administration has been associated with chromatin remodeling (Kumar et al., 2005), receptor-mediated synaptic enhancement (Borgland et al., 2004), and the insertion of new plasma membrane receptors in dopamine-responsive neurons (Schilstrom et al., 2006). Acute cocaine also promotes the expression of immediate early genes and their associated protein products (Koob and LeMoal, 2006), some of which are regulated by dopaminergic receptor activation (Zhang et al., 2004). These genes and their protein products can trigger a variety of intracellular cascades (e.g., cAMP, PKA) leading to changes in gene expression (e.g., CREB, Jun, etc) that can initiate transient to relatively long-lasting changes in protein synthesis and cellular function (Koob and LeMoal, 2006).

Cocaine activates a number of discrete neuroanatomical targets that together compose a distributed neural circuitry responsive to cocaine's motivational and psychomotoric properties. Systemic or intracerebroventricular cocaine elevates synaptic dopamine levels in all terminal regions of ascending dopaminergic projections arising

from the ventral tegmental area (VTA) and/or substantia nigra pars compacta (SNc), including the nucleus accumbens (NAc), striatum, amygdala, and frontal cortex. While not all cocaine-induced activity is mediated by dopamine (Koob and LeMoal, 2006), dopamine release does appear to mediate NAc activity in response to cocaine and cocaine-predictive stimuli in the NAc (Carelli et al., 2000; Carelli and Ijames, 2001; Carelli, 2002, 2004; Roitman et al., 2005, 2008). Recent work revealed that preference for a cocaine-paired chamber increases cFos protein expression in the NAc, basolateral amygdala (BLA), prefrontal cortex (PFC) and medial preoptic area (MPOA) (Mattson and Morrell, 2005), indicating that overlapping neural substrates can be activated both by cocaine and cocaine-associated stimuli (Carelli et al., 2000; Carelli and Ijames, 2001; Carelli, 2002, 2004). Region-specific cellular and molecular contributions to cocaine's reward value, incentive value, and psychomotoric properties are still being explored.

Neurobiological circuitry supporting general motivation

The major ascending monoaminergic systems in the brain originate from anatomically discrete nuclei: dopaminergic neurons from the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), serotonergic neurons from the raphe nuclei, and noradregenergic neurons from the locus coeruleus. A band of ascending and descending dopaminergic, serotonergic, and noradrenergic fibers, known as the medial forebrain bundle, traverses the lateral hypothalamus and is highly rewarding when stimulated (see Wise and Rompre, 1989). It was thought that this "reward pathway" and its regions of origin and termination were responsive to naturally-rewarding stimuli participating importantly in evolutionary survival (i.e., food, water, sexual reproduction, parental behavior). Researchers proposed that drugs of abuse "hijacked" components of

this system to evoke feelings of euphoria, which encouraged continued drug use that often resulted in subsequent dependency/abuse.

Though dopaminergic neurons compose a very small but functionally significant proportion of cells in the human brain (approx. 300,000-400,000; Nestler et al., 2001), early studies revealed dopamine (DA) to be uniquely responsive to rewarding stimuli and drugs of abuse (e.g., Wise, 1980). Since then, DA has remained the subject of extensive research related to motivation, reward, and effortful behavior. Two ascending DA pathways have been identified, based on neuroanatomical and functional studies: the nigrostriatal system, which projects from SNpc to striatum (Nestler et al., 2001; Koob and LeMoal, 2006), and the mesocorticolimbic system, which projects from VTA to limbic and forebrain structures. While nigrostriatal DA plays a critical role in voluntary movement and psychostimulant sensitization (Nestler et al., 2001; Koob and LeMoal, 2006), mesocorticolimbic DA has been associated with various cognitive and emotional functions associated with reward-related processing.

Importantly, recent analyses revealed that mesocorticolimbic DA plays a critical role in goal-directed behavior, effortful decision-making and the attribution of incentive value to stimuli (Ikemoto and Panksepp, 1999; Salamone and Correa, 2002; Salamone 1996; Salamone et al. 2007; Berridge, 2007; Berridge et al., 2009). Rather than mediating hedonia or reward, as originally thought, DA release in NAc fluctuates in response to both rewarding and aversive stimuli (Salamone 1994, 1996; Salamone et al. 2007), and reward value remains unaffected by site-specific or global DA manipulations (Berridge, 2007; Berridge et al., 2009). Instead, DA mediates the attribution of incentive salience to a stimulus, or the *wanting* aspects of behavior (Berridge, 2007; Berridge et

al., 2009). Recent work indicates that DA release in the NAc participates in the attribution of incentive value to both natural and pharmacological stimuli (Carelli et al., 2000; Carelli, 2002; Tzschentke, 2000) and their predictive cues (Carelli, 2002; Yun et al., 2004). Manipulating DA transmission in NAc directly affects the incentive value attributed to a stimulus without affecting the reward value or learning associated with that stimulus (Peciña and Berridge, 2005; Berridge, 2007; Peciña, 2008; Berridge et al., 2009). Thus, changes in DA activity occurring during experiences with these stimuli are proposed to shape an animal's motivated stimulus-seeking behavior for the stimuli and their associated cues (Ikemoto and Panksepp, 1999; Salamone and Correa, 2002; Schultz, 2002).

As conditioned place preference (CPP) is considered to reflect the attribution of incentive value to stimuli, it is likely that mesocorticolimbic DA plays an important role in the expression of CPP for a stimulus-associated chamber. Motivation can be strongly influenced by a subject's current physiological state, prior learning, and experience with a particular stimulus. Thus, it is possible that key mesocorticolimbic substrates differentially affect the responsivity of the DA system when confronted with natural or pharmacological rewarding stimuli (Carelli et al., 2000).

Interactions between motivation-related neurobiology and maternal responsivity

In the maternally responsive female rat, neural activity across a broad network of substrates participates in the unique expression of maternal caregiving behavior and maternal motivation. The medial preoptic area (MPOA) is critical to the integration of polysensory pup-related input and the performance of maternal behavior (Lee et al.,

2000; Numan and Insel, 2003; Numan, 2007; Numan and Stolzenberg, 2008; Pereira et al., 2008). MPOA activity increases dramatically during the expression of maternal behavior, while lesioning the MPOA completely eliminates maternal responsivity (Lee et al., 2000; Numan and Insel, 2003; Perrin et al., 2007). Other regions critical to the expression of maternal behavior include the bed nucleus of the stria terminalis (Lonstein et al., 1998; Numan and Insel, 2003), lateral habenula (Corodimas et al., 1992; Lonstein et al., 1998) and lateral septum (Lonstein and Gammie, 2002), all of which strongly express cFos (a protein marker of neuronal activation) during a female's physical interaction with her pups (Lonstein et al., 1998). A number of regions inhibit maternal responsivity, including the medial amygdala (MeA) (Sheehan et al., 2001; Numan and Insel, 2003), ventromedial nucleus of the hypothalamus (VMN) (Sheehan et al., 2001), and periaqueductal gray (caudal ventrolateral portion; Lonstein and Gammie, 2002).

The balance between inhibitory and facilitatory influences on maternal behavior provides interesting insight into the neural substrates driving initial appetitive behaviors directed at pups. In the non-maternal female, pups are extremely aversive stimuli: females will avoid, bury or attack pups and will not act maternally toward them. In these females, pup-associated odors stimulate MeA, which strongly inhibits maternally responsive brain regions (Numan and Insel, 2003). Eliminating olfactory input or lesioning MeA facilitates maternal behavior by disinhibiting the maternal response (Sheehan et al., 2001), at which point females will readily approach and interact with pups. In the natural state, this disinhibition is mediated by gestational and parturitional hormones that ensure the female is fully maternally responsive by the time she gives

birth. Without hormones, disinhibition can also occur via repeated exposure to pups over time.

In the maternally responsive female, recent work has also revealed that the MPOA is critical to the expression of maternal motivation to seek out and interact with offspring. MPOA inactivation reduces a maternal female's motivation to reunite with offspring (Perrin et al., 2007), bar-press for access to offspring (Lee et al., 2000) and even prefer a pup-paired chamber (Pereira and Morrell, in preparation). More MPOA neurons express cFos associated with preference for a pup-paired chamber than preference for a cocaine-paired chamber or for a neutral (control) environment (Mattson and Morrell, 2005), suggesting that intact MPOA function plays an integral role in motivated responses for natural, biologically relevant stimuli such as pups.

Numan (2007) has proposed an interaction between the neural systems mediating maternal responsivity, i.e. maternal behavior and motivation directed specifically toward offspring, and dopaminergic systems mediating general motivation and goal-directed behavior. Regions activated during maternal motivation, centering on MPOA but also including BLA and PFC (Mattson and Morrell, 2005), selectively increases the female's responsiveness to specific maternal stimuli, which promotes the expression of maternal behavior toward pups. The general system promotes the female's attention and facilitates the attribution of incentive value to a broad range of stimuli (Ikemoto and Panksepp, 1999; Salamone and Correa, 2002; Salamone, 1996; Salamone et al., 2007; Berridge, 2007).

An emerging body of work indicates that maternally responsive circuitry communicates with mesocorticolimbic circuitry to promote maternal-specific motivation. Maternal interactions with pups transiently increase DA release in NAc (Hansen et al., 1993; Champagne et al., 2004), whereas blocking DA transmission abolishes both pup licking and motivated pup retrieval (Pereira et al., 2008; Numan and Stolzenberg, 2008). Stimulation of D1-like DA receptors also promotes the expression of preference for a pup-paired chamber (Pereira et al., 2008), whereas DA blockade impairs the acquisition of pup CPP (Fleming et al., 1994). Thus, pups and pup-related stimuli dynamically engage both MPOA and NAc function.

Evidence suggests that pup-specific information accesses the mesocorticolimbic system via direct projections from MPOA to VTA, as MPOA neurons that are activated by maternal behavior project directly to the VTA (Numan and Numan, 1997). Once this maternal input reaches the VTA, a variety of mesocorticolimbic processes are activated to energize goal-directed responses toward pups. One of these, proposed by Numan (2007; Numan and Stolzenberg, 2008), suggests that pup-relevant input to VTA stimulates NAc, which subsequently inhibit the ventral pallidum to facilitate goal-directed behavior toward pups as biologically relevant stimuli with strong incentive value.

A key component of this proposed circuitry, the MPOA-VTA projections, has remained completely unexplored in relation to maternal motivation. It is likely that maternal motivation relies critically on intact VTA function as a necessary component of the feed-forward circuitry mediating pups' incentive value.

Dissertation goals

The experiments described in this dissertation are designed to characterize the motivational state of the postpartum female rat. The specific aims of these experiments are to 1. challenge the strength of maternal motivation by presenting postpartum females with a choice between pups and another highly salient stimulus, 2. investigate the incentive value attributed to cocaine at discrete points across the postpartum period, 3. assess whether maternal motivation is modulated by exposure to and/or interaction with pups, and 4. determine whether a key component of the general motivational circuitry of the brain, the ventral tegmental area, participates critically in motivated behavior directed toward natural (pup) and/or drug stimuli.

CHAPTER 1

Increasing the incentive salience of cocaine challenges preference for pup- over cocaine-associated stimuli during early postpartum: place preference and locomotor analyses in the lactating female rat

Abstract

Rationale/Objectives: Prior studies using a dual-choice conditioned place preference (CPP) procedure revealed that postpartum female rats (dams) strongly prefer chambers associated with pups over those associated with subcutaneously administered cocaine almost exclusively during early, but not late, postpartum (Mattson et al., 2001). The present study examines whether early postpartum dams retain strong pup-associated chamber preference when contrasted with a cocaine stimulus of greater incentive salience (intraperitoneal injections with brief conditioning sessions). Locomotor rate was measured during conditioning (stimuli-present) and test (stimulus-absent) sessions. *Methods:* A 3-chambered CPP apparatus was used to compare preferences for chambers associated with intraperitoneal (IP) cocaine versus age-matched pups. Unconditioned stimuli were systematically assigned to the least-preferred chamber of separate groups of dams before conditioning. Control dams verified that unconditioned stimuli were necessary for CPP and stimulus-associated locomotion. *Results:* Compared with most late postpartum dams (60%), only 31% of early postpartum dams preferred the cocaine-associated chamber ($P<0.05$). Substantially more dams preferred the pup-associated chamber during early postpartum (27%) than late postpartum (5%) ($P<0.05$). Locomotor sensitization emerged across cocaine-conditioning sessions in cocaine-preferring but not pup-preferring dams ($P<0.05$). Locomotor rates were consistently lower in preferred versus non-preferred chambers during test. *Conclusions:* After increasing cocaine's incentive salience, more early postpartum dams prefer the cocaine-associated chamber than previously reported (Mattson et al., 2001). However, pup-associated chamber preference was still higher in early versus late postpartum. Pup- and cocaine-preferring

dams expressed differences in the induction phase of locomotor sensitization across cocaine-conditioning, but expressed similar motoric patterns in their preferred chambers at test.

Introduction

Our previous work used a dual-choice conditioned place preference (CPP) procedure to contrast preference for chambers associated with pups versus subcutaneously (SC) administered cocaine in the postpartum female rat (dam). While most dams tested during late postpartum (day 16) preferred the cocaine-associated chamber, most dams tested during early postpartum (day 8) strongly preferred the pup-associated chamber (Mattson et al., 2001). To assess cocaine's absolute reward value in early postpartum, independent of the pup contrast, subsequent CPP studies compared dams' preference for cocaine-versus saline-associated chambers (Wansaw et al., 2003a; Seip et al., 2005). While SC cocaine elicited only moderate chamber preference in both postpartum groups, intraperitoneally (IP) administered cocaine (tested over a range of doses and conditioning lengths) elicited strong chamber preference equally in early and late postpartum.

These recent data led us to question whether early postpartum dams would continue to prefer the pup-associated chamber over a cocaine-associated chamber when the perceptual and motivational properties contributing to cocaine's incentive salience (i.e. route of administration and conditioning session length) were manipulated to enhance cocaine's attractiveness (Robinson and Berridge, 1993; Berridge and Robinson, 1998; Salamone and Correa, 2002). The present study uses dual-choice CPP to assess the

strength of pup-associated chamber preference in early and late postpartum dams when contrasted with a chamber associated with a cocaine stimulus of high incentive salience (IP cocaine with short conditioning sessions).

The reward value of cocaine has been well-established in animal models (Pickens and Thompson, 1968; Nomikos and Spyraki, 1988; Johanson and Fischman, 1989; Mayer and Parker, 1993; Durazzo et al., 1994; Russo et al., 2003a, 2003b) and human studies (Javaid et al., 1978; Verebey and Gold, 1988; Gawin, 1991; Volkow et al., 2000; Nelson et al., 2006). While cocaine's reward value is relatively unexplored across postpartum, early postpartum dams bar-press for cocaine at lower rates than virgin females (Hecht et al., 1999). Together with previous CPP work (Mattson et al., 2001), evidence suggests that early postpartum dams may be in a unique state of reward responsivity during which cocaine's incentive salience is actively challenged by pups.

The incentive salience or reward value of a drug can be materially altered by the route of administration (Nomikos and Spyraki, 1988; Mayer and Parker, 1993; Durazzo et al., 1994; Busse et al., 2005). Chamber preference associated with IP cocaine is well-established (Mayer and Parker, 1993; Durazzo et al., 1994; Bardo et al., 1995; Russo et al., 2003b) and can be stronger than preference associated with SC cocaine (Mayer and Parker, 1993; Bardo et al., 1995). While concentrations of drug in the blood and/or brain may be one of many components mediating drug reward, the extremely rapid increase in concentrations of drug in plasma and/or brain following IP administration (Lau et al., 1991; Festa et al., 2004) compared to SC administration (Vernotica and Morrell, 1998; Wansaw et al., 2005) is considered to elicit greater incentive salience, in accord with human subjective reports (Javaid et al., 1978; de Wit et al., 1992; Kollins et al., 1998).

Due to the importance of rapidly increasing plasma concentrations of drug after administration, the length of drug-conditioning sessions can be a critical variable in CPP procedures (Cunningham and Prather, 1992; Mayer and Parker, 1993; Bardo et al., 1995; Tzschentke, 1998; Seip et al., 2005). Compared to shorter sessions, longer sessions that include falling concentrations of drug can even elicit place aversion responses (Cunningham and Prather, 1992). The present study thus uses 30-minute IP cocaine-conditioning sessions to maximize chamber exposure during peak plasma concentrations of drug (Lau et al.,1991; Festa et al., 2004).

Pups, the second unconditioned stimulus used in the present study, can be uniquely and highly rewarding to the postpartum dam (Wilsoncroft, 1969; Hauser and Gandelman, 1985; Fleming et al., 1994; Magnusson and Fleming, 1995; Lee et al., 2000; Wansaw et al., 2003b; Ferris et al., 2005). Early postpartum dams prefer chambers paired with pups over pup-sized neutral rubber objects following just 15-minutes of deprivation from their pups (Wansaw et al., 2003b), and voluntarily stay in the nest for the longest periods of time (Grota and Ader, 1969, 1974; Ader and Grota, 1970; Stern and Keer, 2002); in contrast, late postpartum dams strongly prefer chambers paired with alternative non-pup stimuli, even after 12-hours of pup deprivation, and leave their pups for increasingly longer intervals. While extending the length of pup deprivation prior to conditioning dramatically increases pups' incentive salience even to late postpartum dams (23 hrs, Fleming et al., 1994; 22hrs, Wansaw et al., 2003b), the present study deprives dams of pups for 2 hours prior to conditioning to exploit the naturally prominent differences in maternal motivation between early and late postpartum. As longer pup-conditioning session lengths elicit stronger pup-associated CPP (Fleming et al., 1994), pup-

conditioning session lengths of 2 hours were chosen to allow dams' full expression of pup-directed maternal behaviors and repeated bouts of nursing (Mattson et al., 2001, 2003).

Control dams were conditioned and tested in an identical procedure, except that pups and cocaine were not present in conditioning chambers. Controls were used to confirm that pup and cocaine stimuli, but not conditioning lengths (2 hours versus 30 minutes), elicited CPP.

The present study uses a custom-designed place preference apparatus composed of three equal-sized chambers. This study also incorporates useful features of both biased and unbiased methods of stimulus-chamber assignment (Bardo et al., 1995; Tzschentke, 1998), to further challenge the strength of each stimulus-associated preference. Following a pre-conditioning baseline session, dams were either categorized as lacking chamber preference or as preferring one of the three apparatus chambers. Dams exhibiting a pre-existing chamber preference were assigned to receive either pups or cocaine in the opposite, least-preferred chamber for conditioning, so that post-conditioning preference for that chamber would require a substantial increase in time spent in the chamber against which they were "biased" (Nomikos and Spyraki, 1988; Calcagnetti and Schechter, 1993; Campbell et al., 2000; Cunningham et al., 2003; Le Foll and Goldberg, 2005). Dams that lacked chamber preference were randomly assigned to receive each stimulus in one of the conditioning chambers. As each resulting stimulus-assignment group included both dams exhibiting and lacking pre-existing preference, expression of a post-conditioning preference for each stimulus-associated chamber required a substantial increase in chamber time in at least half of the dams.

Locomotor rates were recorded during cocaine- and pup-conditioning (stimulus-present) sessions to confirm increased locomotion following cocaine injections and to explore how pups may affect dams' locomotion in the CPP chamber; to our knowledge, the latter is a novel contribution to CPP literature. Locomotion during the post-conditioning session characterized locomotion in the absence of both pups and cocaine, as yet unreported using dual-choice CPP.

Methods

Animals

Subjects (*n*=57) were postpartum female Sprague-Dawley rats (aged 90-120 days) of stock originally obtained from Charles River Laboratories (Wilmington, MA) and raised in the Laboratory Animal Facility (LAF) at Rutgers University (Newark, NJ) accredited by the American Association for Accreditation of Laboratory Animal Care. All procedures comply with "Principles of laboratory animal care" and "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council 2003). Females were individually housed in opaque shoebox cages (25.5cm W x 47cm L x 23cm H) with woodchip bedding and food and water ad libitum, and were maintained on a 12hr:12hr light:dark cycle with lights on at 0700. All females were nulliparous before mating and remained healthy across pregnancy, parturition, and the entire experiment. After parturition dams and pups were undisturbed for 24 hours; all litters were co-mingled on the day before the procedure began, and eight pups were redistributed as each dam's homecage litter. Details in Mattson et al. (2001).

Experimental groups of early (*n*=26) and late (*n*=20) postpartum dams were exposed to unconditioned stimuli (pups and cocaine) during conditioning between postpartum days 4-7 or 12-15 and tested on day 8 or 16, respectively. Control dams (early *n*=6; late *n*=5) were similarly conditioned and tested, but were not exposed to unconditioned stimuli during conditioning.

Experimental procedure

Apparatus   The conditioned place preference apparatus consisted of three lidded clear Plexiglas chambers custom-designed to be of equal size (27.5 cm W x 21cm L x 20.5 cm H). The center chamber not only acted as a passageway but as a neutral alternative space to the two stimulus-associated side chambers to prevent post-conditioning preferences based on forced choice (Seip et al., 2006a, 2006b). Center chamber time is thus considered fully in all primary analyses, before comparisons specific to the stimulus-associated side chambers.

Each side chamber contained unique contextual cues of wallpaper (horizontal or vertical black-and-white stripes) and tactile flooring, either small paper squares (ALPHA-dri, Shepherd Specialty Papers, Kalamazoo, MI) or small corn cobs (Bed-o'cobs, The Andersons, Maumee, OH) scattered on solid grey floors. The center chamber had white wallpaper and a solid grey floor. Chambers were connected by manually operated guillotine doors, which could be closed. Luminance (Konica Minolta Luminance Meter LS-100, Japan) was equal in each side chamber.

Five infrared beams traversed the floor of each chamber. Beam breaks made by the dam's body were recorded using an automated interface (Med Associates Inc., St.

Albans, Vermont) configured using MED-PC® Version IV Research Control & Data

Acquisition System. Beam breaks recorded the dam's time in each chamber. Locomotor

activity was defined operationally as new beam breaks; repeated breaks of the same

beam were excluded from analysis. Locomotor activity could not be measured in the

center chamber due to the manufacturer's limitations.

Pre-conditioning baseline session   Each dam was exposed to the apparatus for a single

baseline session prior to conditioning, either on day 1 (early) or 9 (late). Each dam was

placed into the center chamber and allowed to freely access all three chambers for

60min.

Conditioning phase Experimental dams were separately confined to each cue-associated

side chamber containing one of two unconditioned stimuli, pups or cocaine, once a day

for four consecutive days. Control dams did not receive unconditioned stimuli during

equivalent chamber exposures. Daily ordering of pup- and cocaine-conditioning trials did

not affect CPP (Mattson et al., 2001, 2003). The cocaine-conditioning session was last

each day, ensuring at least 12 hours for residual cocaine to clear from dam's circulation

before the next pup-conditioning session.

Pups. Pups were 4-7 or 12-15 days old, age-matched to the early and late postpartum

dams, respectively. Two hours before each pup-conditioning session, pups were removed

from each dam's homecage and placed into an adjacent cage so that dams were unable to

see or physically interact with pups but were exposed to pups' auditory and olfactory

stimuli. Pups were demanding of maternal care immediately before pup-conditioning

(Pereira and Ferreira, 2006). Dams were confined to a side chamber containing five pups for 2hrs, starting at 1000.

Cocaine. No pup-deprivation preceded cocaine-conditioning, eliminating potential deprivation-related confounds associated with the non-cocaine stimulus. Cocaine hydrochloride in highly purified powdered form was obtained from the National Institute of Drug Abuse (Research Triangle Park, NC, USA) and injected intraperitoneally (10mg/kg in 0.9% saline) into each dam. Dams were immediately confined to the opposite side chamber for 30 minutes, starting at 1500. Cocaine did not produce pathology, confirmed by postmortem examination of the peritoneal cavity.

Post-conditioning test session   Dams were tested for CPP on the day after the final conditioning session, either postpartum day 8 or 16. Dams were placed into the center chamber and allowed to freely access all three chambers for 60 minutes. No unconditioned stimuli were present.

Analyses and statistics

Statistical analyses were performed using SAS for Windows (Version 9.1; SAS Institute Inc, Cary, NC) (Cody and Smith, 1997) or manually (Siegel, 1956; Bruning and Kintz, 1987), with $P<0.05$ as significance level. Non-parametric tests were used when necessary. Preference data are presented as proportions of a population. Chamber times (minutes) and locomotor scores are presented as means and standard errors of the mean (sem).

Conditioned place preference   The time spent in each chamber (chamber time) during pre- and post-conditioning sessions was used to identify each dam's chamber preference

(Mattson et al. 2001, 2003). Dams that spent at least 30 minutes in one chamber and 25% more time in that chamber than either other chamber were categorized as preferring that chamber. Dams that spent equal amounts of time in each chamber were categorized as lacking preference. Four preference categories resulted: preference for the left, center, or right chamber, or no preference. Chamber times were also averaged across all preference categories to represent the entire population.

Within-group comparisons. Empirical pre-conditioning preferences were compared to theoretical preferences distributed equally amongst all preference categories (25:25:25:25), using a chi-square goodness-of-fit test for specified proportions. Empirical pre- and post-conditioning preferences were then compared using another chi-square goodness-of-fit test, followed by one-tailed tests for significance of a proportion. Pre- and post-conditioning times were compared with a two-way ANOVA (chamber and session as repeated measures) and select paired *t*-tests, or a one-way ANOVA (chamber as repeated) and Tukey's honestly significant difference post-hocs.

Between-group comparisons. Preferences were compared using Fisher's exact tests or one-tailed tests for significance of difference between two proportions. Times were compared with a two-way ANOVA (chamber as repeated) and select independent *t*-tests.

Pre-conditioning chamber preferences and times. Most early (38%) and late (70%) postpartum dams lacked pre-conditioning chamber preference. Dams exhibiting a preference were similarly distributed across left (early 35%, late 15%), center (15%, 5%), and right (12%, 10%) preference categories. These empirical preferences differed from a theoretical distribution [χ^2 (3, N=46)= 20.96, P<0.0001]. Chamber preferences

and times were similar across postpartum and grouped for presentation (Figure 1a-b). Side chamber times were equal, with center time slightly less than left [$F(2,90)=5.01$, $P<0.01$; Tukey, $P<0.05$]. Data verify the apparatus' relative neutrality.

Assignment of unconditioned stimuli to conditioning chambers. Dams lacking pre-conditioning chamber preference were randomly assigned to receive either pups or cocaine in each side chamber for conditioning. Dams exhibiting a preference were assigned to receive pups (early $n=8$, late $n=4$) or cocaine (early $n=8$, late $n=2$) in their least-preferred side chamber for conditioning. Importantly, both dams that lacked *and* expressed pre-conditioning preference were included in each final stimulus-assignment group, in which either pups (early $n=16$, late $n=12$) (Figures 2a and 3a) or cocaine (early $n=10$, late $n=8$) (Figures 2b and 3b) had been assigned to the least-preferred side chamber in a strong subset of dams.

Locomotion New beam breaks in each side chamber were summed during the post-conditioning session and divided by chamber time to produce a locomotor score (rate) for each chamber. To equate conditioning sessions of unequal length (30 minutes versus 2 hours), beam breaks during the first 30 minutes of each conditioning session were divided by 30 minutes. Conditioning scores thus represent the entire cocaine-conditioning session and the first quarter of the pup-conditioning session. During the remainder of the pup-conditioning session, dams were typically nursing or crouching over their pups; scores did not change after the first 30 minutes (see Feigley et al., 1972; Roth and Katz, 1979).

Locomotor scores were separated by dams' chamber preference. Scores were similar across postpartum and grouped (Picazo and Fernandez-Guasti, 1993; Wansaw et al., 2005). Scores from cocaine-preferring (*n*=20) and pup-preferring dams (*n*=8) were analyzed; dams preferring the center or lacking preference were excluded. All control dams' scores were analyzed.

Locomotor scores during conditioning were compared between-groups using two-way ANOVAs (day and chamber as repeated measures) and within-groups using one-way ANOVAs (chamber as repeated measure). Post-conditioning scores from all groups were compared using a one-way ANOVA (group as repeated measure). ANOVAs were followed by Tukey's HSD tests.

Results

Conditioned place preference

Evidence of conditioning   Pre-conditioning chamber preferences (Figure 2a-b) and times (Figure 3a-b) were compared to those of post-conditioning (Figures 2c and 3c, respectively), to provide evidence of conditioning in each postpartum and stimulus-chamber assignment group.

In early postpartum, pre- and post-conditioning preferences and times differed substantially in groups where pups [$\chi^2(3, N=16)=27.57, P<0.0001$; chamber x session interaction, $P>0.05$] and cocaine [$\chi^2(3, N=10)=86.59$; chamber x session interaction, $F(2,18)= 3.65$; both $P<0.05$] had been assigned to the least-preferred chamber (Figure 2-

3). Across both groups, most (60%) early postpartum dams preferred either the pup- or cocaine-associated chamber following conditioning. More dams for which pups had been least-preferred exhibited pup-associated chamber preference following conditioning (z=-2.16, P<0.05), while more dams for which cocaine had been least-preferred exhibited cocaine-associated chamber preference (z=-2.46, P<0.05). Neither pup- nor cocaine-associated chamber times differed after conditioning, due to the relatively bimodal distribution of dams preferring these chambers post-conditioning.

In late postpartum dams, chamber preferences and chamber times also differed following conditioning, both when pups [χ^2(3, N=12)=10.08; chamber x session interaction, F(2,22)=5.49; both P<0.05] and cocaine [χ^2(3, N=8)=194.56; chamber x session interaction, F(2,14)=4.18; both P<0.05] had been assigned to the least-preferred chamber (Figure 2-3). More dams preferred the cocaine-associated chamber post-conditioning, both when pups (z=2.06, P<0.05) and even cocaine (z=-3.26, P<0.05) had been least-preferred. Few dams preferred the pup-associated chamber; dams collectively spent less time in that chamber post-conditioning, even when time was already low [t(12)=3.18, P<0.01; cocaine in least-preferred, t(8)=-2.17, P=0.067].

Within each postpartum group, pre-conditioning stimulus-chamber assignment groups produced similar post-conditioning preferences and times and were grouped for further analysis.

Comparison of early and late postpartum dams   More dams preferred the cocaine-associated chamber in late postpartum (60%) than in early postpartum (31%) (z=2.28, P<0.05), while more dams preferred the pup-associated chamber in early postpartum

(27%) than in late postpartum (5%) (z=-1.95, P<0.05) (Figure 2c). Overall time in the pup-associated chamber was greater in early versus late postpartum [group x chamber interaction, $F(2,88)$=5.63, P<0.01; Tukey: P<0.05] (Figure 3c). Overall time spent in the cocaine-associated chamber was substantially higher in late versus early postpartum. Late postpartum dams also spent more time in the cocaine- than pup-associated chamber [$t(20)$=-4.11, P<0.001]; this difference was not seen in early postpartum.

Locomotion

Conditioning. During cocaine-conditioning, locomotor scores of dams exhibiting pup- or cocaine-associated chamber preference (pup- and cocaine-preferring dams, respectively) were significantly higher than those of control dams [cocaine-preferring: main group effect, $F(1,29)$=17.73, group x day interaction, $F(3,87)$=5.82, both P<0.01; pup-preferring: main group effect, $F(1,17)$=22.24, P<0.001; group x day interaction, $F(3,51)$=2.64, P=0.059; all Tukey: P<0.05] (Figure 4a). Across conditioning, scores increased in cocaine-preferring dams [$F(3,57)$=8.11, P<0.001; Tukey: P<0.05], did not change in pup-preferring dams, and decreased in control dams [$F(3,30)$=6.06, P<0.01; Tukey: P<0.05]; see Figure 4a for details.

On select pup-conditioning days, scores of cocaine- and pup-preferring dams were higher than those of control dams [cocaine-preferring: main group effect, $F(1,29)$=15.12, P<0.001; Tukey: P<0.05; pup-preferring: group x day interaction, $F(3,51)$=2.90, P<0.05; Tukey: P<0.05]; see Figure 4c for details. Scores were lower in pup- than cocaine-preferring dams on the first two conditioning days [main group effect, $F(1,26)$=8.05, P<0.01; Tukey: P<0.05]. Across conditioning, scores decreased in cocaine-preferring

[$F(3,57)=10.45$, $P<0.0001$; Tukey: $P<0.05$] and control dams [$F(3,30)=16.19$, $P<0.0001$; Tukey: $P<0.05$], but not pup-preferring dams.

Post-conditioning session. In the cocaine-associated chamber, scores were substantially lower in cocaine-preferring dams than both pup-preferring and control dams [$F(2,36)=11.36$, $P<0.001$; Tukey: $P<0.05$] (Figure 4b). Similarly, in the pup-associated chamber, scores were significantly lower in pup-preferring dams than cocaine-preferring and control dams [$F(2,36)=6.29$, $P<0.01$; Tukey: $P<0.05$] (Figure 4d). Scores were consistently lower in dams' preferred versus non-preferred chamber [cocaine-preferring: $t(20)=5.68$; pup-preferring: $t(8)=-2.93$; both $P<0.05$], the latter of which did not differ from controls. Controls' scores were equal in each side chamber.

Control group

Pre-conditioning session. Chamber preferences and times did not differ across postpartum and were grouped. Most (64%) control dams lacked pre-existing chamber preference; the remaining dams preferred the center chamber (Figure 5a); thus, dams responded differently than an equal theoretical distribution [$\chi^2(3, N=11)=12.02$, $P<0.01$]. Side chamber times were equal and lower than in the center chamber [$F(2,20)=4.21$, $P<0.05$; Tukey: $P<0.05$] (Figure 5b). Session lengths of 2 hours or 30 minutes were randomly assigned to side chambers for conditioning.

Post-conditioning session. Post-conditioning preferences and times did not differ across postpartum and were grouped. There was no conditioned effect for either side chamber. A few more dams preferred the center chamber, and one dam preferred the empty side chamber paired with cocaine-conditioning length (30 minutes) [$\chi^2(2, N=11)=363.02$,

P<0.0001] (Figure 5a). Side chamber times remained equal and, in the empty side chamber paired with pup-conditioning length (2hrs), lower than the center chamber [$F(2,20)=4.63$, *P*<0.05; Tukey: *P*<0.05] (Figure 5b).

Discussion

Conditioned place preference

We have increased the incentive salience of cocaine, one of two stimuli presented in this relative reinforcement procedure, to challenge the strength of maternal motivation previously reported in early postpartum dams (Mattson et al., 2001). The present study reveals a novel difference in the strength of maternal motivation in two distinct groups of early postpartum dams. A considerable proportion of early postpartum dams retain strong pup-associated preference when contrasted with a cocaine stimulus of considerable incentive salience; this group of maternally-motivated dams is found exclusively in early, but not late, postpartum. The present study also reveals a novel subset of cocaine-preferring early postpartum dams, for which the relative reward value of pups was selectively compromised. The bimodal distribution of early postpartum dams' stimulus-associated preferences contrasts sharply with the almost-exclusive preference for the cocaine-associated chamber characterizing late postpartum. Results reveal not only that maternal motivation wanes as the postpartum period progresses but that the strong maternal motivation characterizing early postpartum may be compromised in a subset of dams if pups are presented alongside an alternate stimulus eliciting strong incentive salience.

The dual-choice nature of the present study addresses the relative reinforcement value of both stimuli (Flaherty, 1996), which describes a dynamic component of the unique reward state of postpartum. Recent work from our lab reveals that, in absolute comparisons, nearly all early and late postpartum dams strongly prefer IP cocaine over saline (Wansaw et al., 2003a; Seip et al., 2005). In the relative comparison of the present study, however, less than half of the early postpartum dams preferred the same IP cocaine stimulus over pups, while most late postpartum dams still preferred IP cocaine. While pups may present an attractive alternative to the incentive salience of cocaine exclusively in early postpartum, some early postpartum dams remain vulnerable to increasingly salient cocaine stimuli: nearly three times as many (31%) dams preferred IP cocaine over pups than preferred SC cocaine over pups (11%; Mattson et al., 2001). While relative preference for IP cocaine in early postpartum dams was still substantially lower than in absolute comparisons, late postpartum dams' preference remained consistently high despite administration route or contrasting stimulus' identity (Wansaw et al., 2003a).

As expected, control dams did not express meaningful preference for either side chamber, confirming that unconditioned stimuli, not conditioning session length, produced substantial side chamber preferences. Controls' center chamber preferences at post-conditioning may be attributable to a novelty-induced preference (Bardo et al., 1989; Klebaur and Bardo, 1999).

Intentional stimulus-chamber assignments following the pre-conditioning session revealed strong conditioning effects to pup and cocaine stimuli within independent groups of experimental dams, as expected (Nomikos and Spyraki, 1988; Calcagnetti and

Schechter, 1993; Cunningham et al., 2003; Le Foll and Goldberg, 2005). The few center-preferring experimental dams clarify that pup- and cocaine-preferring dams were not exhibiting side chamber preference due to a forced choice, validating use of the three-chambered apparatus.

Endocrinological changes across the postpartum period may importantly contribute to the incentive salience of pups to the postpartum dam. Early postpartum, the period of strongest overall maternal motivation (Mattson et al., 2001; Wansaw et al., 2003b), is characterized by relatively low levels of estrogen (E), increasing levels of progesterone (P), and high levels of prolactin compared to late postpartum (Grota and Eik-Nes, 1967; Smith and Neill, 1977; Taya and Greenwald, 1982). As the postpartum period progresses, rising E, decreasing P, and strikingly reduced prolactin gradually return to levels of a cycling female (Grota and Eik-Nes, 1967; Smith and Neill, 1977; Taya and Greenwald, 1982), coinciding with substantially decreased maternal motivation. While these changes occur over a period of days, the two postpartum time points examined in the present study represent fundamentally different endocrinological states that may contribute substantially to the expression of pup-associated chamber preference. While E and P are necessary for maternal motivation in ovariectomized virgin female rats (Fleming et al., 1994), however, a contingent relationship between endocrine levels and maternal motivation has yet to be demonstrated in the postpartum female. Further, it remains to be determined whether these endocrinological substrates are distinct from or overlap with those mediating cocaine's reward value (Hecht et al., 1999; Russo et al., 2003a; Carroll et al., 2004; Jackson et al., 2006).

Other non-endocrinological, stimulus-bound properties may also contribute to maternal motivation. While the present study used age-matched pups to mimic the stimuli present in the dam's natural environment, changing physical characteristics accompanying normal pup development may include properties that elicit less incentive salience and thus evoke less maternal motivation. Pups' increasing size and functional capacity coincides with a dramatic reduction in maternal behavior (Bridges et al., 1972; Reisbick et al., 1975; Stern and Mackinnon, 1978), while young pups increase the frequency and quality of maternal behavior (Noirot, 1964a, 1964b, 1965; Stern and MacKinnon, 1978) and activate dopaminergic reward circuits in the brain (Ferris et al., 2005). However, maternal behavior and motivation do not co-vary in the postpartum dam (Hauser and Gandelman, 1985; Mattson et al., 2003). As dams prefer either young or old (age-matched) pups equally in a dual-choice CPP procedure (Wansaw and Morrell, unpublished observations), maternal motivation may be driven not by pups but by undetermined physiological or behavioral changes in the dam that subsequently affect her responsivity to pups across multiple developmental time points.

Locomotion during the place preference procedure

Locomotion has been analyzed infrequently but usefully within CPP studies (Brockwell et al., 1996; Martin-Iverson et al., 1997; Shimosato and Ohkuma, 2000). To our knowledge, locomotor analyses across a dual-choice CPP procedure and within a chamber paired with natural salient stimuli (pups) contribute uniquely to CPP literature.

Rodents experience increased locomotion following cocaine injections (Wise and Bozarth, 1987; Yeh and Haertzen, 1991; Shimosato and Ohkuma, 2000) and may exhibit

locomotor sensitization after repeated injections (Post and Rose, 1976). The present study not only confirms cocaine-induced locomotion during each cocaine-conditioning session but reveals a unique time course of the induction phase of sensitization in distinct preference populations. Sensitization was induced in cocaine-preferring dams by the third conditioning day, but not in pup-preferring dams by the final (fourth) day. Pup-preferring dams may require additional drug exposures for induction to occur. This relationship between cocaine's motoric and reward (incentive) value accords with and extends others' work (Wise and Bozarth, 1987; Berridge and Robinson, 1995).

Across pup-conditioning, locomotion decreased consistently amongst cocaine-preferring and control dams. A similar reduction may emerge in pup-preferring dams with additional pup-exposures. Dams' reduced locomotion across repeated exposures to pups may represent dams' habituation to pups despite pups' ongoing development.

During the post-conditioning session, locomotor rates were consistently lower in each dam's preferred chamber than her non-preferred chamber, regardless of the identity of the stimulus (pups or cocaine) associated with each chamber. Compared to locomotion measured in single-choice CPP procedures (Brockwell et al., 1996; Russo et al., 2003a), this is the first report of a highly consistent locomotor pattern in chambers paired with two distinct stimuli. Conditioned locomotion did not emerge in the pup- or cocaine-associated chamber, the latter despite substantial locomotor sensitization on the final conditioning day. Important procedural differences may explain others' reported conditioned locomotion (Martin-Iverson et al., 1997). Locomotion across CPP thus helps to characterize the incentive salience of distinct stimuli and may predict dams' responsivity to drugs with abuse potential (Wise and Bozarth, 1987; Piazza et al., 1989).

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Figure Captions

Figure 1. Proportion of dams exhibiting preference for each chamber or lacking a chamber preference (a) and mean times spent in each chamber (b) of the three-chambered conditioned place preference (CPP) apparatus during the pre-conditioning baseline session, prior to assigning dams to different stimulus-chamber assignment groups. Both graphs include data from both postpartum groups. Proportions of dams placed into each preference category differed significantly from each other; only times in the left and center chambers differed significantly (#); both $P<0.05$.

Figure 2. Proportion of dams exhibiting preference for each chamber or lacking a chamber preference in the CPP apparatus. Dams were conditioned and tested either during early (*striped bars*) or late (*solid bars*) postpartum. Pre-conditioning chamber preferences are presented separately for each stimulus-chamber assignment group, in which either pups (a) or cocaine (b) were assigned to the least-preferred chamber in most dams. Post-conditioning chamber preferences were similar regardless of stimulus-chamber assignment group and were grouped (c). Statistically significant differences are designated with * for between-group comparisons and *corresponding numbers* for comparisons between respective pre-and post-conditioning groups; all $P<0.05$.

Figure 3. Mean time (+/- sem) spent in each chamber of the CPP apparatus by dams conditioned and tested either during early (*striped bars*) or late (*solid bars*) postpartum. Pre-conditioning chamber times are presented separately for each stimulus-chamber assignment group, in which either pups (a) or cocaine (b) were assigned to the least-preferred chamber in most dams. Post-conditioning chamber times were similar

regardless of stimulus-chamber assignment group and were grouped (c). Statistically significant differences are designated with * for between-groups and # for within-groups, and *corresponding numbers* for respective pre-and post-conditioning groups; all $P<0.05$ except (5) where $P<0.07$.

Figure 4. Locomotor scores were calculated during all four cocaine-conditioning (a) and pup-conditioning (c) sessions, and during the post-conditioning session in the side chamber associated with cocaine (b) and with pups (d). On select conditioning days and during the test session, scores differed significantly between control dams and cocaine-preferring dams (x) or pup-preferring dams (y), and between cocaine- and pup-preferring dams (*). Within each group, conditioning scores that differed from those on another day of conditioning are marked with the number of the day from which they differ, e.g. (1) if different from day 1. All $P<0.05$.

Figure 5. Proportion of control dams exhibiting preference for each chamber or lacking a chamber preference (a) and mean times spent in each chamber (b) of the CPP apparatus during the pre-conditioning (*solid bars*) and post-conditioning (*hatched bars*) sessions. Control dams were not exposed to unconditioned stimuli in the conditioning chambers, but were conditioned for identical lengths as experimental dams, either 2 hours ("pup-associated length") or 30 minutes ("cocaine-associated length"). Graphs include data from both postpartum groups. Proportions of dams placed into each preference category differed significantly from each other and differed between sessions; chamber times were equal between side chambers during both sessions, but selectively differed from the center chamber (#); all $P<0.05$.
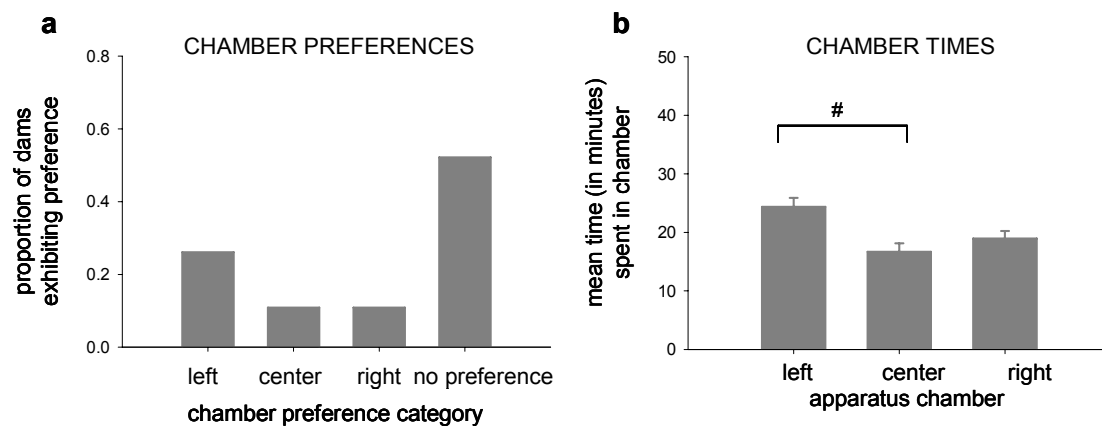
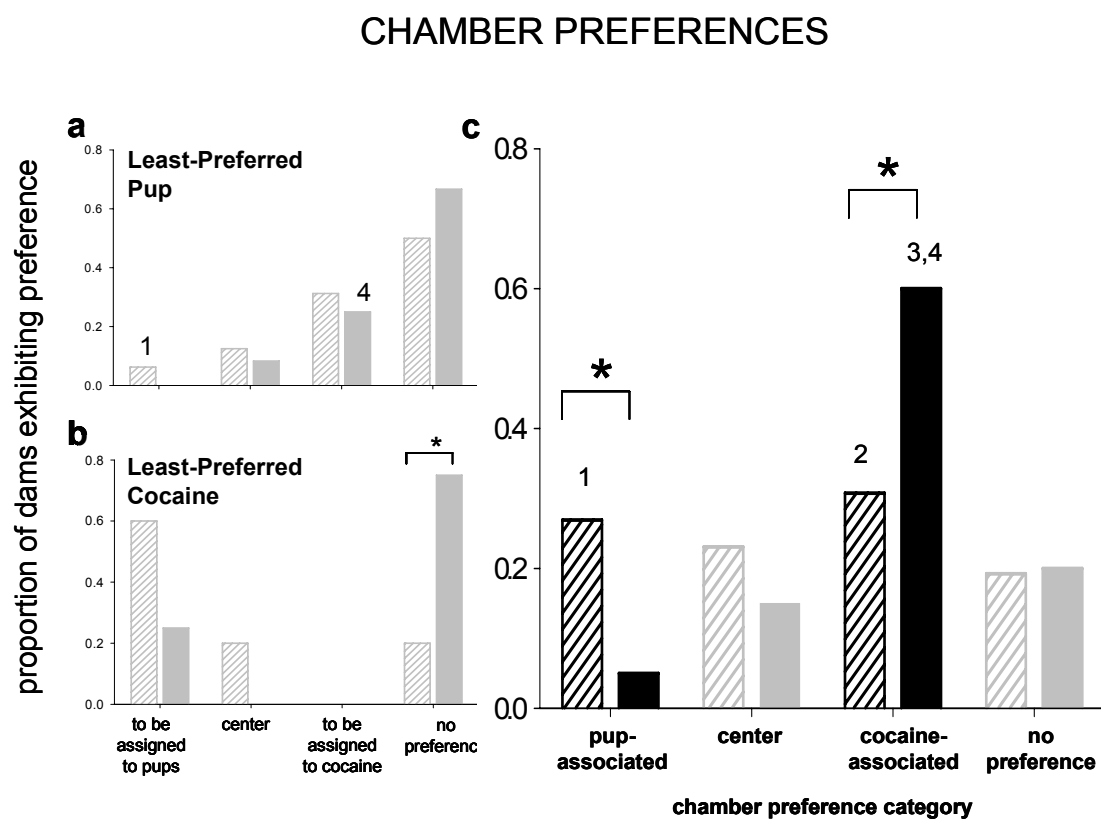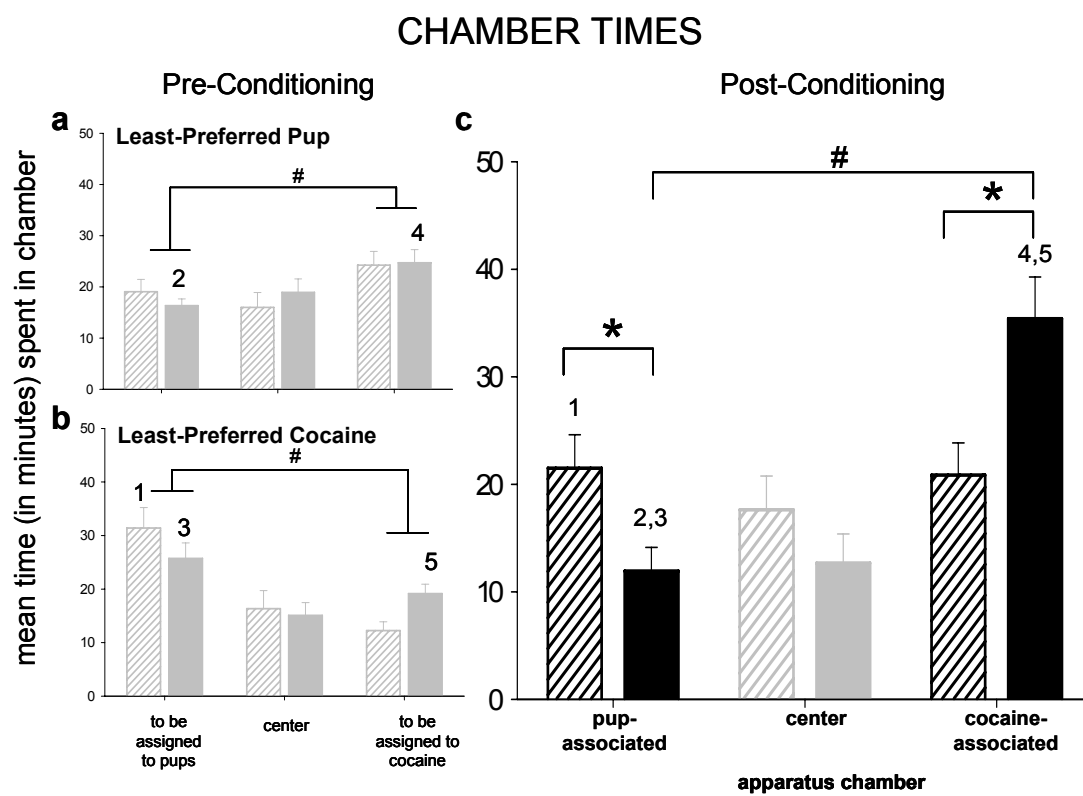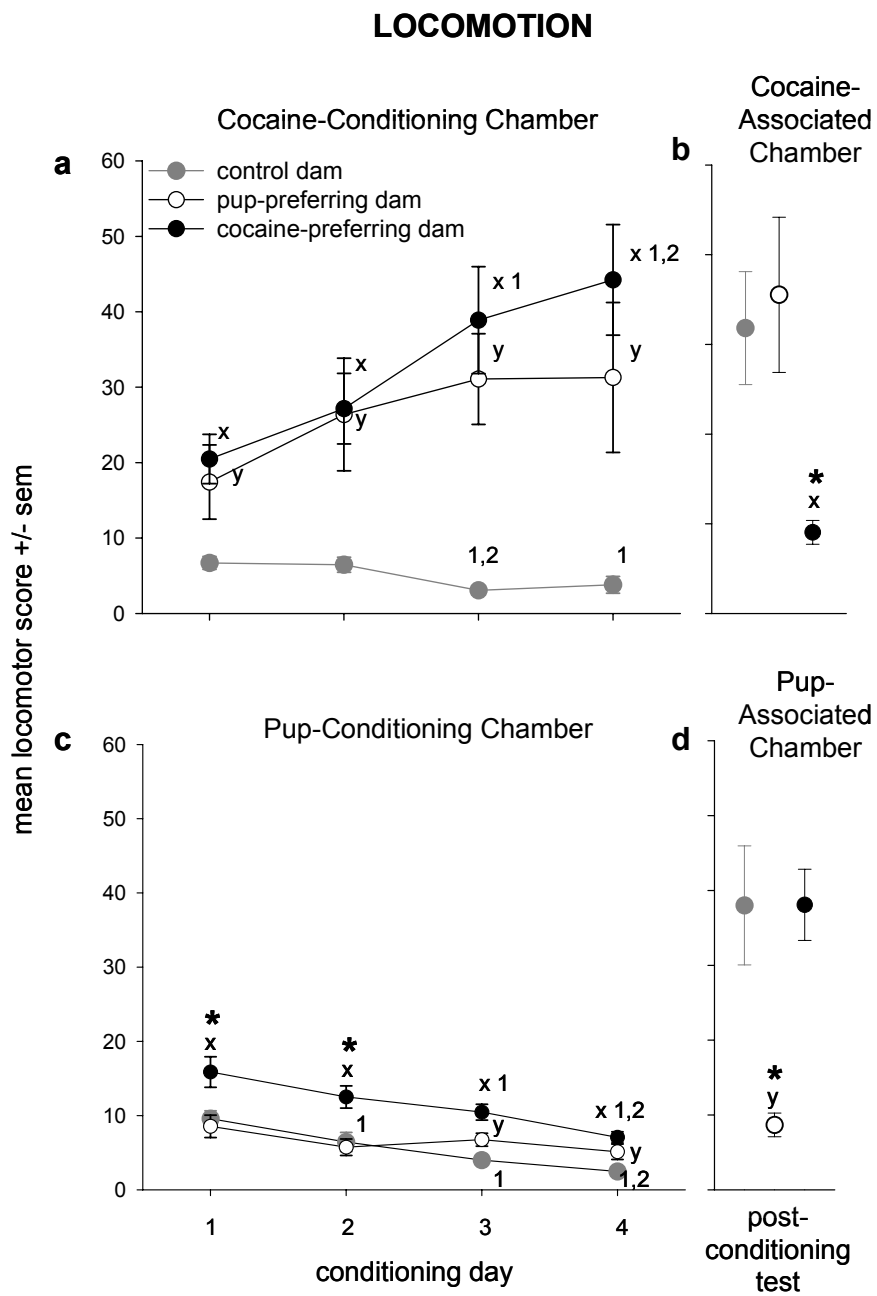Figure 1.

Figure 2.

CHAMBER PREFERENCES

Figure 3.

Figure 4.

LOCOMOTION

Figure 5.
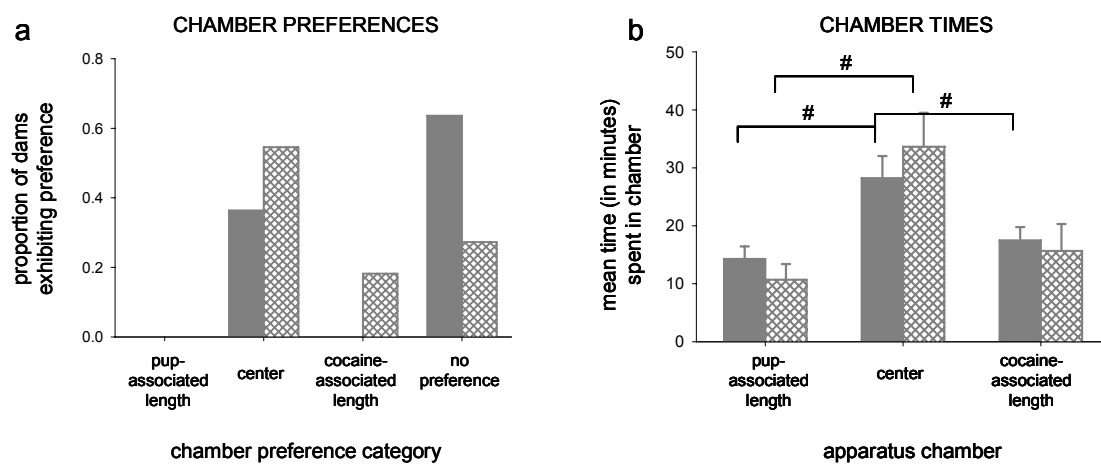
CHAPTER 2

Incentive salience of cocaine across the postpartum period of the female rat

Abstract

Rationale/Objectives: Our prior conditioned place preference (CPP) work demonstrates that late (day 16) postpartum female rats consistently prefer cocaine- over pup-associated chambers, whereas far fewer early (day 8) postpartum females prefer the cocaine-associated chamber. The present study examines early and late postpartum females' preference for a cocaine-associated chamber when contrasted with a chamber associated with saline (rather than pups). *Methods:* Postpartum females were tested for conditioned preference for chambers associated with cocaine [10mg/kg subcutaneous (SC) or 0.5, 5, 10, or 20mg/kg intraperitoneal (IP) injections] versus saline; preferences of virgin female and male rats for select cocaine stimuli (10mg/kg SC or IP) were also tested. Locomotion was recorded during CPP conditioning and testing. *Results:* Early and late postpartum females expressed strikingly similar preference for the cocaine-associated chamber across all administration routes and doses. IP cocaine produced an orderly, inverted U-shaped dose-preference curve, with preference peaking at the 5mg/kg dose (83% of females). While many postpartum females preferred 10mg/kg cocaine administered either SC or IP, both virgin females and males expressed strong aversion to SC cocaine and, while virgin females strongly preferred IP cocaine, males remained relatively indifferent. Across 10mg/kg IP cocaine-conditioning sessions, locomotor sensitization occurred exclusively in cocaine- but not saline-preferring postpartum females. Locomotor rate was lower in preferred versus non-preferred chambers at CPP test. *Conclusions:* Early and late postpartum females may be equally and uniquely susceptible to sampling and/or abuse of modestly salient doses of cocaine (10mg/kg SC; 5mg/kg IP) compared to virgin females and/or males.

Introduction

Previous dual-choice conditioned place preference (CPP) studies revealed that most late postpartum female rats consistently prefer cocaine- over pup-associated chambers, whereas few early postpartum females prefer the cocaine-associated chamber, even when cocaine's incentive salience is optimized (Mattson et al., 2001; Seip and Morrell, 2007). It remains unknown, however, whether this postpartum difference is driven by a fundamentally different response to pups, cocaine, or both stimuli in relative contrast to each other. In the present study, we closely examine postpartum preference for cocaine by presenting early and late postpartum females with a simplified CPP choice between a chamber associated with cocaine and one associated with saline, a relatively neutral stimulus with little incentive salience.

CPP is a widely used Pavlovian conditioning procedure used to assess the motivation or incentive value attributed to various natural or pharmacological stimuli, like cocaine (Mucha et al., 1982; Tzschentke, 1998, 2007; Bardo and Bevins, 2000). In CPP, the incentive value of a stimulus is represented by a subject's conditioned response to seek out the stimulus in an environment where the stimulus was previously presented but is not currently available (Schechter and Calcagnetti, 1998; Tzschentke, 2007). Thus, CPP can provide important insight into the initial phases of the postpartum female's motivated response toward cocaine while in a drug-free state.

To manipulate cocaine's incentive salience, we varied two stimulus parameters, administration route and dose, that have been demonstrated to affect conditioned preference for a drug-associated CPP chamber (Nomikos and Spyraki, 1988; Mayer and

Parker, 1993; Durazzo et al., 1994; Tzschentke, 1998; Brabant et al., 2005). Initial

cocaine stimuli were identical to those used in our prior dual-choice CPP studies

contrasting CPP for cocaine versus pups (Mattson et al., 2001; Seip and Morrell, 2007)

and were then systematically extended across a range of doses (SC 10mg/kg; IP 0.5, 5,

10, or 20mg/kg). To ensure that conditioning occurred during the most reinforcing aspect

of cocaine's temporal profile (Mayer and Parker, 1993; Ettenberg et al., 1999; Seip et al.,

2005), the length of conditioning sessions with SC and IP cocaine coincided with rising

concentrations of cocaine in serum and brain (Lau et al., 1991; Festa et al., 2004;

Wansaw et al., 2005; Niyomchai et al., 2006). Conditioning session lengths thus lasted

2hr for SC cocaine and 30min for IP cocaine. Select postpartum preferences were

compared to those of virgin female and male rats to establish key points of comparison

with well-documented CPP literature.

To provide an additional dependent measure of postpartum females' responsivity

to cocaine, locomotion was recorded during CPP conditioning and test sessions. Our

characterization of cocaine-induced locomotion and the induction phase of motoric

sensitization in the postpartum female during conditioning sessions with IP cocaine

builds upon existing dose-response locomotor analyses in males and cycling female rats

(Sell et al., 2000; Hu and Becker, 2003; Todtenkopf and Carlezon, 2006). Locomotion

during CPP testing characterizes subjects' behavioral responsivity to cocaine- and saline-

associated chambers during their expression of conditioned chamber preference, offering

unique insight into individual differences in motivation (Seip and Morrell, 2007) and

extending prior CPP work using other abused drugs (Parker, 1992).

By comparing postpartum females' incentive motivation and motoric responses to various cocaine stimuli, we can explore how the postpartum female's unique endocrinological, physiological, and behavioral status may modulate cocaine CPP. While cocaine CPP is influenced by sex (Russo et al., 2003b; Carroll et al., 2004; Jackson et al. 2006), estrus cycle stage (Roberts et al., 1989; Hecht et al., 1999), and exogenous administration of ovarian hormones (Russo et al., 2003a, 2008), little research exists on cocaine CPP across the postpartum period, a complex phase characterized by subtle fluctuations in endogenous ovarian hormones and neuroactive peptides and influenced uniquely by biologically relevant stimuli (i.e. pups). The present study constitutes the first systematic examination of cocaine's incentive value across this unique period in the natural lifecycle of the female rat.

Methods

Subjects

Virgin male (*n*=30) and female (*n*=161) Sprague-Dawley rats (90-120 days old; Charles River Laboratories, Wilmington, MA) were raised in an animal colony maintained at the Laboratory Animal Facility at Rutgers University (Newark, NJ), which is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All procedures are in compliance with the "Principles of Laboratory Animal Care" and follow the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council, 2003). Details of animal husbandry, housing, and care are described previously (Mattson et al., 2001; Seip and Morrell,

2007). All subjects remained healthy across the experiment. Postmortem examination of the peritoneal cavity, dermis/subderma, and (in postpartum females) mammary glands confirmed no cocaine-induced pathologies (Scott et al., 1997).

Females in postpartum groups (*n*=137) remained with their pups for 24hrs after parturition, without interruption or manipulation. Each litter was culled to eight pups on the morning that CPP began. Litters of early and late postpartum females that received cocaine injections all gained weight (Figure 1) and weighed the same as litters of age-matched control females that did not receive cocaine [pooled age-matched experimental and control litter weights: early, $F(4,248)=1851.9$, $P<0.0001$; late, $F(4,224)=1704.2$, $P<0.0001$; both Tukey: $P<0.05$]. Behavioral observations in females' homecages confirmed that pup retrieval, maternal nest building, and nursing proceeded normally in all postpartum females. Postmortem visual inspection of mammary glands verified that no pathological effects of cocaine developed over the course of the experiment. Brief separations of females from pups during CPP conditioning and test sessions mimicked time periods naturally spent away from the nest by mother rats (Grota and Ader, 1974; Pereira et al., 2008) and did not affect litter growth or development (Wansaw et al., in press). Thus, neither our cocaine administration parameters nor our brief female-pup deprivations negatively affected females or their litters, in accord with our prior work (Vernotica and Morrell, 1998; Mattson et al., 2001; Seip and Morrell, 2007).

Postpartum females were conditioned and tested either in early (days 4-8) or late (days 12-16) postpartum. Virgin females (*n*=24) were conditioned and tested without identifying estrous stage, as vaginal lavage can elicit CPP and attenuate females' motoric response to cocaine (Walker et al., 2002; Nazarian et al., 2004). The typical four-day

estrous cycle coincides with the length of our four-day conditioning phase, so all cycle
stages were represented equally.

Groups. Subjects either received SC (males n_{10mg} =14, virgin females n_{10mg} =8, early
postpartum n_{10mg} =18, late postpartum n_{10mg} =15) or IP injections (males n_{10mg} =16,
virgin females n_{10mg} =16, early postpartum $n_{0.5mg}$=8, n_{5mg}=12, n_{10mg}=23, n_{20mg}=12; late
postpartum $n_{0.5mg}$=6, n_{5mg}=12, n_{10mg}=22, n_{20mg}=10).

Conditioned place preference procedure

Apparatus. The custom-designed conditioned place preference apparatus (Med
Associates Inc., St. Albans, Vermont, with MED-PC$^{®}$ Version IV Research Control &
Data Acquisition System) consisted of three lidded clear Plexiglas chambers of equal
size (27.5cm W x 21cm L x 20.5cm H) that were each decorated with unique contextual
cues. Details in Seip and Morrell (2007).

Chamber preference criterion. Our prior CPP work reveals that individual rats typically
spend most of their time in one of the three apparatus chambers, after learning to
associate each chamber with a unique unconditioned stimulus. Individual categorization
of each subject's chamber preference reveals important information about how
individuals comprising a population respond to each stimulus-associated chamber.
Standard measures of chamber times, averaged across subjects with different chamber
preferences, cannot accurately represent these individual differences. We have therefore
developed a quantitative preference criterion to objectively identify each individual
subject's chamber preference (for additional details see Mattson et al., 2001; Seip and
Morrell, 2007). To meet the criterion for chamber preference, subjects must spend at

least 30min in one chamber and 25% more time in that chamber than any other chamber. Subjects that do not meet preference criterion are categorized as having no preference. We also present the mean times spent in each chamber, averaged across all subjects, to allow direct comparisons with well-established CPP literature (Tzschentke, 1998; Bardo and Bevins, 2000).

Pre-conditioning baseline session. To identify any pre-existing chamber preferences, subjects were exposed to the apparatus for a single baseline session before conditioning (day 1 or 9 for early and late postpartum females, respectively). Each subject was placed into the center chamber and allowed free access to all three chambers for 60 minutes. Neither pre-conditioning preferences nor times differed across any groups of subjects (n=191), so all data were pooled for the following pre-conditioning analyses. Prior to conditioning, most subjects (45%) lacked chamber preference and were randomly assigned to receive either stimulus (cocaine or saline) in one or the other side chambers. Of subjects preferring a side chamber, cocaine was typically assigned to their least-preferred chamber so that few subjects (16%) preferred the chamber where they would receive cocaine and more subjects (27%) preferred the chamber where they would receive saline. Subjects spent more time in the chamber where they would receive saline than in any other chamber [$F(2,191)$=10.81, P<0.0001; Tukey: P<0.05].

Conditioning phase. Subjects received a separate conditioning session with each stimulus, cocaine or saline, once a day for four consecutive days. Immediately prior to each session, subjects were injected with saline or cocaine, either subcutaneously (SC) into the dorsal flank or intraperitoneally (IP), and confined to the appropriate conditioning chamber. Conditioning sessions lasted either 2hr (SC) or 30min (IP) and

were identical for cocaine and saline sessions. Subjects were returned to their home cages after each conditioning session. Conditioning sessions started at 10:00 for saline and 15:00 for cocaine, ensuring that circulating concentrations of cocaine returned to baseline levels before subsequent conditioning sessions (Lau et al., 1991; Wansaw et al., 2005). Cocaine hydrochloride (National Institute of Drug Abuse, Research Triangle Park, NC) was freshly dissolved in daily doses of 0.5, 5, 10, or 20mg/kg cocaine (calculated as a salt) in a 0.9% saline solution. Postpartum females received an IP dose of 0.5-20mg/kg or SC dose of 10mg/kg; virgin females and males received IP or SC doses of 10mg/kg.

Post-conditioning test session. Subjects were tested for CPP for 60min on the day after the final conditioning session (for postpartum females, day 8 or 16). No cocaine or saline injections occurred. Conditioning was confirmed if, using within-subjects comparisons, subjects were differently distributed across chamber preference categories or spent different amounts of time in each chamber during the pre- and post-conditioning sessions.

Analyses and statistics

All analyses were performed as in Seip and Morrell (2007). The significance level was $P<0.05$. A Levine's test for equality of variance and Kolmogorov-Smirnov test preceded all parametric tests; non-parametric tests were used as needed.

Conditioned place preference. Preference data are presented as the percentage of individual subjects categorized into one of four preference categories: preference for the cocaine-associated, center, or saline-associated chamber, or no preference. Chamber

times (in minutes) were averaged across all subjects in each preference category and are presented as mean percentages of total session time, with standard errors of the mean (sem). *Within-group comparisons.* Pre- and post-conditioning preferences were compared within-subjects using a chi-square goodness-of-fit test for specified proportions, then a one-tailed test for significance of a proportion. Pre- and post-conditioning times were compared using two-way ANOVAs (chamber, session as repeated) or one-way ANOVAs (chamber as repeated) with Tukey's honestly significant difference post-hoc tests. Cocaine- and saline-associated chamber times pooled across dosage groups were compared using a Wilcoxon signed rank test. *Between-group comparisons.* Preferences were compared using Fisher's exact test or one-tailed tests for significance of difference between two proportions. Times were compared using one-way ANOVAs (chamber as repeated) with Tukey's post-hoc tests. Male and virgin female data was compared to pooled postpartum data, which did not differ.

Locomotion. Locomotion was recorded during CPP for IP cocaine in postpartum females. New infrared beam breaks in each side chamber were summed during each conditioning session and divided by 30min to produce a locomotor score (rate) for that session. Beam breaks in each chamber during the post-conditioning session were summed and divided by total chamber time; scores were similar across females conditioned with different IP doses and were pooled. During each conditioning and test session, scores did not differ across postpartum and were pooled.

Locomotor scores were separated by postpartum females' post-conditioning preference for the cocaine- or saline-associated chamber (cocaine-preferring females: $n=59$; saline-preferring females: $n=16$). In the 10mg/kg group, saline-conditioning scores

failed to record on day 1 in seven cocaine-preferring females and day 3 in a saline-preferring female. Scores in each chamber during conditioning were compared within-groups using two-way ANOVAs (day and chamber as repeated). Post-conditioning scores were compared between-groups using Mann-Whitney U tests and within-groups using Wilcoxon signed rank tests.

Results

Postpartum females

Subcutaneous cocaine

Confirmation of conditioning. In both early and late postpartum females, chamber preferences changed significantly between the pre- and post-conditioning session, indicating successful conditioning [early: χ^2(3, N=18)=10.59; late: χ^2(3, N=15)=10.95; both P<0.05]. Mean chamber times did not change after conditioning in either postpartum group.

Post-conditioning preferences. Following conditioning, chamber preferences were strikingly similar in early and late postpartum females. Most late (53%) and early (39%) postpartum females preferred the chamber associated with 10mg/kg SC cocaine; postpartum groups did not differ (Figure 2a). Postpartum females spent similar amounts of time in each chamber (Figure 2b).

Intraperitoneal cocaine

Confirmation of conditioning. Both before and after conditioning, chamber preferences and times did not differ between early and late postpartum females; data were

subsequently pooled. In all postpartum females, chamber preferences and times changed

significantly between the pre- and post-conditioning session, confirming conditioning in

all dosage groups [preferences: 0.5mg/kg: χ^2(3, N=14)=9.75; 5mg/kg: χ^2(3,

N=24)=393.67; 10mg/kg: χ^2(3,N=45) =31.09; 20mg/kg: χ^2(3, N=22)=437.64; all

P<0.0001 except P<0.05 for 0.5mg/kg; chamber time x session interactions: 0.5mg/kg:

F(2,26)=4.00; 5mg/kg: F(2,46)=45.29; 10mg/kg: F(2,88)= 14.50; 20mg/kg:

F(2,42)=13.10; all P<0.05]. After conditioning, more postpartum females preferred the

cocaine-associated chamber [0.5mg/kg: z=-2.19; 5mg/kg: z=-10.39; 10mg/kg: z=-3.68;

20mg/kg: z=-4.25; all P<0.0001 except P<0.05 for 0.5mg/kg] and, in all dose groups but

0.5mg/kg, females spent more time in the cocaine-associated chamber (Tukey: all

P<0.01).

Post-conditioning preferences. Regardless of dose, more postpartum females preferred

the cocaine-associated chamber than the saline-associated chamber (z=-6.45, P<0.0001)

(Figure 3a). More postpartum females (83%) preferred the chamber associated with

5mg/kg cocaine than any other dose (Fisher's exact: P<0.05; 0.5mg/kg: z=-2.56;

10mg/kg: z=-3.14; 20mg/kg: z=-2.69; all P<0.01). Females also spent more time in the

cocaine-associated chamber than the saline-associated chamber (Wilcoxon signed rank =

1718, P<0.0001) (Figure 3b), spent substantially more time in the cocaine-associated

chamber paired with 5mg/kg than with 0.5mg/kg, and spent less time in the saline-

associated chamber in the 5mg/kg group than the 0.5mg/kg or 20mg/kg groups [chamber

x group interaction: F(6, 202)=2.91, P<0.01; Tukey: all P<0.05] (Figure 3b).

Selective preference re-testing. Some postpartum females were retested once for cocaine

CPP. Most (80%) cocaine-preferring females retained their initial chamber preference

when retested either after three ($n_{20mg/kg}$=24; z=1.66, P=0.05) or seven days ($n_{5mg/kg}$=5; P>0.05).

Subcutaneous versus intraperitoneal cocaine

More early postpartum females preferred chambers associated with IP cocaine (52%) than SC cocaine (39%) at 10mg/kg (z=-10.98, P<0.0001), whereas late postpartum females equally preferred chambers associated with IP (59%) and SC (53%) cocaine. Postpartum females spent more time in the cocaine-associated chamber and less time in the saline-associated chamber when cocaine was injected IP versus SC [main route effect: early, $F(1,39)$=147.0; late, $F(1,35)$=56.0; both P<0.00001; Tukey: P<0.001].

Locomotor responses to intraperitoneal cocaine

Conditioning. Across conditioning sessions with 0.5 or 5mg/kg cocaine, locomotor scores were similar regardless of whether females were injected with cocaine or saline and regardless of eventual chamber preference (Figure 4a-b). Across conditioning sessions with 10 or 20mg/kg cocaine, locomotor scores were higher following cocaine injections than saline injections in cocaine-preferring females [10mg/kg: chamber x day interaction $F(3,54)$=5.88, P<0.01; Tukey: all P<0.001; 20mg/kg: main chamber effect $F(1,9)$=40.08, P<0.001; Tukey: all P<0.05] but higher in saline-preferring females only with 20mg/kg cocaine [main chamber effect, $F(1,5)$=37.97, P<0.01; Tukey: P<0.05] (Figure 4c-d). Following injections of 10mg/kg cocaine, scores of cocaine-preferring females increased substantially across the four conditioning sessions (Tukey: all P<0.001), while scores of saline-preferring females remained constant (Figure 4c).

Post-conditioning test. Locomotor scores of postpartum females administered various IP cocaine doses during cocaine-conditioning were similar in each stimulus-associated chamber and thus pooled. Cocaine-preferring females' scores were substantially lower in the cocaine- than saline-associated chamber (T=20.0, P<0.0001), whereas the converse was true of saline-preferring females' scores (T=10.0, P<0.01) (Figure 5). In the cocaine-associated chamber, cocaine-preferring females' scores were lower than those of saline-preferring females (U=159.0, P<0.0001), whereas the converse was true in the saline-associated chamber (U=68.0, P<0.0001).

Males and virgin females

Subcutaneous cocaine

Confirmation of conditioning. After conditioning, preferences changed significantly in both male and virgin female rats [males: χ^2(3,N=14)=111.92; females: χ^2(3,N=8)=1106.80; both P<0.001]. Chamber times only changed in males [chamber x session interaction, F(2,26)=5.20, P<0.05].

Post-conditioning preferences. No males or virgin females preferred the cocaine-associated chamber at test (Figure 6a). More males (79%) than females (38%) preferred the saline-associated chamber (z=1.93, P=0.05). Males also spent less time in the cocaine-associated chamber and more time in the saline-associated chamber than virgin females [main group effect, F(1,20)=7.0; P<0.05, Tukey: all P<0.001] (Figure 6c). Males spent more time in the saline- than cocaine-associated chambers [chamber x session interaction, F(2,26)=5.20, P<0.05; Tukey: P<0.001].

Comparison to postpartum females. More postpartum females preferred the chamber associated with 10mg/kg SC cocaine than did males or virgin females [males: z=-3.02; females: z=-2.37; both $P<0.01$] (Figure 6a). Fewer postpartum females than males preferred the saline-associated chamber (z=19.44, $P<0.0001$), and postpartum females spent more time in the cocaine-associated chamber and less time in the saline-associated chamber than males [chamber x group interaction: $F(2,90)$=7.78; $P<0.001$; Tukey: $P<0.05$] (Figure 6c). Chamber times differed between virgin and postpartum females, but no interaction emerged [main group effect: $F(1,39)$=31.92; $P<0.0001$].

Intraperitoneal cocaine

Confirmation of conditioning. Chamber preferences and times differed after conditioning in male and virgin females [males: $\chi^2(3, N=16)$=1560.41, $P<0.0001$; main session effect, $F(1,15)$=7.52, $P<0.05$; females: $\chi^2(3, N=16)$=273.14, $P<0.0001$; chamber time x session interaction, $F(2,30)$=9.00, $P<0.01$]. More virgin females preferred the cocaine-associated chamber (z=-2.62, $P<0.01$) and spent more time in that chamber after conditioning (Tukey: $P<0.05$).

Post-conditioning preferences. More virgin females (56%) than males (19%) preferred the cocaine-associated chamber (z=-2.19, $P<0.05$) (Figure 6b). Virgin females spent more time in the cocaine- than saline-associated chamber and spent more time than males in the cocaine-associated chamber [chamber x group interaction, $F(2,60)$=3.00, $P<0.05$; Tukey: all $P<0.001$] (Figure 6d).

Comparison to postpartum dams. More postpartum females than males preferred the chamber associated with 10mg/kg IP cocaine (Fisher's exact: $P<0.05$; z=-2.55, $P<0.01$)

(Figure 6b). Postpartum females also spent more time in the cocaine-associated chamber and less time in the center chamber than males [$F(2,118)=5.86$, $P<0.01$; Tukey: $P<0.05$] (Figure 6d). Virgin and postpartum females expressed remarkably similar chamber preferences and times (Figure 6b,d).

Subcutaneous versus intraperitoneal cocaine

In both males and virgin females, post-conditioning chamber preferences and times differed as a result of cocaine's administration route (SC versus IP) (Fisher's exact: males, $P<0.001$; females, $P<0.05$; chamber time x route interaction: males, $F(2,56)=13.11$; females, $F(2,44)=8.56$; both $P<0.001$]. Both males and virgin females spent more time in the chamber associated with IP cocaine than with SC cocaine (Tukey: both $P<0.001$).

Discussion

Early and late postpartum females expressed strikingly similar patterns of cocaine-associated chamber preference. Postpartum preference for cocaine was substantial, with many postpartum females preferring cocaine whether administered SC or IP (10mg/kg) and across a wide range of IP doses (0.5-20mg/kg). More postpartum females preferred SC cocaine than did males or virgin females; postpartum females' robust preference for IP cocaine was similar to that of virgins but superseded that of males at 10mg/kg. Postpartum preference persisted for at least a week. Together, our results reveal an unexpected and unique vulnerability to cocaine's incentive value in the postpartum female that remains remarkably stable across the postpartum period.

The present study also reveals a notable, inverted U-shaped cocaine dose-CPP response curve in postpartum females. Nearly all postpartum females (83%) expressed robust chamber preference for 5mg/kg IP cocaine, but only approximately half of the postpartum females preferred the cocaine-associated chamber associated with higher doses (10-20mg/kg) and the lowest (0.5mg/kg) doses tested. Although CPP has been considered to be relatively insensitive to dose manipulations (Bevins, 2005; Bevins and Cunningham, 2006), the present study supports previous reports of consistent relationships between varied drug doses and CPP or CPP effect size (Bardo et al., 1995; Roma et al., 2006; Davis et al., 2007; Rezayof et al., 2007).

That postpartum females expressed strikingly similar preferences for cocaine suggests that the distinct hormonal profiles characterizing early and late postpartum, including dynamically fluctuating concentrations and unique combinations of gonadal and lactogenic hormones, do not impact cocaine CPP. Early postpartum is characterized by very low concentrations of estrogen and high concentrations of progesterone and prolactin; concentrations gradually shift back to the normal cycling state during late postpartum (Grota and Eik-Nes, 1967; Smith and Neill, 1977; Taya and Greenwald, 1982). Although low estrogen concentrations are associated with reduced cocaine self-administration (Roberts et al., 1989; Hecht et al., 1999) and high progesterone concentrations with attenuated cocaine CPP (Russo et al., 2003a, 2008), cocaine CPP remains nearly identical in early and late postpartum, suggesting that cocaine CPP may be relatively insensitive to subtle hormonal changes occurring across the postpartum period. Collectively, however, we believe that the unique endogenous combinations and concentrations of gonadal and lactogenic hormones present across postpartum may

establish a prolonged period that subtly alters and increases the incentive value of cocaine.

Postpartum females are substantially more likely than virgin females or males to attribute incentive value to cocaine. While many postpartum females strongly preferred SC cocaine, both males and virgin females expressed dramatic place aversion to this cocaine stimulus. These findings accord with prior work indicating that male rats prefer cocaine-associated chambers only at relatively low SC doses (0.32-3.2mg/kg) (Mayer and Parker, 1993; Durazzo et al., 1994) and, along with virgin females, find higher SC doses aversive (5-32mg/kg) (van Haaren and Hughes, 1990; Busse et al., 2005). That a striking proportion of our postpartum females preferred SC cocaine at 10mg/kg may point to heightened responsivity to the reinforcing properties of cocaine or blunted response to the aversive qualities of cocaine that is unique to the postpartum state.

It is important to note that, while IP cocaine was preferred equally by both postpartum and virgin females, our strict preference criteria identified very few males that strongly preferred IP cocaine, representing a select point at which cocaine's incentive salience is dissociable by gender. Prior work confirms that males display place aversion or very modest preference for 10mg/kg IP cocaine but strongly prefer higher IP doses (20mg/kg) (Nomikos and Spyraki, 1988; Mayer and Parker, 1993; Russo et al., 2003b), whereas postpartum and virgin females prefer IP cocaine at lower doses (5-10mg/kg) (Russo et al., 2003a, 2003b; present study). This sharp gender contrast may be partially attributable to global endocrinological, physiological and/or pharmacokinetic differences between males and females, previously proposed to underlie cycling females' heightened vulnerability to cocaine's motoric and motivational effects (Becker, 1999;

Kuhn et al., 2001; Chin et al., 2002; Hu and Becker, 2003; Russo et al., 2003a, 2003b; Carroll et al., 2004; Festa et al., 2004; Festa and Quinones-Jenab, 2004). Notably, cocaine pharmacokinetics remain strikingly consistent across postpartum and cycling states following cocaine administration parameters similar to those used here (Vernotica and Morrell, 1998; Wansaw et al., 2005; see Dwivedi et al., 1993 for recently parturient females).

One remaining factor contributing uniquely to the incentive-motivational state of the postpartum female, however, is the continuous presence of offspring (pups). Pups are a critical and salient biological stimulus present exclusively in the postpartum females' environment. The postpartum female is uniquely and highly responsive to pups: she vigorously performs maternal caregiving behaviors toward her pups across the postpartum period and attributes considerable incentive salience to pups during early postpartum (Wilsoncroft, 1969; Lee et al., 2000; Mattson et al., 2001). Pup-directed maternal behaviors substantially increase dopaminergic activity in the nucleus accumbens (Hansen et al., 1993; Champagne et al., 2004), a mesolimbic region closely tied to motivational processing, goal-directed behaviors (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Salamone and Correa, 2002) and, recently, cocaine-associated chamber preference (Mattson and Morrell, 2005). As dynamic endogenous changes in the female's endocrine state also modulate mesolimbic responsivity (Bazzett and Becker, 1994; Bakowska and Morrell, 1995), both pup-induced and endocrine-associated changes in dopaminergic tone across postpartum may contribute to cocaine's unique incentive salience in the postpartum female.

The present findings contribute meaningfully to an emerging body of evidence suggesting that pups may actually reduce females' preference for cocaine exclusively during early postpartum. When postpartum females were offered a choice between chambers associated with age-matched pups or with one of two cocaine stimuli examined in the present study (10mg/kg SC, Mattson et al., 2001; 10mg/kg IP, Seip and Morrell, 2007), substantially fewer early postpartum preferred cocaine than when the same cocaine stimuli was contrasted with saline (present study); late postpartum preference for cocaine remained unchanged. We posit that the high incentive value of young pups, naturally present in the early postpartum female's environment, actively competes with cocaine's incentive value during the postpartum period, a critical time in which offspring are extremely dependent on motivated caregiving by the female.

Locomotion across the CPP procedure

The present analyses importantly extend our characterization of cocaine-induced locomotion in the postpartum female (Vernotica and Morrell, 1998; Wansaw et al., 2005) during the CPP procedure, providing important points of comparison with the well-documented behavioral effects of cocaine in virgin female and male rats (Sell et al., 2000; Chin et al., 2002; Festa and Quinones-Jenab, 2004; Festa et al., 2004). Following 10mg/kg IP cocaine injections, locomotor sensitization emerged rapidly in cocaine-preferring but not saline-preferring postpartum females after only four exposures, replicating the induction phase of sensitization reported by others (Post and Rose, 1976) and supporting proposed correlations between cocaine's motoric and motivational effects (Wise and Bozarth, 1987). This locomotor sensitization emerged using short (30min) conditioning sessions, indicating that longer (60-120min) exposures are not necessary to

produce sensitization to 10mg/kg IP cocaine (Todtenkopf and Carlezon, 2006). Locomotor sensitization did not emerge in cocaine-preferring females given 20mg/kg IP, possibly due to the number of subjects (i.e. power) or relatively large variability.

Interestingly, a clear dissociation between cocaine's motoric and motivational (i.e. CPP) effects emerged exclusively at low IP doses. While 5.0mg/kg IP cocaine produced little locomotor activation, it elicited the strongest preference in the majority of postpartum females. This dissociation may be partially attributable to dopaminergic activity. D2-like dopamine receptors in the nucleus accumbens and caudate-putamen are thought to be involved in cocaine-induced locomotion but not cocaine CPP (Baker et al., 1996), but the reverse is true for D1-like receptors (Baker et al., 1998). We speculate that changes in D2-like dopamine receptor activity associated with the maternal state may limit cocaine-induced locomotion without affecting CPP.

During the post-conditioning test session, locomotor scores of postpartum females were dramatically lower in their preferred chamber, as in prior work (Parker, 1992; Seip and Morrell, 2007), and regardless of dose. Females displaying large cocaine-induced increases in locomotion (10-20mg/kg) did not display conditioned locomotion during the test, contrary to others' findings (Martin-Iverson et al., 1997). These scores provide a novel means of characterizing conditioned place preference for natural (Seip and Morrell, 2007) and pharmacological stimuli (Parker, 1992) across a range of doses, and may help predict preference for stimuli such as drugs of abuse.

We posit that these findings reveal a unique postpartum vulnerability to cocaine's incentive value that remains remarkably stable across the entire postpartum period.

Notably, more postpartum females attribute incentive value to various cocaine stimuli than do their virgin female and male counterparts, suggesting that the maternal state is not sufficient to offer natural protection against competing drug stimuli available in a female's environment. We posit that the unique hormonal, physiological, and behavioral factors characterizing the maternal state may contribute to the postpartum females' heightened motivation to seek out and interact with drugs with abuse potential. While the subtle hormonal fluctuations occurring across postpartum may preclude a notable difference in cocaine CPP, our present data, together with that of others, suggest that specific combinations of estrogen and progesterone may be one critical determinant of cocaine's incentive value. Identifying additional dynamic factors that contribute to postpartum females' heightened attribution of incentive salience to various cocaine stimuli, e.g. dopaminergic tone and offspring presence (Pereira et al., 2008), gain added urgency in order to protect this critical period in which the female's behavior directly helps or harms her offspring.

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Figure Captions

Figure 1. Percentage of average weight gained by each 8-pup litter of early (*circles*) and late (*triangles*) postpartum females. Mean weights (in grams) and standard error of the mean recorded on the morning of each day are listed for litters of experimental females (*black shapes*) and age-matched litters of control females, which received no injections but were deprived of pups for similar amounts of time (*grey shapes*). Weights of experimental and control litters did not differ on any day, but all weights increased significantly on each day in each group (*). All $P<0.05$.

Figure 2. Preferences and times for chambers associated with **subcutaneous cocaine** in the postpartum female rat. The percentage of early (*light grey*) and late (*dark grey*) postpartum females categorized into each of four chamber preference categories (a) and mean percentage of total session time that females spent in each apparatus chamber (b) during the post-conditioning session, after conditioning with subcutaneous (SC) injections of 10mg/kg cocaine and saline. No differences existed across postpartum.

Figure 3. Preference and times for chambers associated with **intraperitoneal cocaine** in the postpartum female rat. The percentage of early (*light grey*) and late (*dark grey*) postpartum females categorized into each of four chamber preference categories (a) and mean percentage of time that females spent in each apparatus chamber (b) during the post-conditioning session, after conditioning with intraperitoneal (IP) injections of cocaine and saline. During conditioning, IP cocaine was administered at one of four

doses, either 0.5, 5, 10, or 20mg/kg; doses are listed on bars representing cocaine-associated preference, with graphical order of doses remaining consistent across all chambers and preference categories. No differences existed across postpartum at any dose. * denotes significant difference between doses; # denotes significant difference between chambers; all $P<0.05$.

Figure 4. Locomotor response to four doses of intraperitoneal cocaine across conditioning sessions in the postpartum female rat. Mean locomotor scores during each conditioning session within each conditioning-chamber, calculated separately for cocaine-preferring postpartum females (*black shapes*) ($n_{0.5mg/kg}=4$; $n_{5mg/kg}=20$; $n_{10mg/kg}=25$; $n_{20mg/kg}=10$) and saline-preferring postpartum females (*white shapes*) ($n_{0.5mg/kg}=5$; $n_{5mg/kg}=1$; $n_{10mg/kg}=4$; $n_{20mg/kg}=6$). Circles represent cocaine-conditioning sessions (COC) and triangles represent saline-conditioning sessions (SAL). * denotes significant differences between cocaine- and saline-conditioning sessions within the same preference group, while numbers denote significantly higher scores than those on day 1 and 1-2, respectively; all $P<0.05$.

Figure 5. Locomotor response to intraperitoneal cocaine during the post-conditioning (test) session. Scores did not differ across postpartum group or cocaine dose and were pooled. Mean locomotor scores in each stimulus-associated chamber during the 1hr post-conditioning test session were calculated separately for cocaine-preferring postpartum females *(black)* ($n=59$) and saline-preferring postpartum females *(white)* ($n=16$). # denotes significantly differences between-groups and * for within-groups; all $P<0.05$.

Figure 6. Preference and times for chambers associated with **subcutaneous or intraperitoneal cocaine** in postpartum female, virgin female, and male rats. No differences existed across postpartum, so data from all postpartum females were pooled for these comparisons. The percentage of males (*striped*), virgin females (*white*), and postpartum females (*black*) categorized into each of four chamber preference categories (a,c) and mean percentage of time that males, virgin females, and postpartum females spent in each apparatus chamber (b,d) during the post-conditioning session, after conditioning with either subcutaneous (a-b) or intraperitoneal (c-d) injections of 10mg/kg cocaine and saline. Significant differences are marked with * for between-groups; # for within-groups; all $P<0.05$.
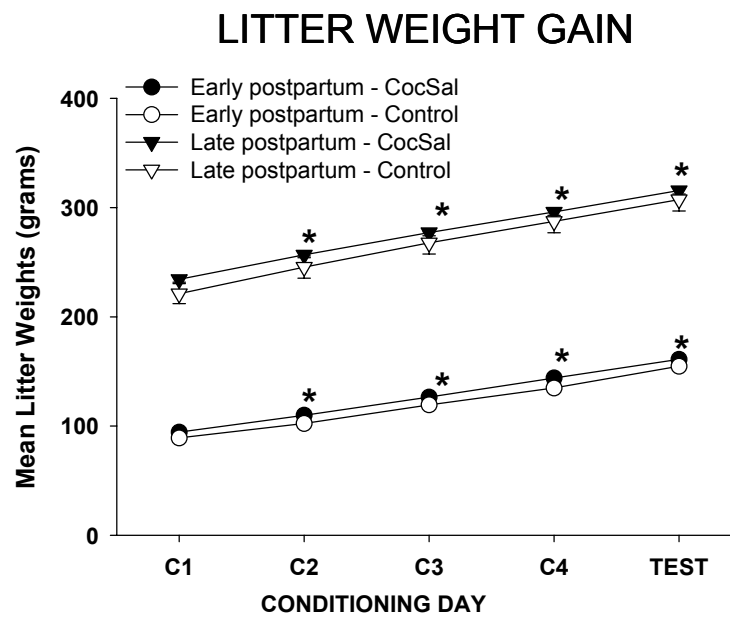
Figure 1.

LITTER WEIGHT GAIN

Figure 2.

a. **CHAMBER PREFERENCES**

b. **CHAMBER TIMES**

Figure 3.

CHAMBER PREFERENCES

a.

Y-axis: Percentage of subjects (0, 20, 40, 60, 80, 100)

Legend: Early postpartum / Late postpartum

Bar labels: 0.5, 5, 10, 20

X-axis: Chamber preference category — Cocaine-associated, Center, Saline-associated, No preference

CHAMBER TIMES

b.

Y-axis: Mean percentage of time +/- SEM (0, 20, 40, 60, 80)

Bar labels: 0.5, 5, 10, 20

X-axis: Apparatus Chamber — Cocaine-associated, Center, Saline-associated

Figure 4.

LOCOMOTION across CONDITIONING

Figure 5.

LOCOMOTION during PREFERENCE TESTING
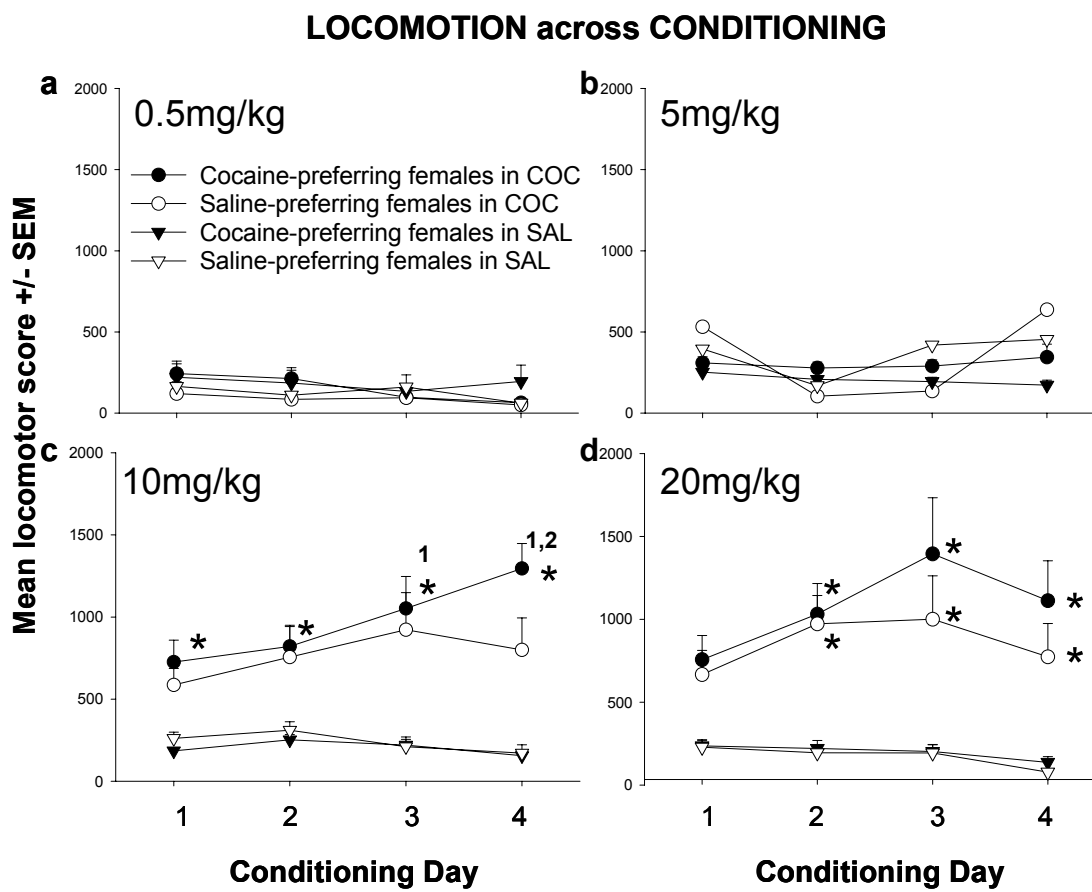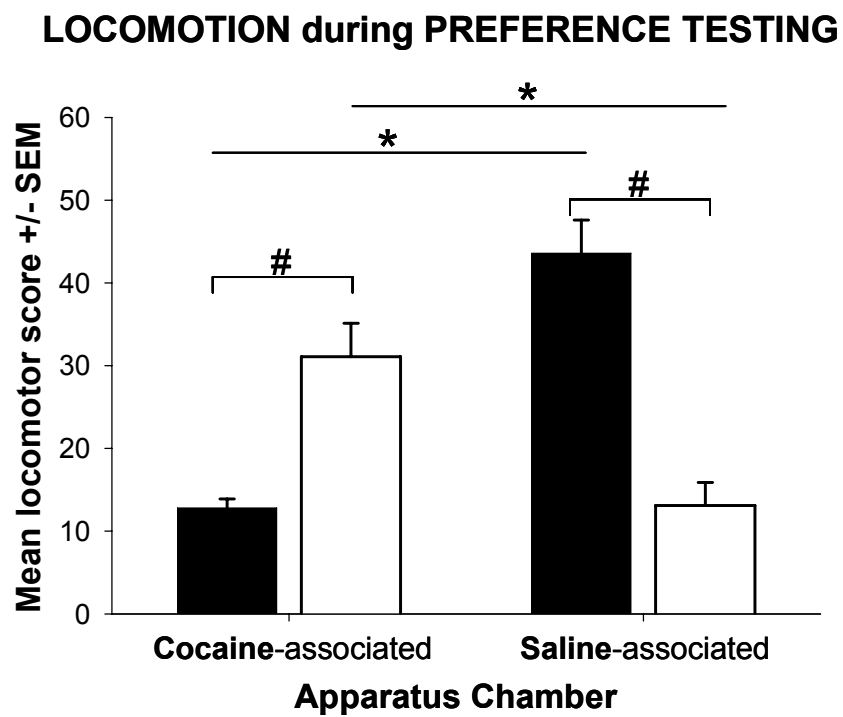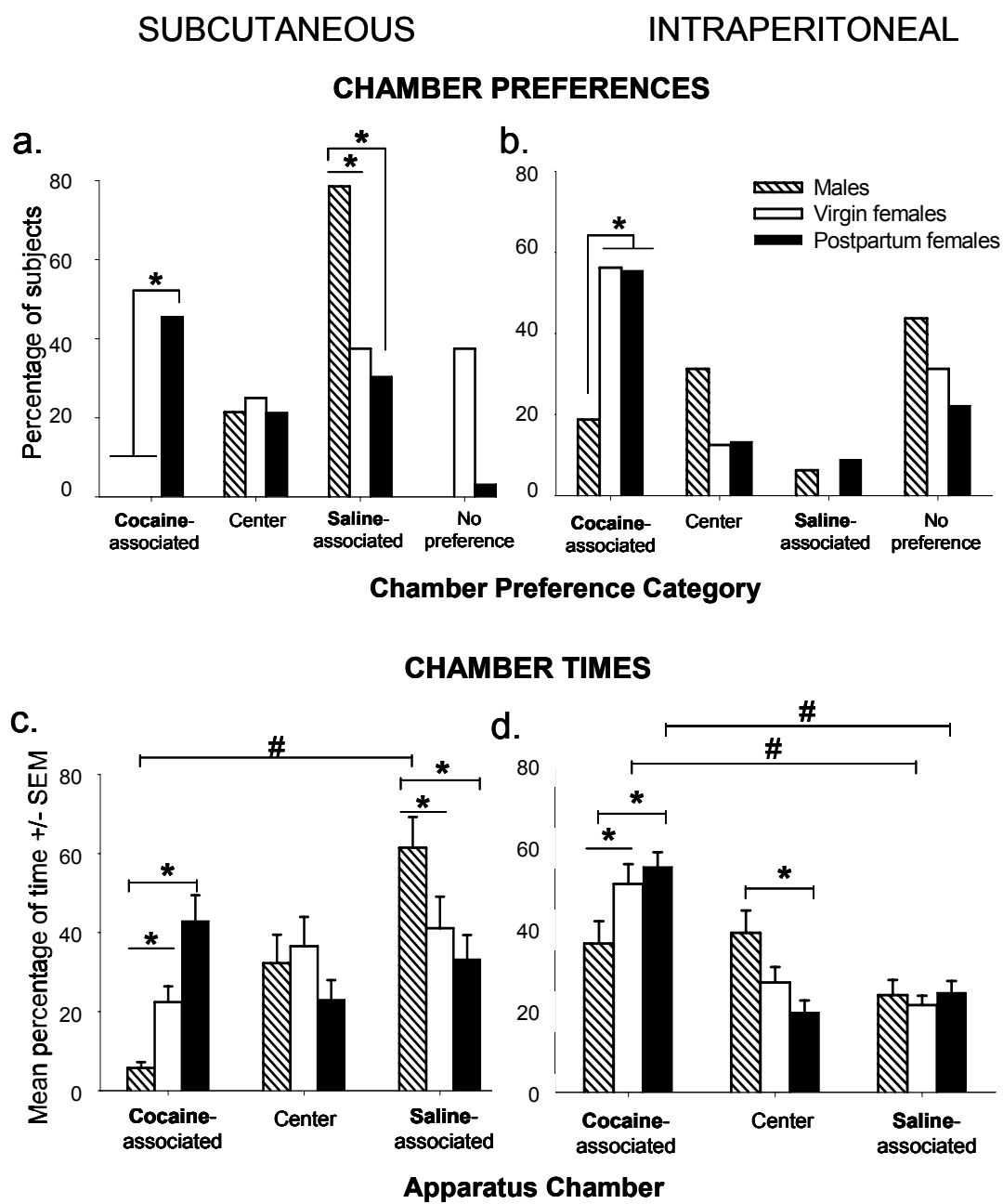
Figure 6.

CHAPTER 3

Exposure to pups influences the strength of maternal motivation in virgin female rats

Abstract

Following repeated exposure to foster pups, virgin female rats acquire and eventually express a full spectrum of maternal caretaking behaviors directed toward pups. Though these behaviors are vigorous, these females are reportedly less motivated to seek out and interact with pups (i.e. maternally motivated) than parturient females during early postpartum. The present study systematically assesses how the length of pup exposure and nature of interactions between the female-pup dyad affect maternal motivation in the virgin female rat. Virgin females were exposed to young pups consistently (24h/day) across a prolonged period (21days), briefly (1h/day) across a relatively brief period (7days), or distally (pups inaccessible in mesh bag). During final pup-exposure days, females were conditioned and tested for their preference for a pup-associated chamber (e.g. maternal motivation) using conditioned place preference. Early postpartum females provided a comparison group. Fully maternal behavior only emerged in females given prolonged pup-exposure; this behavior improved significantly over time and was maximally expressed for a duration equivalent to early postpartum. Females given brief pup-exposure expressed only emergent maternal behaviors initiated by pups; distal pup-exposure evoked pup-avoidance. Virgin females given prolonged or brief pup-exposure expressed substantial pup-associated chamber preference, with more females preferring the pup-associated chamber following longer pup-exposures in a subtle stepwise relationship. Maternal motivation was strikingly similar in prolonged pup-exposure virgin and early postpartum females. Females given distal pup-exposure completely lacked maternal motivation. Maternal behavior did not predict chamber preference.

Results suggest that pup-exposure, regardless of length, is sufficient to support strong maternal motivation, whereas parity is not required.

Introduction

Offspring (pups) elicit strongly motivated, rigorous caregiving behaviors from the maternally responsive female rat (Numan and Insel, 2003). In the postpartum female, the performance of these maternal behaviors coincides with a strong drive to seek out and interact with pups, or maternal motivation. Early postpartum females voluntarily spend the vast majority of their time with their pups (Pereira et al., 2007, 2008; Grota and Ader 1969, 1974; Reisbick et al., 1975), bar-press insatiably for access to pups (Wilsoncroft, 1969; Lee et al., 2000), prefer a pup-associated context over a cocaine-associated context (Mattson et al., 2001; Seip and Morrell, 2007), and readily retrieve pups from anxiety-provoking areas (Bridges et al., 1972; Stern and Mackinnon, 1976; Pereira et al., 2005). The complex motivational state of the early postpartum female not only promotes the expression of maternal behaviors critical to the development and survival of her offspring, but also promotes vigorous pup-seeking behavior when her pups are absent or unavailable. This maternal motivation is highly adaptive during the early postpartum period and thus may only emerge in females that have recently given birth.

Maternal caregiving behaviors, however, can be induced in virgin (nulliparous) female and male rats following continuous exposure to pups (Rosenblatt, 1967; Terkel and Rosenblatt, 1971; Fleming and Rosenblatt, 1974). Constant (24h/day) exposure to young pups typically induces stable maternal behavior in the adult, gonadally intact

virgin female after 2-9 days (Rosenblatt, 1967; Terkel and Rosenblatt, 1971; Bridges et al., 1972; Fleming and Rosenblatt, 1974; Mayer and Rosenblatt, 1979; Mayer et al., 1979; Fleming et al., 1994; Olazabal et al., 2002); these females do not need to experience gestation, parturition, or lactation (i.e. parity) to behave maternally (Numan and Insel, 2003; Rosenblatt, 1967; Terkel and Rosenblatt, 1971; Fleming and Rosenblatt, 1974). Importantly, the pup-induced maternal behavior expressed by these maternally responsive virgin females includes most components of the naturally occurring maternal behavior displayed by postpartum females (Bridges et al., 1972; Lonstein et al., 1999) and can remain in place over extended periods of time (Fleming and Sarker, 1990). The present study uses the pup-exposed virgin female as a unique model to explore whether pup-exposure also promotes maternal motivation outside of the influences of parity.

Limited evidence suggests that maternal motivation is substantially weaker in the maternally responsive virgin female than in the postpartum female. Maternally responsive virgins retrieve pups more slowly in both familiar and anxiety-provoking environments (Bridges et al., 1972; Stern and Mackinnon, 1976; Pereira et al., 2005; Lonstein et al., 1999) and lick pups less frequently than do early postpartum females (Lonstein et al., 1999). In the absence of pups, maternally responsive virgin females express less pup-seeking behavior than do early postpartum females, only preferring a pup-associated chamber after extensive deprivation from pups (Fleming et al., 1994); such extensive pup-deprivation periods bolster maternal motivation even in females in late postpartum that spend little voluntary time around pups (Grota and Ader 1969, 1974; Reisbick et al., 1975; Wansaw et al., 2008).

To assess the virgin females' motivation to seek out and interact with pups in their absence, we use a well-established conditioned place preference (CPP) procedure to compare virgin and postpartum females' conditioned preference for a pup-associated chamber, considered to reflect the incentive-motivational value of pups and thus a female's maternal motivation (Mattson et al., 2001; Seip and Morrell, 2007; Wansaw et al., 2008). Work by Fleming and colleagues suggests that both virgin and postpartum females must be exposed to pups in their homecage and express fully maternal behavior in order to prefer the pup-associated chamber (Fleming et al., 1994); further, females must be allowed to interact physically with pups during CPP conditioning in order to prefer the pup-associated chamber (Magnusson and Fleming, 1995). However, the length of pup exposure and nature of the direct physical interactions between the female-pup dyad have not been systematically assessed with respect to maternal motivation. We posit that extending the length of time that virgin females are exposed to pups will enhance the incentive-motivational value of pups (Salamone and Correa, 2002; Berridge, 2004, 2007), resulting in strong preference for the pup-associated chamber in females given prolonged pup-exposure and little chamber preference in females given limited or no pup-exposure.

Virgin females were exposed to young pups using one of three pup-exposure regimens: either consistently across a prolonged period (24h/day for 21 days), briefly across a relatively brief period (1h/day for 7 days), or distally, with pups remaining inaccessible in a mesh bag (1h/day for 4 days). The novel prolonged pup-exposure regimen closely replicates the consistent homecage exposure to pups and the intensive time naturally spent with pups during the highly motivated early postpartum period

(Pereira et al., 2008); accordingly, prolonged-exposure females were only deprived of pups for minimal, behaviorally relevant lengths of time prior to CPP. The brief pup-exposure regimen represents an important, early behavioral transition from pup avoidance to tolerance but prior to the emergence of full maternal behavior, addressing for the first time whether a motivational transition occurs concurrently during this early exposure period. Distal pup-exposure evokes neither maternal behavior nor motivation, replicating previous work (Magnusson and Fleming, 1995). All females were scored daily on pup retrieval, nest-building, and crouching/hovering over pups, to characterize the expression of maternal behaviors across pup-exposure regimens and during the CPP procedure.

To our knowledge, this is the first study to systematically manipulate the length of pup exposure in virgin females, relative to conditioned preference for a pup-associated chamber, and is only the second (Fleming et al., 1994) study to assess maternal motivation in virgin females using CPP.

Methods

Animals

Subjects (*n*=56) were 90-120 day-old female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) raised in an animal colony maintained at the Laboratory Animal Facility at Rutgers University, accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All procedures comply the "National Institutes of Health Guide for Care and Use of Laboratory Animals" [29] and

are approved by the Rutgers University Animal Care and Facilities Committee. Females were moved into a quiet testing suite 2-5 days prior to the initial pup exposure (virgins females, *n*=32) or parturition (primiparous females, *n*=24). Each female was housed individually in opaque shoebox homecages (25.5cm W x 47cm L x 23cm H) with woodchip bedding and food and water ad libitum. Virgin and parturient females' homecages were adjacent so that virgin females were exposed to olfactory and auditory stimuli associated with pups as their parturient neighbors experienced parturition and initiated maternal caregiving. A 12hr:12hr light:dark cycle was maintained (lights on 0700). Females remained healthy and pups gained weight and developed normally across the experiment (Seip and Morrell, 2007; Seip et al., 2008). Females were not tested for estrous cycle stage, as vaginal lavage can elicit CPP (Walker et al., 2002).

Pup-exposure procedures.

Virgin females were assigned to receive either prolonged (*n*=12), brief (*n*=10), or distal (*n*=8) exposure to pups prior to the conditioned place preference (CPP) procedure. Pups were provided by parturient, lactating females (*n*=10) and, by carefully controlling the precisely timed births of foster litters, always ranged between 1-8 days old to elicit maximal maternal responsivity from females (Stern and Mackinnon 1978; Pereira et al., 2008). Pup age did not affect onset or quality of any behavior. To limit females' neophobic response and prevent pup attacking/killing, only two freshly nourished pups were given to females on each pup-exposure day. Females that attacked/killed pups were removed from the study (*n*=2).

Prolonged pup-exposure. Two 1-day old pups were placed in each virgin female's homecage, opposite her nest. Pups remained with virgin females for 12h before being returned to lactating females for nursing and care; two fresh pups were immediately given to each virgin female for the subsequent 12h. These 12h rotations allowed pups to be nursed and cared for by lactating females more frequently than typical 24h rotations (see Afonso et al., 2008), with vocalization rate, corporal cooling, and hunger reaching a relatively steady-state between 12-24h (Hofer and Shair, 1978; Blumberg and Alberts, 1991). Continuous (24h/day) pup-exposure continued across CPP for a total of 21 days.

Brief pup-exposure. Two 1-day old pups were presented for 1h daily for two days prior to the CPP procedure. During CPP, virgin females received 1h daily exposures during each CPP pup-conditioning session. The brief (1h/day) pup-exposures occurred over a total of 7 days.

Distal pup-exposure. Virgin females were exposed to distal pup stimuli during each 1h CPP pup-conditioning session. Two pups were enclosed in a mesh bag that allowed females to smell, hear, and see pups but not to interact physically with them; females were unable to mouth, carry, or lick the pups. The bag was promptly removed from the chamber if the female attacked the bag; one female attacked on the first conditioning day and no attacks were observed on any other day.

Early postpartum pup-exposure. Parturient, lactating females that did not donate pups to virgin females (*n*=14) remained with culled litters of eight pups. Every day, pups were collected from all females, commingled, and redistributed to ensure all lactating and virgin females were similarly exposed to "foster" pups.

Maternal behavior observations and scoring

All subjects were observed and scored daily on three maternal behaviors: retrieval of pups, nest-building, and crouching/hovering over pups. A female met the criterion for full maternal behavior if she achieved a retrieval score of 1 or higher on two consecutive days and a crouch/hover score of 1 or higher on one of those days.

Retrieval. Two freshly-nourished pups were placed in the corner of the cage opposite each female's nest site and preferred corner. Criterion for pup retrieval required a female to pick up the pup with her mouth and transport the pup to her nest. Scores ranged from 0-2, corresponding to the number (0-2) of pups retrieved to the nest; scores of 0.5 and 1.5 reflected retrieval of one or both pups to a non-nest location, respectively. Females were observed for 10min.

Crouching/hovering. After retrieval was scored, females were scored on crouching/hovering postures on a scale ranging from 0-3: 0= lack of definitive hovering; 1 = active hovering over pup(s) or lying/resting on top of pup(s); 2 = low crouch over pup(s) or prone nursing posture; 3 = active/high rigid crouch (kyphosis) over pup(s).

Nest building. Nests were scored immediately after crouching/hovering, on a scale ranging from 0-4: 0 = no nest, nest material scattered across cage; 1 = poor, a flat nest without walls built without using all nest material; 2 = fair, a flat nest without walls built using all nest material; 3 = good, nest with low/medium walls constructed using all material; 4 = excellent, nest with high walls built using all material. Scores of 2 or higher were considered maternal. After scoring, nests were destroyed. Nest material (shredded paper towels) was changed every 3-4 days.

For all subjects exposed to pups in their homecage, maternal behaviors were scored between 0700-0900h; pups were first removed from homecages (if necessary) and placed into small boxes adjacent to each homecage for 1h prior to testing. Homecage nest-building and crouching/hovering scoring began eight days into the prolonged pup-exposure regimen, at their first emergence. All subjects were also observed during each 1hr CPP pup-conditioning session.

Conditioned place preference procedure

Apparatus. The custom-designed conditioned place preference (CPP) apparatus consisted of three equal-sized clear Plexiglas chambers (27.5 cm W x 21cm L x 20.5 cm H) each decorated with unique contextual cues (details in Seip and Morrell, 2007; Seip et al., 2008) and containing infrared beams that traversed the floor of each chamber. Beam breaks recorded time spent and locomotion within each chamber.

Pre-conditioning baseline session. Each female was placed into the center chamber and allowed access to all three chambers for 1h. This session occurred on pup-exposure day16 (prolonged), day2 (brief), prior to pup-exposure (distal), or on postpartum day 1 (postpartum group). The day of this session, particularly with respect to postpartum, does not influence CPP (Wansaw and Morrell, unpublished observations).

Conditioning phase. Females received a separate conditioning session, one with young pups and one with nothing (empty), once a day for four consecutive days. Pups were 4-7 days old on conditioning days 1-4, respectively, and thus age-matched to the postpartum day of the early postpartum females. At 0800, pups were removed from each female's homecage (if necessary) and freshly-nourished pups were placed into small, adjacent

cages so that subjects could not see or physically interact with pups but were exposed to pups' auditory and olfactory stimuli. At 1000, females were confined to one cue-decorated side chamber containing pups (*pup-associated chamber*) that had been deprived of female contact for 2h and were thus hungry and demanding of maternal care (Pereira and Ferreira, 2006). Two pups were given to each virgin female given brief or prolonged pup-exposure, to mimic homecage exposures and minimize pup attacks; similarly, two pups were confined in each mesh bag given to each virgin female given distal pup-exposure. Five pups were given to each postpartum female (Pereira et al., 2008; Seip and Morrell, 2007). Females and pups remained in the conditioning chamber for 1h (Fleming et al., 1994) to allow sufficient time for female-pup interactions; females' behavior was recorded every 5min. Females were then returned to homecages for 4h, and pups were returned to early postpartum and prolonged pup-exposure females. At 1500, females were confined to the opposite cue-decorated side chamber, which remained empty (*empty chamber*), for 1h.

Post-conditioning test session. Females were tested for conditioned chamber preference on the day after the final conditioning session. Females were allowed access to all chambers for 1h. Pups were not present. Virgin females were tested on pup-exposure day 21 (prolonged), day 8 (brief), or after 4 days of exposure to the mesh bag containing pups (distal). Postpartum females were tested on postpartum day 8.

Analyses and statistics

Statistical analyses were performed as before (Seip and Morrell, 2007; Seip et al., 2008), with $P<0.05$ as significance level. Preferences are presented as the percentages of

individual subjects within each population that met criteria for each of the four preference categories. Chamber times (averaged across all subjects) and behavior scores are presented as means and standard errors of the mean (sem).

Conditioned place preference. The time spent in each chamber (chamber time) during pre- and post-conditioning sessions was used to identify each subject's chamber preference (Mattson et al., 2001; Seip et al., 2007; Seip et al., 2008; Wansaw et al., 2008). Subjects spending at least 30 minutes in one chamber and 25% more time in that chamber than either other chamber were categorized as preferring that chamber; those failing to meet criteria were categorized as having no preference. Thus, each individual subject was categorized into one of four preference categories: preference for the pup-associated, center, or empty chamber, or no preference. *Within-groups.* In each group, pre- and post-conditioning chamber preferences were compared using a chi-square goodness-of-fit test for specified proportions. Pre- and post-conditioning chamber times were compared with a two-way ANOVA (chamber and session as repeated measures). *Between-groups.* Chamber preferences within a session were compared using Fisher's exact tests or one-tailed tests for significance of difference between two proportions, and chamber times within a session were compared using a two-way ANOVA (chamber as repeated).

Maternal behavior scores. The mean latency to express fully maternal behavior was calculated as a percentage of the total number of pup-exposure days, in order to standardize data across pup-exposure groups, and compared using a one-way independent ANOVA followed by Tukey's posthoc tests. After CPP testing, prolonged and brief pup-exposure females were separated by their preference for the pup-associated

(prolonged *n*=5; brief *n*=3) or empty chamber (prolonged *n*=4; brief *n*=4) and mean

scores within each preference group were analyzed using two-way ANOVAs (day as

repeated) and Tukey's posthocs. Preference groups did not differ on any behavioral

measure, so scores were pooled and compared across days using a one-way ANOVA

(day as repeated) and Tukey's posthocs.

Locomotion during conditioning. Infrared beam breaks in each CPP chamber provided a

measure of subjects' locomotion during each conditioning session. Data from prolonged

and brief pup-exposure females that eventually preferred the pup-associated chamber or

empty chamber were compared to identify any relationships between conditioning

session locomotion and conditioned chamber preference. Locomotion did not differ by

chamber preference category and was pooled within each pup-exposure group.

Locomotion in the empty chamber was similar across pup-exposure groups and pooled

for presentation. Locomotion in the pup-associated and empty chambers was compared

across pup-exposure groups using a two-way ANOVA (day as repeated) and within each

pup-exposure group across chambers using paired t-tests.

Results

Expression of full maternal behavior

All postpartum females expressed full maternal behavior immediately after parturition

(Figure 1). No virgin female expressed full maternal behavior upon initial exposure to

pups. Virgin females only expressed full maternal behavior after 8-15 days of prolonged

pup-exposure, whereas females never met criterion for full maternal behavior after brief

or distal pup-exposure (Figure 1). The latency to express full maternal behavior (Figure 2) was substantially shorter in postpartum females than any virgin female group; amongst virgin females, prolonged pup-exposure resulted in a shorter latency to fully maternal behavior than seen in brief or distal pup-exposure, during which fully maternal behavior was never expressed ($F(3,38)=446.19$, $P<0.0001$; Tukey's: $P<0.05$).

Conditioned place preference

Pre-conditioning session. Chamber preferences and times were similar across all females and pooled. Females were equally distributed across chamber preference categories ($P>0.05$), with the majority of females lacking a chamber preference (36%) or preferred the empty side chamber (34%) (Figure 3a). Females spent equal amounts of time in each chamber ($P>0.05$) (Figure 3b).

Confirmation of conditioning. Conditioning was confirmed in each group of females if the distribution of chamber preferences and/or the mean time spent in each chamber changed significantly between pre- and post-conditioning sessions. In all females, chamber preferences changed between sessions [distal exposure: χ^2 (3, $N=8$)=20.10; brief: χ^2 (3, $N=10$)=96.92; prolonged: χ^2 (3, $N=12$)=13.44; early postpartum: χ^2 (3, $N=14$)=18.32; all $P<0.01$]. In prolonged pup-exposure females, chamber times also changed [chamber x session: $F(2,22)=3.73$, $P<0.05$].

Post-conditioning session. After conditioning, chamber preferences were strikingly similar between early postpartum females and virgin females given prolonged pup-exposure. Specifically, substantial preference for the pup-associated chamber emerged in early postpartum females (50%) and in virgin females given prolonged pup-exposure

(42%) (Figure 4a). Pup-associated chamber preference was slightly reduced in virgin females given only brief pup-exposure (33%). No (0%) virgin females preferred the pup-associated chamber following distal pup-exposure, which was significantly less than any other group (Fisher's: $P<0.05$; prolonged: $z=2.12$; brief: $z=1.70$; postpartum: $z=2.40$; all $P<0.05$); instead, most (64%) of these females lacked chamber preference, more than in any other group (pooled groups, as did not differ: $z=-2.96$; $P<0.01$). No differences emerged between chamber times in any group (Figure 4b).

Data from all females were pooled and separated by preference for additional analyses. Females preferring the pup-associated chamber ($n=15$) spent substantially more time in that chamber than the empty chamber [$F(2,28)=80.36$, $P<0.0001$], whereas females preferring the empty chamber ($n=16$) spent more time in that chamber than the pup-associated chamber [$F(2,30)=113.30$, $P<0.0001$] (Figure 4c).

Emergence and strength of individual maternal behaviors

Virgin females given prolonged or brief pup-exposure expressed a range of individual maternal behaviors toward pups (Figure 5). Retrieval scores of females given prolonged pup-exposure improved significantly across the pup-exposure period [retrieval on days 1-13; $F(12,96)=2.22$, $P<0.05$; Tukey: $P<0.05$] and retrieval and nest-building scores were consistently maternal across the final 11-13 days of pup-exposure. Females given brief pup-exposure never attained consistently maternal scores on any behavior, though inconsistent retrieval, nest-building, and crouching/hovering behaviors were observed on various days. All daily scores were reliably and substantially higher in females given prolonged pup-exposure than those given brief pup-exposure [main group

effect: retrieval, $F(1,17)=163.94$; crouch/hover, $F(1,17)=10.37$; nest, $F(1,17)=50.98$; all $P<0.05$; Tukey: all $P<0.05$] (Figure 5a-c).

To examine the relationship between the expression of individual behaviors and the subjects' post-conditioning chamber preference, daily maternal behavior scores were separated by each female's preference for the pup-associated or empty chamber (Figure 5a-c). Both preference groups achieved similar scores on each behavior and did not differ in onset (i.e. first day of expression) of behavior, regardless of pup-exposure length.

Locomotion during conditioning

As expression of maternal behaviors differed dramatically in females given prolonged and brief pup-exposure across CPP conditioning, locomotion within the pup-associated chamber during the first and last conditioning sessions was analyzed in these groups. On the first conditioning day, locomotion in the pup-associated chamber was higher in females given prolonged pup-exposure than those given brief pup-exposure [$F(1,12)=119.37$, $P<0.0001$] and was higher than in the empty chamber only in the prolonged pup-exposure group [$t(9)=4.96$, $P<0.01$] (Figure 6). On the last conditioning day, locomotion in the pup-associated chamber was similar, regardless of pup-exposure length, and was higher than in the empty chamber [$t(16)=3.07$, $P<0.01$]. Between the first and last conditioning days, locomotion in the empty chamber decreased in both pup-exposure groups [$t(16)=3.17$, $P<0.01$] whereas locomotion in the pup-associated chamber decreased only in females given prolonged pup-exposure [$t(9)=2.88$, $P<0.05$].

Maternal behavior during conditioning

Select females were observed and their behaviors recorded every 5min throughout the first and last pup-conditioning sessions. Behavior raster plots were compiled for three representative females, typifying a fully maternal individual (prolonged and early postpartum), a non-maternal individual with limited pup-exposure (brief), and a non-maternal individual lacking full exposure to pups (distal) (Figure 7). Across both conditioning sessions, maternally responsive females (prolonged and early postpartum) expressed mostly pup-directed, maternal behaviors, whereas non-maternal females (distal pup-exposure) avoided the mesh bag containing pups. Non-maternal females given brief pup-exposure shifted notably from avoidant to pup-tolerant behaviors between the first and last conditioning day. In all pup-exposure groups, behavioral patterns during conditioning did not correspond with female's eventual chamber preference.

Discussion

Virgin females will strongly prefer a pup-associated chamber after being exposed to pups for prolonged periods of time (24h/day for 21 days), rivaling the robust pup-associated chamber preference seen in parturient females during the early postpartum period, when maternal motivation is strongest (Mattson et al., 2001; Seip et al., 2007; Numan, 2007; Wansaw et al., 2008). Fewer virgin females (33%) exposed to pups for relatively brief periods of time (1h/day for 7 days) preferred the pup-associated chamber, compared to virgin females given prolonged pup-exposure (42%) and, particularly, early postpartum females (50%), revealing a subtle stepwise relationship between length of pup exposure and strength of maternal motivation. No females lacking full exposure to

pups and receiving only distal (auditory and olfactory) pup-cues preferred the pup-associated chamber. This work reveals, for the first time, that even brief exposure to pups is sufficient to promote maternal motivation in a strong subpopulation of virgin females and that this maternal motivation can be enhanced to postpartum levels by increasing the length of exposure to pups.

Importantly, our prolonged pup-exposure regimen is the closest published replication of the natural behavioral patterns of the parturient female during the first eight days of the postpartum period, when the strength of her maternal motivation peaks (Mattson et al., 2001; Seip et al., 2007; Numan, 2007; Wansaw et al., 2008). First, the maternal female expresses vigorous, consistent maternal behaviors toward her pups during the early postpartum period (Numan and Insel, 2003). In the present study, virgin females given prolonged pup-exposure expressed maternal behavior that improved significantly over time and was maximally established by CPP testing. That nearly half of these virgin females preferred the pup-associated chamber at CPP test suggests that maximizing females' maternal responsivity may increase her attribution of incentive value to pups.

Second, the parturient female spends the majority of her time with her pups during the early postpartum period (Pereira et al., 2008; Grota and Ader 1969, 1974; Reisbick et al., 1975; Wansaw et al., 2008). In the present study, females were only deprived of pups for relatively brief, biologically relevant periods (2h) prior to pup-conditioning (Seip and Morrell, 2007; Wansaw et al., 2008). Our data demonstrates, for the first time, that dramatic pup-deprivation is not necessary for virgin females to strongly prefer a pup-associated chamber (Fleming et al., 1994). As even parturient

females will not prefer a pup-associated chamber following brief pup-deprivations in late postpartum (Wansaw et al., 2008), when increasing amounts of time are spent away from pups and maternal care declines, maternal motivation in virgin females can actually surpass that of the naturally parturient female in late postpartum.

Together, our data demonstrate that, by mimicking the natural behavioral patterns seen in early postpartum, virgin females can express strong maternal motivation that rivals that of the parturient female. We conclude that maternal motivation is not established exclusively by the complex neuroendocrine states of gestation, parturition, and postpartum, or by the physiological, behavioral, and polysensory experiences of parity.

Additionally, many virgin females given brief pup-exposure, which never expressed fully maternal behavior, expressed strong preference the pup-associated chamber. These non-maternal females expressed a markedly reduced locomotor response during the first pup-conditioning session compared to the fully maternal females given prolonged pup-exposure and expressed mostly freezing and/or avoidant behavior toward pups. By the last conditioning session, brief pup-exposure females were increasingly tolerant of pup-initiated physical interaction and no longer expressed freezing/avoidant behaviors, and locomotor response to pups matched that of prolonged pup-exposure females. We posit that the emergence of these inconsistent, maternal-like behaviors represents a transitional state of maternal responsivity during which pups shift from an aversive to a neutral or slightly attractive stimulus to the female. That females given brief pup-exposure did not express locomotor habituation across pup-conditioning sessions, as did prolonged pup-exposure females, indicating that the females' perception of the pup

stimulus differed dramatically across these four exposures (Mazur, 2005). We posit that the initial tolerance for pup-initiated contact (e.g. rooting) represents an emerging transition in the motivational state of the female that is potentiated by the changing incentive-motivational status of pups and ultimately expressed as a drive to initiate contact and interact with pups.

Notably, the motivational transition observed in virgin females given brief pup-exposure was *not* observed in females given only distal exposure to a limited range of pup-related stimuli. While both groups of females were completely non-maternal, the olfactory and auditory cues provided by distal pup-exposure were insufficient to initiate a similar shift in the incentive-motivational value of pups to the distally exposed female. We posit that the limited time spent in the pup-associated chamber by these distally exposed females reflects their sub-threshold attraction to the novel odors and vocalizations of pups enclosed within the mesh bag (Bardo et al., 1989).

Together, these findings suggest that pup-associated chamber preference in the virgin female requires a basal level of physical interaction between the female and pups, which distal pup-exposure prevents. Pups confined inside a mesh bag were prevented from initiating physical contact with these non-maternal virgin females, e.g. rooting at females' ventrum and nipples, whereas pup-initiated contact occurred in all other groups of females, even those given brief pup-exposure, with contact reciprocated and supplemented by maternally responsive females. Prior work by Fleming and colleagues (Lee et al., 2000; Magnusson and Fleming, 1995) suggests that maternal motivation is contingent upon direct female-pup interactions: bar-pressing for access to pups declines dramatically if pups cannot be retrieved upon delivery (Lee et al., 2000) and postpartum

females will not prefer a pup-associated chamber if prevented from interacting with pups in that chamber (Magnusson and Fleming, 1995). Our data importantly extend these findings by revealing that physical interactions can be initiated by the maternally responsive female (i.e. during prolonged pup-exposure) or by pups (i.e. during brief pup-exposure) and must include a complete range of polysensory input in order to promote maternal motivation in both virgin and parturient females.

It is also important to note that the expression maternal behavior, an important correlate to our appetitive-motivational CPP measures, remained remarkably consistent between virgin females preferring the pup-associated or empty chamber. At no point did any female's maternal behavior in the homecage or behavioral patterns recorded during pup-conditioning predict or correlate with her preference for the pup-associated or empty chamber; this dissociation was evident across our novel, prolonged pup-exposure regimen, including the 15 days prior to CPP, the six days of CPP (Figure 5), and during each pup-conditioning session (data not shown; see Figure 7 for exemplar). Strongly motivated, 'active' maternal behaviors (retrieval and nest-building) (Stern and Mackinnon, 1976; Lonstein et al., 1999; Pereira et al., 2005; Numan, 2007), which were expressed consistently after prolonged pup-exposure and were emerging after brief pup-exposure, did not predict any females' preference for the pup-associated chamber. Thus, even well-established, motivated caretaking behaviors do not predict a virgin females' maternal motivation and that behavior and motivation remain distinguishable components of the maternal state, in accord with others' work (Fleming et al., 1994; Lee et al., 2000; Mattson et al., 2003).

As females are the primary providers of parental care in rodents and most other small mammals, many factors may mediate the shift the incentive-motivational value of pups from aversive to attractive, initiate the emergence of a drive to interact with pups in the non-maternal female, and promote the expression of a range of maternal behaviors that further strengthen maternal motivation in the female (Numan and Insel, 2003; Pereira et al., 2008). These factors include neurobiological, environmental, and experiential factors, which act in concert to regulate maternal responsivity in the female rat. The present work uniquely addresses how two of these critical factors, postpartum status and exposure to pups, affect maternal motivation in the female rat, a model that is uniquely evolutionarily primed to respond to changes in the incentive-motivational value of pups.

It has been proposed that females' motivated behavior may be influenced by dynamic differences in levels of gonadal hormones and lactogenic peptides in cycling and postpartum females. While preference for a pup-associated chamber is strongest in gonadally intact (versus ovariectomized) females (Fleming et al., 1994), this preference is likely attributable to hormonally facilitated female-pup interactions (Fleming and Rosenblatt, 1974; Fleming and Sarker, 1990; Fleming et al., 1994; Numan and Insel, 2003). In the present study, pup-exposed virgin and postpartum females expressed comparable preference for the pup-associated chamber, despite dramatic neuroendocrine differences (Grota and Eik-Nes, 1967; Smith et al., 1975; Smith and Neill, 1977; Marinari and Moltz, 1978; Taya and Greenwald, 1982). Furthermore, CPP is relatively insensitive to differences across the estrus cycle (Seip et al., 2008), suggesting that any perseverative changes in gonadal hormone levels induced by pups (Marinari and Moltz,

1978) do not influence CPP, either. Mere exposure to pups can up-regulate long-form prolactin receptor mRNA in both virgin and postpartum females (Sugiyama et al., 1996); circulating prolactin levels are substantially higher in females expressing pup-induced maternal behavior than in non-maternal females exposed briefly to pups (Bridges et al., 1974; Marinari and Moltz, 1978). Prolactin has been proposed as a likely factor promoting maternal motivation (Pereira et al., 2008), and it is possible that pup-exposed virgin females that prefer the pup-associated chamber express higher levels of centrally acting prolactin.

The mesolimbic dopamine (DA) system also contributes importantly to the expression of highly motivated maternal behaviors (Gaffori and LeMoal, 1979; Giordano et al., 1990; Hansen et al., 1991a, 1991b; Keer and Stern, 1999; Silva et al., 2001; Byrnes et al., 2002; Numan et al., 2005; Pereira and Ferreira, 2006; Pereira et al., 2007; Afonso et al., 2008) and, as emerging evidence suggests, the attribution of incentive-motivational value to pups in the parturient female (Fleming et al., 1994; Pereira et al., 2007). Dopamine release into the nucleus accumbens increases markedly during bouts of licking/grooming (Hansen et al., 1993; Champagne et al., 2004), while blocking dopamine transmission impairs both licking and motivated pup retrieval (Keer and Stern, 1999; Silva et al., 2001; Byrnes et al., 2002; Numan et al., 2005; Pereira and Ferreira, 2006; Pereira et al., 2008). Recent work indicates that dopamine antagonism blocks the acquisition of pup-associated chamber preference in postpartum females (Fleming et al., 1994), whereas stimulation of D1 receptors can promote it (Pereira et al., 2007, 2008). Thus, we posit that individual differences in D1-receptor responsivity may modulate the incentive value attributed to pups by the maternally responsive female. This hypothesis

was supported by a recent study (Afonso et al., 2008) revealing that accumbal dopamine is chronically elevated in maternally responsive virgin females; we posit that this persistent increase in mesolimbic dopamine may contribute to strong maternal motivation in our virgin females given prolonged pup-exposure. Also, as reunion with pups transiently increases accumbal dopamine in the maternal female (Hansen et al., 1993; Champagne et al., 2004), we further posit that such increases in dopamine may also enhance the incentive-motivational value of pups during each pup-conditioning session, facilitating the shift in incentive-motivational value of pups in our virgin females given brief pup-exposure.

It is possible that pup-induced elevations in dopaminergic transmission may have enhanced associative learning in all pup-exposed females (Alcaro et al., 2007), thereby preventing most females given only distal pup-exposure from strongly preferring a conditioning chamber. However, pup-associated stimuli (e.g. odors, vocalizations) and novel stimuli, such as the mesh bag containing pups, elicit arousal in females and depends upon mesolimbic dopamine activity (Saigusa et al., 1999; Rebec et al., 2007). Thus, whether in the form of vigorous caretaking behaviors (maternal females) or as mild anxiety (non-maternal females) (Numan and Insel, 2003), we posit that attention and learning of conditioned associations (e.g. CPP) was similarly enhanced in all females in this study (Yerkes and Dodson, 1908).

Ultimately, the present study reveals, for the first time, striking levels of maternal motivation in the virgin female rat. This motivation is contingent upon physical interactions between the female and pups, including full exposure to a broad spectrum of polysensory stimuli associated with pups. Furthermore, maternal motivation can be

enhanced by systematically extending the length of time that females are exposed to pups. Early work revealed that consistent, repeated exposures to a stimulus can increase the attractiveness of that stimulus (Zajonic, 1968); we posit that the systematic increases in maternal motivation seen in the present study may be attributable to the systematic increases in pup-exposure length and, as a consequence, the quality of maternal behavior. This critical shift in incentive-motivational value of pups from aversive to attractive may be attributable to endocrinological, neurochemical and behavioral changes emerging during initial pup-exposures. Importantly, this motivational shift contributes critically to pups' survival and health by promoting motivated caregiving and, as revealed in the present study, can occur equally in both virgin and parturient female rats.

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Figure Captions

Figure 1. Cumulative percentage of fully maternal virgin females given brief, prolonged, or distal exposure to young pups and percentage of fully maternal postpartum females beginning immediately after parturition.

Figure 2. Latency, presented as the percentage of total days of pup-exposure, to express full maternal behavior in virgin females given brief, prolonged, or distal exposure to pups and in early postpartum females toward their own pups beginning immediately after parturition. The latency to express full maternal behavior was significantly shorter in postpartum females than all three groups of virgin females (***). Prolonged pup-exposure decreased the latency to full maternal behavior compared to females given brief or distal exposure to pups, which never expressed full maternal behavior (**). All $P<0.05$.

Figure 3. Pre-conditioning chamber preferences (a) and mean time spent in each chamber (b) during the pre-conditioning (baseline) session. Data did not differ across groups of females and were pooled for presentation.

Figure 4. Post-conditioning chamber preferences (a), mean time spent in each chamber by all females (b), and mean time spent in each chamber by only females preferring the pup-associated or empty chambers (c) during the post-conditioning (test) session. Between-group differences within each chamber (*) and within-group differences between chambers (#) are identified; all $P<0.05$.

Figure 5. Mean daily scores on retrieval (a), crouching/hovering (b) and nest-building (c) behaviors of virgin females given prolonged pup-exposure (circles) or brief pup-

exposure (triangles). Scores are separated by females' post-conditioning preference for the pup-associated (filled shapes) or empty chamber (open shapes); within each pup-exposure group, scores were similar across preferences. Retrieval scores increased significantly by day 13 in the prolonged pup-exposure group (#). Scores of females given prolonged and brief pup-exposure differed every day (*). All *P*<0.05.

Figure 6. Mean locomotion within the pup-associated and empty chamber during the first and last conditioning sessions of virgin females given prolonged or brief exposure to pups. Locomotion within the pup-associated chamber (PUP) is separated into females given prolonged (*filled circles*) or brief (*open circles*) pup-exposure. Locomotion within the empty chamber (EMPTY) did not differ across pup-exposure and was pooled (*grey triangles*). Between-group differences within chambers (*) and within-group differences between chambers (#) and within each chamber across days (†) are identified; all *P*<0.05.

Figure 7. Behavior raster plot for three representative females during the first and last conditioning sessions in the pup-associated chamber. Observed behaviors were typical for all maternally responsive (prolonged pup-exposed and early postpartum) females (*light grey*), non-maternal females with brief pup-exposure (*black*) and females with only distal pup-exposure (*white*).
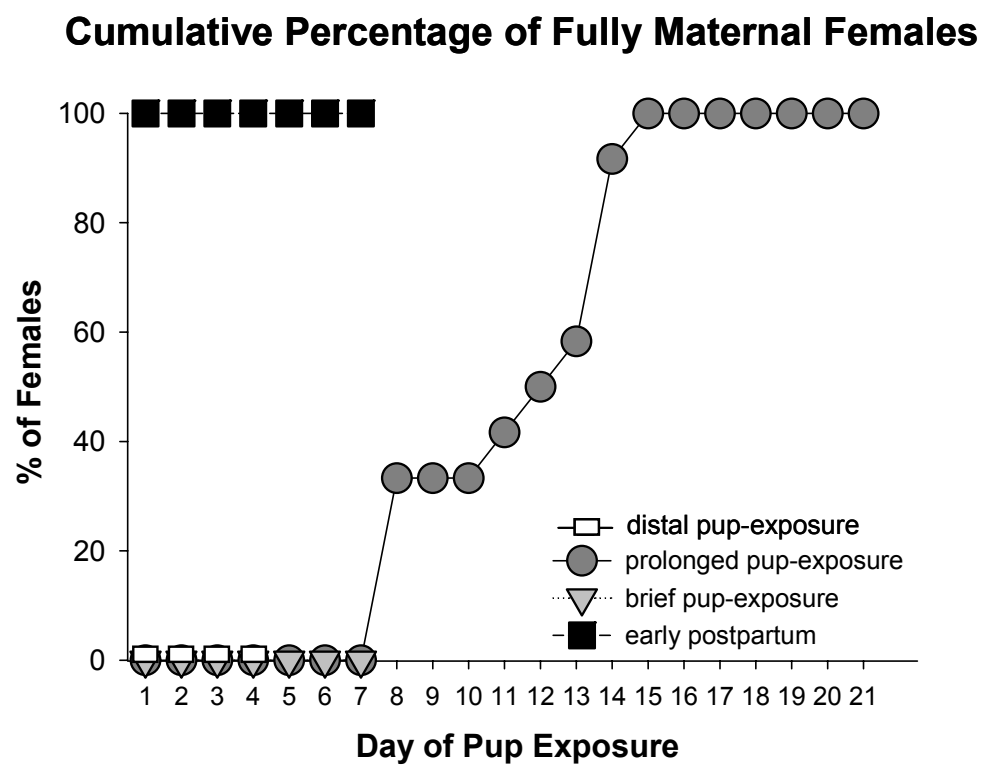
Figure 1.

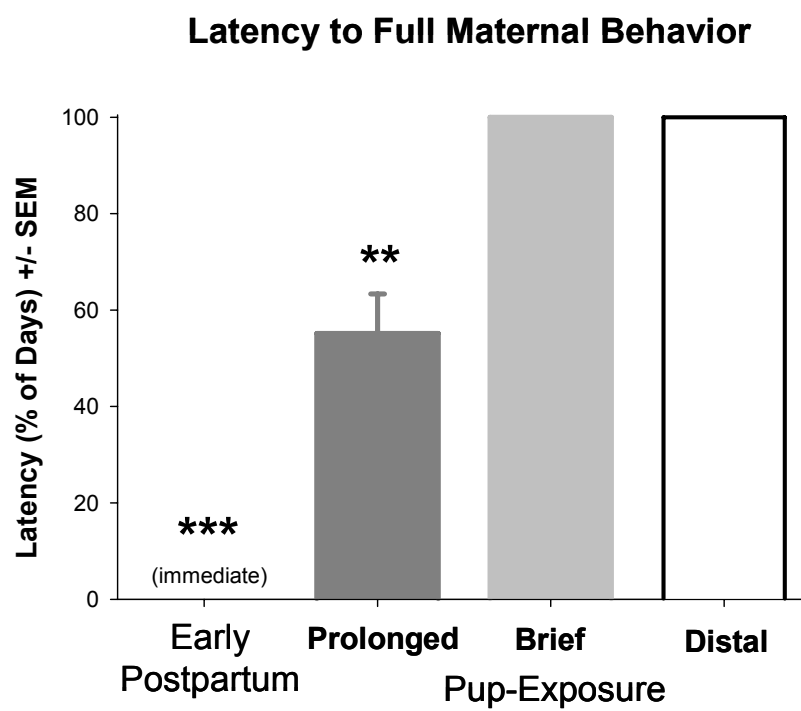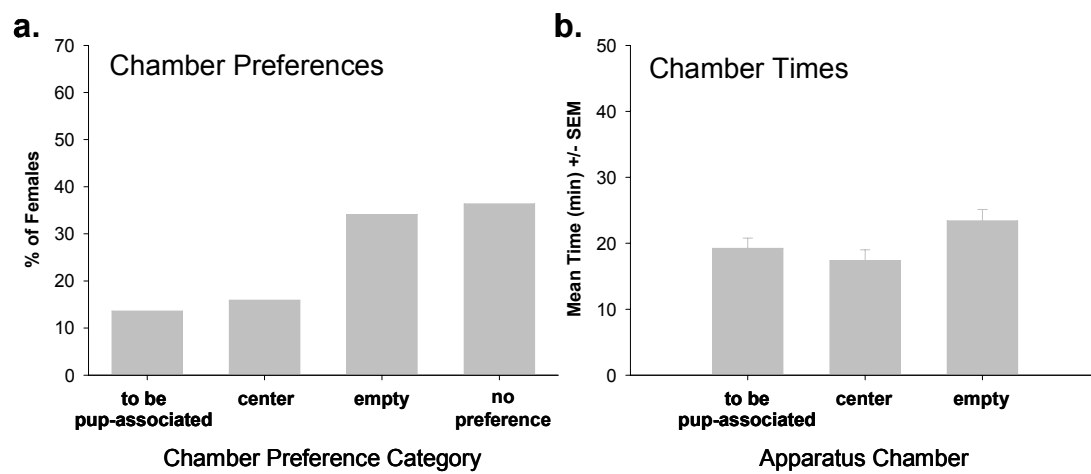**Cumulative Percentage of Fully Maternal Females**

Figure 2.

Latency to Full Maternal Behavior

Figure 3.

a. Chamber Preferences — % of Females vs Chamber Preference Category (to be pup-associated, center, empty, no preference)

b. Chamber Times — Mean Time (min) +/- SEM vs Apparatus Chamber (to be pup-associated, center, empty)

Figure 4.

a.

Chamber Preferences

b.

Chamber Times

- □ no pup-exposure (n=8)
- ▨ brief pup-exposure (n=10)
- ▨ prolonged pup-exposure (n=12)
- ■ early postpartum (n=14)

Chamber Times, by Preference

c.

Females Preferring the Pup-Associated Chamber

Females Preferring the Empty Chamber

Figure 5.

Individual Maternal Behaviors

Figure 6.

Locomotion in Conditioning Chambers
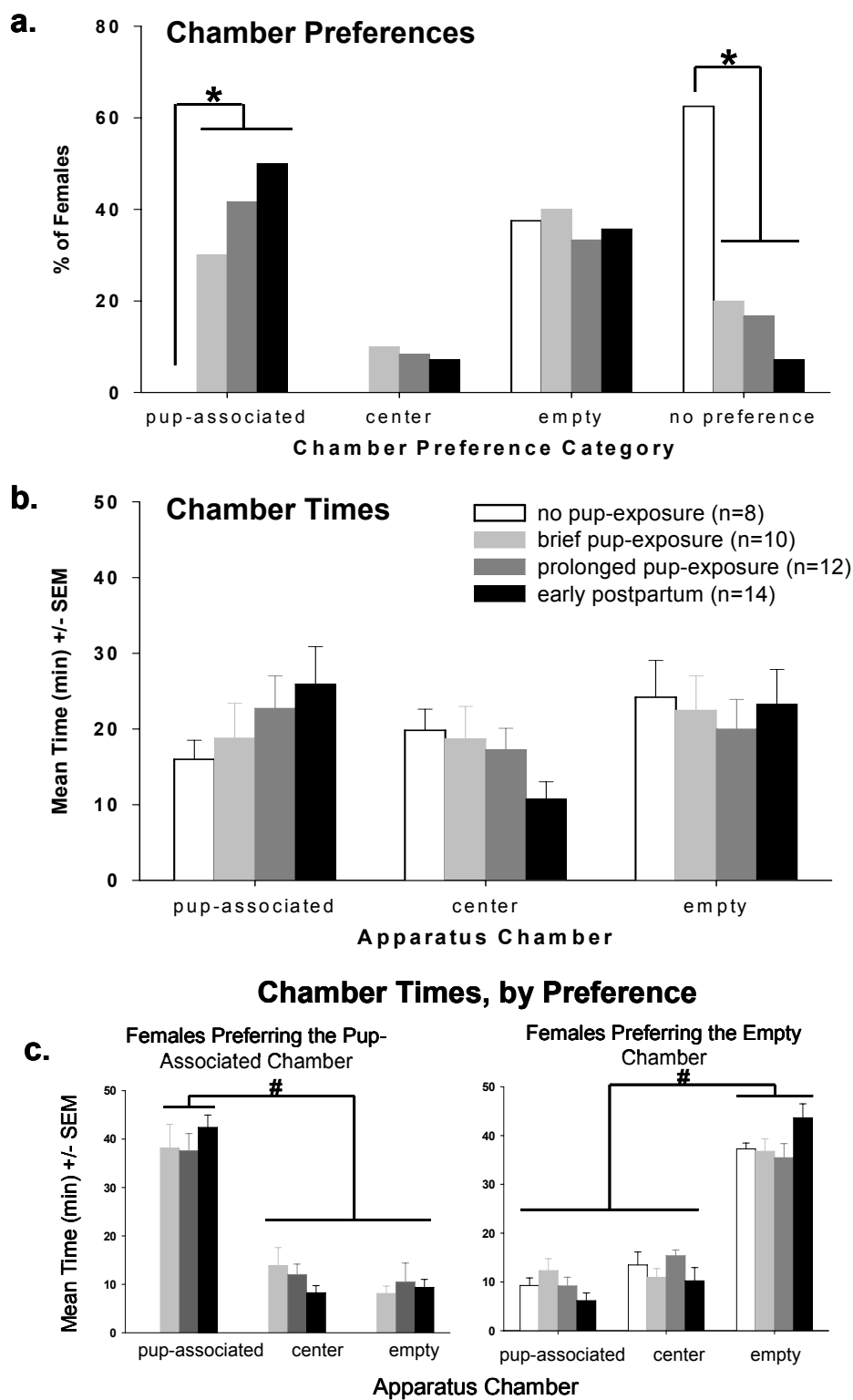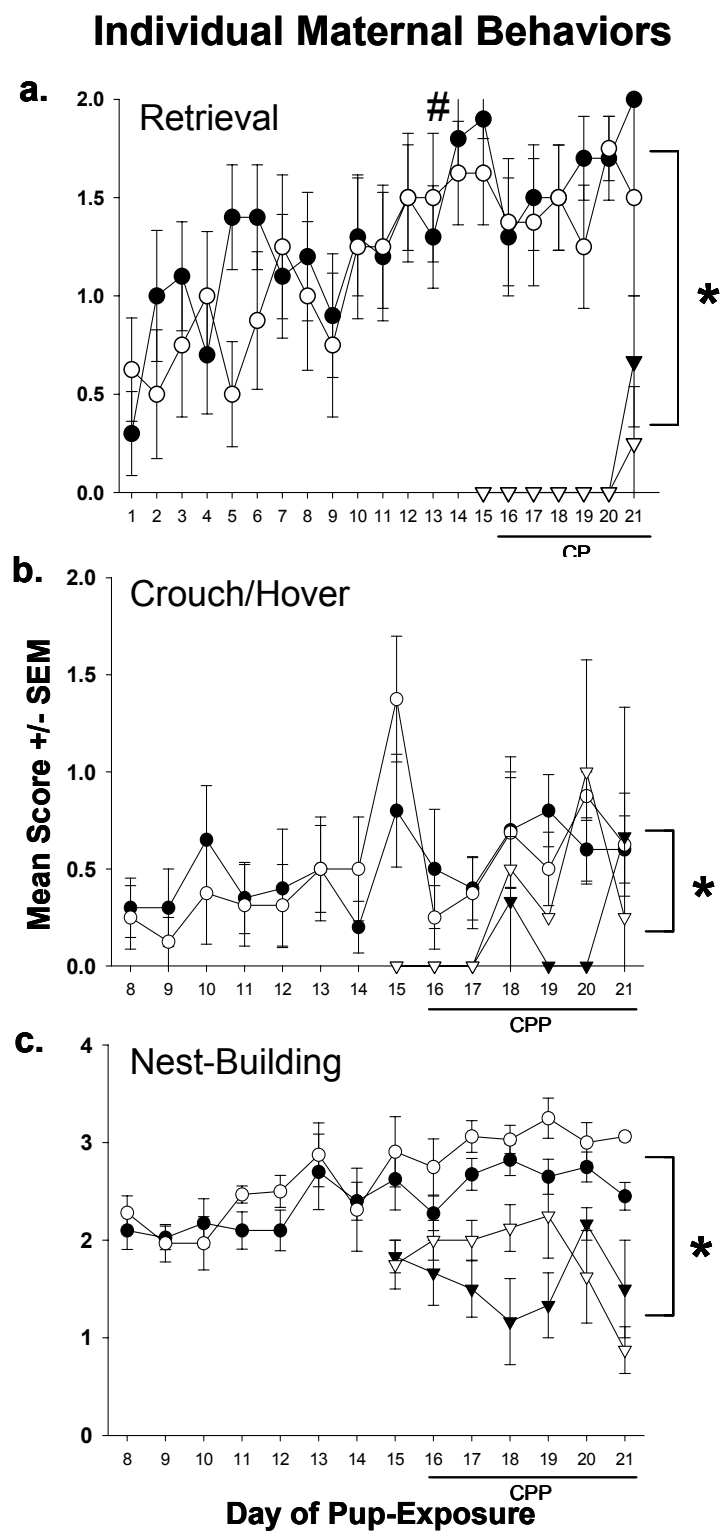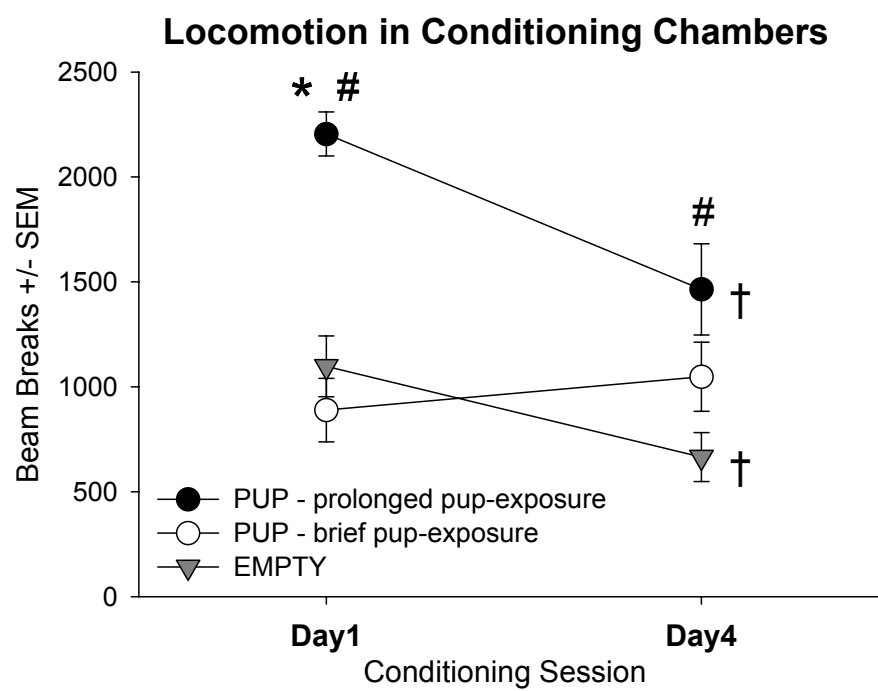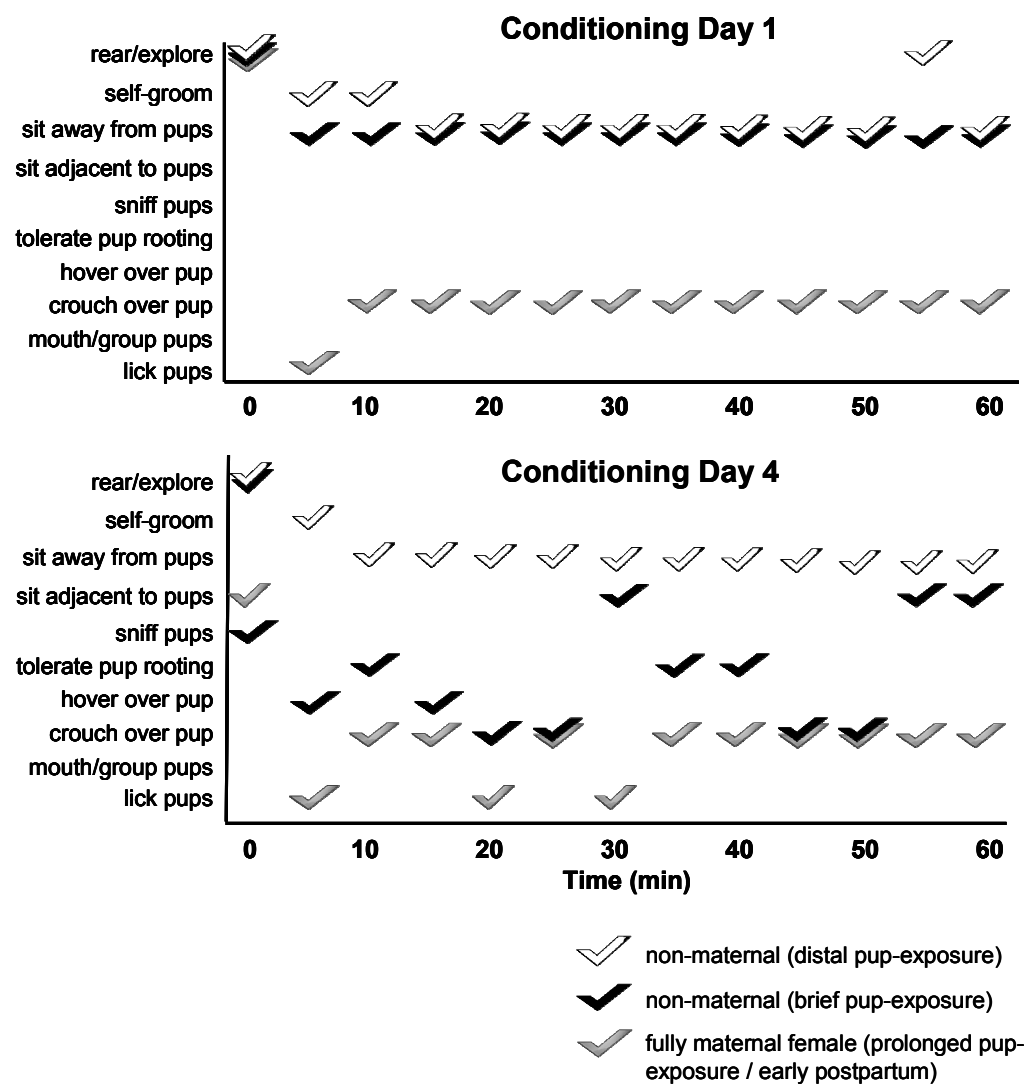
Figure 7.

CHAPTER 4

Transient inactivation of the ventral tegmental area selectively disrupts the

expression of conditioned place preference for pup- but not cocaine-paired stimuli

Abstract

The ventral tegmental area (VTA) plays a critical role in motivated behavior. To determine whether the VTA plays a differential role in motivated behavior directed toward cues paired with natural and pharmacological stimuli, female rats were tested for their conditioned place preference (CPP) for chambers paired with stimuli selected for their strong incentive-motivational value in distinct populations: cocaine (5mg/kg intraperitoneally) in virgin females and young offspring (pups) in postpartum females. Prior to CPP testing, the VTA was transiently inactivated via bilateral microinfusion of bupivacaine; subjects were also tested following saline microinfusion. Preference for the pup-paired chamber was abolished by VTA inactivation but was restored to control levels following saline microinfusion. In separate tests, VTA inactivation also impaired maternal behavior, particularly motivated pup licking and retrieval. Cocaine CPP remained unaffected by VTA inactivation. Locomotion was not affected by VTA microinfusions. Thus, intact VTA function is necessary for the expression of conditioned preference for highly salient natural stimuli but not cocaine.

Introduction

The ventral tegmental area (VTA) and its ascending projections are involved in motivational processes, including the performance of goal-directed behavior, effortful decision-making and the attribution of incentive value to stimuli (Ikemoto and Panksepp, 1999; Wise, 2004; Yun et al., 2004; Salamone et al. 2005, 2007; Fields et al., 2007; Berridge 2007). Neurons in the VTA respond to appetitive stimuli attributed with positive incentive value, including drugs of abuse (Kiyatkin and Rebec, 1997; Brodie et al., 1999), and to cues that predict appetitive stimuli (Fields et al., 2007; Sombers et al., 2009). Intact VTA function is also required for an animal to acquire (Zellner et al., 2009) and express (Yun et al., 2004) effortful stimulus-seeking behavior directed toward predictive cues. Thus, the VTA participates critically in an animal's motivated behavior directed toward appetitive stimuli and their predictive cues (Schultz, 2002; Phillips et al., 2003b; Roitman et al., 2004; Fields et al., 2007).

It remains unknown whether the VTA plays a differential role in motivated behavior directed toward cues paired with natural and pharmacological stimuli. Two such stimuli, cocaine and young offspring (pups), have strong incentive value to virgin and postpartum female rats, respectively, and will elicit motivated stimulus-seeking behavior from these females given only limited exposure to these stimuli (Seip et al., 2008; Seip and Morrell, 2008; Wansaw et al., 2008). The present study explores whether intact VTA function is necessary for an individual to seek out cues paired with pup or cocaine stimuli.

The VTA is required for the performance of motivated behavior directed toward natural stimuli, such as pups (Numan and Stolzenberg, 2008, 2009). In postpartum females, the VTA is activated by somatosensory and auditory stimuli associated with pups (Febo et al., 2005; Hernández-González et al., 2005b), exposure to pups (Lin et al., 1998; Komisaruk et al., 2000) and the expression of maternal behaviors (Hernández-González et al., 2005a). The expression of motivated maternal behaviors, such as pup retrieval and licking, transiently elevates extracellular DA in the nucleus accumbens (NAc) (Hansen et al., 1993; Champagne et al., 2004; Afonso et al., 2008) via the activation of ascending mesolimbic projections arising from the VTA (Hansen et al., 1991). The VTA also receives projections from the medial preoptic area (MPOA), a key region mediating maternal responsivity (Numan and Insel, 2003), that respond to maternal hormones (Fahrbach et al., 1986) and participate in maternal behavior (Numan and Numan, 1997).

The VTA may also participate in postpartum females' motivation to seek out and interact with pups in their absence (i.e., maternal motivation). Maternal motivation is strongest during the early postpartum period, when females will bar-press insatiably for access to pups (Lee et al., 2000) and prefer a pup-paired chamber over neutral or even cocaine-paired chambers (Fleming et al., 1994; Mattson et al., 2001; Seip and Morrell, 2007, 2008; Wansaw et al., 2008). The strong incentive value of pups during early postpartum is mediated, at least in part, by dopamine (DA) (Fleming et al., 1994; Pereira et al., 2008) and thus likely involves the VTA. To date, no work has directly assessed whether intact VTA function is required for maternal motivation.

The VTA and its ascending projections do play a role in motivated behavior directed toward a qualitatively different stimulus, cocaine. Acute cocaine administration and cocaine-seeking behavior can rapidly alter VTA activity (Einhorn et al., 1988; Le Foll et al., 2002; Febo et al., 2004) and transiently increase accumbal DA via a VTA-dependent mechanism (Carelli et al., 2000; Carelli and Ijames, 2001; Carelli, 2002, 2004; Sombers et al., 2009). Vigorous operant and place preference responses to cocaine, such as expressed by virgin females (Hecht et al., 1999; Russo et al., 2003; Seip et al., 2008), also rely on intact DAergic projections from the VTA to the NAc and PFC (Kiyatkin and Gratton, 1994; Tzschentke, 2000; Harris and Aston-Jones, 2003; Sellings and Clarke, 2003; Beninger and Gerdjikov, 2004; Achat-Mendes et al., 2005; Ishikawa et al., 2008; Sombers et al., 2009). Even in the absence of cocaine, neuronal firing rates are higher and bursting activity is more frequent in the VTA of individuals that acquire cocaine self-administration most rapidly (Marinelli and White, 2000). Thus, it is likely that intact VTA function plays a key role in acute cocaine-seeking behavior in the virgin female.

To determine whether the VTA plays a differential role in a subject's motivated stimulus-seeking behavior toward salient natural or drug stimuli, we transiently inactivated the VTA immediately before testing females' conditioned place preference (CPP) for cue-decorated chambers paired with pups (postpartum females) or cocaine (virgin females). The CPP paradigm offers an ideal measure of motivation during the early phases of stimulus-seeking behavior, as subjects only receive limited exposure to these stimuli during CPP conditioning (Seip et al., 2008; Seip and Morrell, 2008). CPP conditioning and testing can also occur within five days, allowing motivation to be tested during the early postpartum period while minimizing the impact of ongoing

physiological and developmental changes in the postpartum female and her pups, respectively. Finally, CPP is considered to reflect an individual's motivation to seek out the chamber cues associated with a desired target stimulus in the absence of that stimulus. Thus, inactivating the VTA prior to testing the expression of CPP provides a discrete window during which an individual has already learned to associate the desired stimulus with a cue-decorated chamber during CPP conditioning, but must express voluntary goal-directed behavior to actively seek out these contextual cues to express CPP during testing.

Depressing VTA activity between CPP conditioning and testing requires transient, rather than permanent, site-specific inactivation. Permanent lesions must be performed prior to CPP conditioning to allow sufficient time for subjects to recover from surgery, affecting both the acquisition and expression of CPP. Transient site-specific inactivation, however, can occur any length of time after surgery and cannula implantation, enabling temporally discrete functional interventions after CPP conditioning but prior to testing the expression of CPP. Such transient inactivation also minimizes long-term compensatory neural and behavioral adaptations induced by permanent site-specific lesions (Luhmann, 1996; Payne et al., 1996).

Site-specific and transient depression of neuronal activity can be achieved using microinjections of the local anesthetic bupivacaine (Floresco et al., 2008; Ghods-Sharifi et al., 2008; Kim and Richardson, 2008). Bupivacaine induces neuronal inactivation within 2-5min post-injection by reversibly blocking voltage-gated sodium (Na+) channels (Hille, 1966; Tucker and Mather, 1998) that, due to prolonged inactivation of the Na+ channel pore (Fukuda et al., 2005), can last up to 75min (Coyle and Sperelakis,

1987; Tucker and Mather, 1998). This period of VTA inactivation allows a range of

complex behaviors (e.g., CPP, locomotion, maternal behavior) to be tested both during

functional blockade and once neuronal activity has recovered. Like the structurally

similar amide anesthetic lidocaine (Sandkühler et al., 1987; Martin and Ghez, 1999;

Tehovnik and Sommer, 1997; Boehnke and Rasmussen, 2001; Pereira de Vasconcelos et

al., 2006), bupivacaine effectively blocks all neuronal activity within and from the

discrete region of infusion (Sandkühler et al., 1987; Martin and Ghez, 1999). Given the

heterogeneity of the neurochemistry and connectivity of VTA neurons (Fields et al.,

2007), complete inactivation of the entire VTA is the first critical step in determining

whether intact VTA function is necessary for motivated stimulus-seeking behavior

directed toward a unique natural stimulus (pups) and toward cocaine.

Methods

Animals. Subjects (n=78) were 90-120 day old female Sprague-Dawley rats raised in a

colony maintained at the Laboratory Animal Facility at Rutgers University (Newark NJ),

which is accredited by the American Association for Accreditation of Laboratory Animal

Care (AAALAC). Subjects were moved into a quiet testing suite two days prior to the

experiment and were handled daily. Virgin females (n=37) were sexually naïve.

Postpartum females (n=41) were primiparous and remained with their culled, eight-pup

litter after parturition. Each subject was housed individually in an opaque shoebox cage

containing nest material and ad lib access to food and water. A 12hr:12hr light:dark cycle

was maintained with lights on at 0700. All subjects remained healthy and pups gained

weight and developed normally across the experiment. Separate groups of subjects were implanted with bilateral cannulae (postpartum, n=22; virgin, n=25) or remained intact, non-surgical controls (postpartum, n=19; virgin, n=12).

Cannula implantation. Subjects were anesthetized with an intraperitoneal injection of ketamine hydrochloride (76.9mg/ml; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA), xylacine (7.69mg/ml; Lloyd Laboratories, Shenandoah, IA) and acepromazine maleate (1.54mg/ml; AmTech Group, Inc., St. Joseph, MO), at a dose of 0.09ml/100g body weight. When surgical anesthesia was reached, the scalp was shaved, scrubbed repeatedly with Betadine, and injected subcutaneously with a 0.5% marcaine solution (0.5ml; Hospira, Inc., Lake Forest, IL) before the subject was secured into a stereotaxic apparatus using non-rupture ear bars (David Kopf Instruments, Tujunga, CA) and bite bar set at +/- 3.3mm. Bilateral stainless steel guide cannulae (22 gauge; Plastics One, Inc., Roanoke, VA) were aimed 1.5mm dorsal to VTA (flat skull; AP -5.6mm from Bregma, +/-1.0mm lateral from midline, and -7.0mm ventral from skull surface and cannula pedestal) and secured to the skull using stainless steel screws and dental cement. Coordinates were obtained from Paxinos and Watson (1998). Bilateral dummy stylets were inserted through guide cannulae to preserve patency. Surgery occurred on postpartum day 1 in postpartum females. Subjects were allowed to recover for a minimum of two days before CPP conditioning began. All subjects gained weight normally, displayed no signs of inflammation or infection at the surgical site, and expressed normal behavior following surgery.

Conditioned place preference.

Apparatus. The custom-designed conditioned place preference apparatus consisted of three equal-sized clear Plexiglas chambers. Each side chamber contained unique contextual cues of differently striped wallpaper and tactile flooring. The center chamber had white walls and a solid grey floor. Chambers were connected by manually operated guillotine doors (details in Seip and Morrell, 2007, 2008; Seip et al. 2008).

Chamber preference criteria. Individual categorization of each subject's chamber preference reveals important information about how individuals comprising a population respond to each stimulus-associated chamber. We use stringent quantitative criteria to objectively identify whether an individual subject prefers one of the three apparatus chambers (Seip and Morrell 2007, 2008; Seip et al. 2008): subjects must spend at least 30min in one chamber and 25% more time in that chamber than any other chamber. Subjects that did not meet criteria for chamber preference were categorized as having no preference. To allow direct comparisons with established CPP literature (Tzschentke, 1998, 2007), the mean time spent in each chamber, averaged across all subjects, is also presented.

Pre-conditioning baseline session. Each female was placed into the center chamber and allowed to freely access all three chambers for 60min, one day prior to conditioning.

Conditioning phase. Subjects were conditioned to associate each cue-decorated side chamber with an unconditioned stimulus once a day for four consecutive days. Unconditioned stimuli were selected based on an established history of eliciting strong CPP in each group (Seip et al., 2008; Seip & Morrell, 2008), with the most salient

stimulus (i.e., cocaine or pups) assigned to the least-preferred side chamber, as identified during pre-conditioning.

Virgin females. Conditioning stimuli were intraperitoneal injections of a cocaine (5mg/kg) or saline solution. Cocaine hydrochloride (National Institute of Drug Abuse, Research Triangle Park, NC) was freshly dissolved in a 0.9% saline solution at a 5mg/kg dose, calculated as a salt. At 1000, females were injected with saline and confined to one cue-decorated chamber for 30min (*saline-paired chamber*), then returned to their homecages. At 1200, females were injected with cocaine and confined to the opposite cue-decorated chamber for 30min (*cocaine-paired chamber*).

Postpartum females. Conditioning stimuli were pups that were age-matched to the postpartum day of the parturient females [postpartum day (PPD) 4-7 on conditioning days 1-4, respectively]. At 0945, all pups were removed from females' homecages and placed in small boxes adjacent to each homecage, so that females could smell and hear pups but not interact physically with them (details in Seip and Morrell, 2007, 2008). At 1000, females were confined to one cue-decorated apparatus chamber that contained no unconditioned stimuli (*empty chamber*) for 1hr. Females were returned to their pup-less homecages for 1hr. At 1200, five pups were placed into the opposite cue-decorated apparatus chamber and females were confined to that chamber for 1hr (*pup-paired chamber*), allowing sufficient time for maternal interactions (Fleming et al., 1994; Seip and Morrell, 2008) and prompting robust maternal care from females, as pups had been deprived from females for 2hrs and were demanding of maternal care (Pereira and Ferreira, 2006).

Post-conditioning test session. Females were tested for conditioned chamber preference on the day after the final conditioning session (e.g., PPD8 for postpartum females). All females were handled and lightly restrained in the experimenter's lap for 5min on the day before CPP testing to habituate females to the infusion procedures used on the test day. One hour prior to CPP testing, all pups were removed from postpartum females' homecages and placed in small boxes adjacent to their homecages.

Microinfusions of bupivacaine and saline. Immediately before CPP testing, females were microinfused with 2% **bupivacaine** (in 0.9% saline solution) or with a neutral **saline** solution. Microinfusions were made via bilateral injector cannulae that projected 1.5mm from the tip of the guide cannulae (8.5mm from guide cannula pedestal; 28 gauge, Plastics One, Inc., Roanoke, VA). Infusions of 0.5μl/side/min were performed using 10μl gastight syringes (1800 Series, Hamilton, Reno, NV) connected to a Harvard infusion pump. The injector cannulae were left in place for 1min after infusions ended. Females were then promptly placed into the center apparatus chamber and allowed free access to all chambers for 60min. Unconditioned stimuli (pups or drug injections) were not present. Females were retested on the following day (PPD9) with the alternate microinfusion (bupivacaine or saline) in a counterbalanced design. Microinfusion order did not elicit any CPP differences, so data were pooled.

Controls. Females that had immovable dummy stylets (i.e., surgical controls) and intact controls were handled and light restrained for 5min prior to CPP testing to mimic infusions.

After CPP testing ended, all females and pups were returned to their homecages. In all subjects, a positive conditioning effect was identified if, within each group, mean chamber times changed from pre- to post-conditioning. In females given microinfusions, the saline CPP test was used as the post-conditioning data.

Locomotion. Locomotion was measured within the CPP apparatus via infrared beams that traverse the chamber floors. For each female receiving microinfusions, new beam breaks were automatically pooled into 5min bins and divided by the time (in seconds) spent in the chamber during those 5min, resulting in a locomotor rate. Locomotor rates calculated during both CPP test sessions were compared to confirm that microinfusions did not impair subjects' motor activity during CPP testing.

Maternal behavior testing. In a time frame independent of CPP conditioning and testing, homecage maternal behavior was observed and scored in a subpopulation of VTA (n=9) and intact (n=3) postpartum females on PPD7-8. Behavioral testing commenced at least 3hrs after postpartum females were reunited with pups in their homecages following CPP. One hour prior to behavioral testing, pups were removed from females' homecages and placed in small boxes adjacent to each homecage (see Seip and Morrell, 2008). VTA females received a microinfusion of bupivacaine or saline (as described above) and were promptly returned to their homecages. Intact controls (PPD8) were handled and lightly restrained for 5min before being returned to their homecages. Eight pups were scattered around each female's homecage and maternal behaviors were observed for 15min. The female's latency to retrieve her first pup to her nest, retrieve all eight pups to her nest, hover over her pups, and assume an immobile (low) crouch position over her pups were recorded. The occurrence of anogenital and corporal licking

of pups, carrying a pup in mouth (non-retrieval back to nest), and non-maternal behaviors (sniffing the air, self-grooming, eating/drinking, resting/sleeping) were also recorded every 10sec. Criteria for these behaviors are as previously defined (see Seip and Morrell, 2008).

Histology. Subjects were deeply anesthetized with Nebutal (1ml) and intracardially perfused with 4% formalin. Brains were removed, sectioned at 30μm using a Bright-Hacker cryostat, and mounted on chrom-alum coated slides. Alternate sections were stained with Cresyl Violet to identify cannulae location, based on neuroanatomy described by Paxinos and Watson (1986), and rinsed with a series of distilled H_2O and alcohol washes before coverslipping with Permount (Fisher Scientific). Tissue located past the injector cannulae tips did not show signs of lesion or scarring, indicating that injected volumes of solution did not permanently damage tissue. *Anatomical location of cannulae.* The most caudal tips of injector cannulae marked the presumed anatomical location of microinfusions. Females receiving injections were separated into groups by anatomical location of injector cannulae termination: bilaterally in the VTA (*VTA females*), unilaterally within or in close proximity to the substantia nigra (*Substantia nigra controls*), or bilaterally rostrally and/or dorsally to VTA (*Injection controls*). Additional groups were formed by females having dummy stylets that could not be removed from guide cannula for microinjections (*Surgical controls*) and by non-surgical females (*Intact controls*). See Figure 1.

Statistics. Statistical analyses were performed as before (Seip and Morrell, 2007, 2008; Seip et al., 2008), with $P<0.05$ as significance level. Chamber preferences are presented as the percentage of individual subjects meeting criteria for each of four preference

categories (preference for either stimulus-paired side chamber, center chamber, or no preference). Chamber times (averaged across subjects), locomotor rates and behavior scores are presented as means and standard errors of the mean (SEMs). Non-parametric tests used as needed. *Within-groups.* Pre- and post-conditioning preferences were compared using a chi-square goodness of fit test for specified proportions or a one-tailed test for significance of a proportion. Pre- and post-conditioning times were compared using two-way ANOVAs (chamber and session as repeated measures) and select t-tests. Locomotor rates were compared using two-way ANOVAs (infusion and time as repeated) and select t-tests. Maternal behavior scores were compared using Wilcoxon signed ranks (*T*) tests. *Between-groups.* Chamber preferences were compared using one-tailed tests of significance of difference between two proportions. Chamber times and locomotor rates within a session were compared using two-way ANOVAs (chamber or time/day as repeated, respectively) and select t-tests. Maternal behavior scores were compared using Mann-Whitney *U* tests.

Results

Verification of anatomical location of cannulae. *VTA females.* The tips of injector cannulae terminated bilaterally in the VTA and microinfusions were patent in eight virgin and ten postpartum females (Figure 1a-b). *Substantia nigra controls.* In two postpartum and two virgin females, injector cannulae terminated unilaterally within or in close proximity to the substantia nigra (SN; Bregma -5.20mm) and unilaterally in VTA (Figure 1c). These females displayed severe locomotor impairment immediately after

bupivacaine microinfusion. Importantly, this locomotor deficit emerged exclusively in these females, confirming that the functional inactivation of a discrete non-VTA site produced observable changes in behavior. As females were unable to move between CPP chambers and remained in the center chamber during the first 15min of the CPP test session, they were omitted from CPP test analyses. *Injection controls*. Injector cannulae terminated rostrally and/or dorsally to VTA in seven females (Figure 1d). These females expressed identical conditioned chamber preferences and times (i.e., CPP) following bupivacaine and saline microinfusions (Figure 2). As both microinfusions elicited similar CPP in the injection control group, data following saline microinfusion was used in pooled comparisons (see below). *Surgical controls*. In ten postpartum and seven virgin females, guide cannula terminated dorsally and/or rostrally to the VTA but dummy cannula could not be removed for microinjections (Figure 1e). *Intact controls*. Nineteen postpartum and twelve virgin females remained intact. *All controls*. Intact, surgical, and injection control groups all expressed identical CPP responses. As conditioned chamber preferences and times were similar in all control females able to perform CPP (i.e., excluding substantia nigra controls), data from all control groups (now referred to as *control females*) were pooled for subsequent analyses.

Conditioned place preference.

Pre-conditioning response and effect of conditioning. Virgin females spent the least amount of time in the chamber to be paired with cocaine [compared to saline chamber: $t(36)=-3.43$, $P<0.01$] and postpartum females spent the least amount of time in the chamber to be paired with pups [compared to center: $t(40)= -4.19$; to saline: $t(40)=-3.04$; both $P<0.01$] (Figure 3). A positive effect of conditioning was confirmed in all virgin

females [VTA females: $F(2,14)=3.22$, $P=0.07$; controls: $F(2,52)=10.98$, $P<0.001$] and in VTA postpartum females [$F(2,18)=4.65$, $P<0.05$].

Post-conditioning test session.

Bupivacaine microinfusion. Amongst virgin females, VTA females microinfused with bupivacaine expressed identical chamber preferences (Figure 4a) and times (Figure 4b) to those of control females. Specifically, preference for the cocaine-paired chamber and time spent within the cocaine-paired chamber remained strikingly similar in VTA and control virgin females. In contrast, while approximately 35% of postpartum control females preferred the pup-paired chamber, no (0%) postpartum VTA female preferred the pup-paired chamber following bupivacaine microinfusion ($z=2.13$, $P<0.05$) (Figure 5a). Most postpartum VTA females preferred the empty chamber or did not express chamber preference. More VTA females than control females lacked chamber preference after bupivacaine microinfusion ($z=2.13$, $P<0.05$).

Saline microinfusion. All virgin females expressed identical chamber preferences (Figure 4a) and times (Figure 4b) to those of control females, with 52% of control females and 50% of VTA females preferring the cocaine-paired chamber following saline microinfusion. Amongst postpartum females, 50% of VTA females strongly preferred the pup-paired chamber following saline microinfusion, which was significantly greater than the pup-paired chamber preference expressed after bupivacaine microinfusion ($z=$-3.16, $P<0.001$). During this saline session, VTA females' preference for the pup-paired chamber also matched that expressed by control females (Figure 5a). Few VTA or control postpartum females did not express a chamber preference, with substantially

fewer VTA females lacking chamber preference following saline microinfusion than following bupivacaine microinfusion (z=-3.15, P<0.001). VTA females also spent significantly more time in the pup-paired chamber after saline microinfusions than after bupivacaine microinfusions [$t(9)$=-2.57, P<0.05] (Figure 5b). Importantly, time spent in the center and empty chambers by VTA females remained identical after each infusion. Following each infusion, chamber times did not differ significantly between VTA and control females.

Locomotion. In all VTA females, locomotion was strikingly similar after bupivacaine and saline microinfusions (Figure 6). Following each infusion, all VTA females moved easily between CPP chambers and visited each chamber during the first 15min of each CPP test session. Regardless of microinfusion, locomotor rate decreased significantly across the CPP test session in both virgin females [main rate effect: cocaine-paired chamber, $F(11,77)$=4.01; saline-paired chamber, $F(11,77)$=2.67; both P<0.01] (Figure 6b,d) and postpartum females [main rate effect: pup-paired chamber, $F(11,99)$=4.03; empty chamber, $F(11,99)$=4.12; both P<0.001] (Figure 6a,c). A significant interaction emerged between session time and microinfusion in the empty side chamber in postpartum females [$F(11,99)$=2.70, P<0.01] and in the cocaine-paired chamber in virgin females [$F(11,77)$=2.89, P<0.01]. Locomotor rates in these chambers were subsequently collapsed across the first half (0-30min) and last half (30-60min) of the session and compared; a significant reduction in locomotor rate only emerged in the empty chamber following bupivacaine infusion [$t(9)$=3.41, P<0.01].

Maternal behavior. In postpartum females, the expression of various maternal behaviors was severely impaired following bupivacaine microinfusion into VTA (Figure

7). Bupivacaine microinfusion into the VTA substantially extended postpartum females' latency to retrieve all eight pups to the nest [T=2.0, P<0.02], hover over pups [T=5.0, P<0.05] and assume a low crouch position over pups [T=5.0, P<0.05] compared to saline microinfusion (Figure 7a). Following bupivacaine microinfusion, VTA females also performed less anogenital licking [T=5.0, P<0.05] and more non-maternal behaviors such as sniffing the air [T=1.0, P<0.01] than they did following saline microinfusion (Figure 7b). VTA females microinfused with bupivacaine also were observed carrying pups in their mouth around their homecage and/or dropping pups in non-nest locations (i.e., disorganized retrieval) more frequently than when microinfused with saline [T=0.0, P<0.01]. Compared to control females, VTA females microinfused with bupivacaine took significantly longer to retrieve their first pup (U=-8.5, P<0.02) and all eight pups to the nest (U=-12.0, P<0.02) and to hover (U=-9.0, P<0.02) and crouch over pups (U=-10.0, P<0.02). Following bupivacaine microinfusion, VTA females also performed less anogenital licking (U'=1.0, P<0.05) and sniffed the air more frequently (U=-13.0, P<0.02) than control females. Following saline microinfusions, all maternal behaviors expressed by VTA females were identical to those of controls.

Discussion

The present study reveals a novel and striking difference in the role of VTA during the expression of CPP for a chamber paired with a natural (pup) stimulus than preference for a chamber paired with a pharmacological (cocaine) stimulus. Specifically, virgin females' preference for the cocaine-paired chamber remained intact despite VTA

inactivation (via bupivacaine), with chamber preference remaining identical regardless of whether bupivacaine or saline was microinfused into the VTA. In contrast, postpartum females' preference for a pup-paired chamber was abolished by bupivacaine microinfusions into the VTA but was identical to that of control females following saline microinfusions into the VTA. Importantly, postpartum females' preference for the center and empty side chambers remained similar regardless of VTA function, confirming that disrupted CPP expression was restricted to the chamber paired with the salient pup stimulus. To our knowledge, this is the first demonstration that intact VTA function is critical to the expression of CPP for a chamber paired with a natural stimulus attributed with strong incentive value but not a chamber paired with highly salient cocaine.

Importantly, females' chamber preferences following intra-VTA saline microinfusions were strikingly similar to those of intact controls, confirming that neither VTA cannulation nor surgery itself altered CPP expression. Potential confounds related to surgery (e.g., anesthesia, post-surgical stress and discomfort) or tissue damage caused by cannula implantation (e.g., Benveniste and Diemer, 1987) occurred prior to the start of the CPP paradigm and did not affect acquisition or expression of CPP, as reported for amygdalar cannulation (Herzig and Schmidt, 2007).

As bupivacaine blocks Na+ channels to induce widespread neuronal inactivation, discrete bupivacaine microinfusions used in the present study presumably inactivated all neuronal types within the VTA. Neurons in the VTA vary widely in their connectivity and neurochemical content: in addition to well-characterized DAergic neurons, distinct subpopulations of VTA neurons contain gamma-amino butyric acid (GABA) (Van Bockstaele and Pickel, 1995; Carr and Sesack, 2000) and glutamate (Yamaguchi et al.,

2007). While DAergic neurons projecting from the VTA to the NAc have received the most experimental attention regarding motivation and goal-directed behavior, it is possible that GABAergic and glutamatergic VTA neurons also participate in motivated behavior and drug-related responsiveness. These distinct subpopulations of VTA neurons send long-range projections to a variety of targets, including the NAc (Van Bockstaele and Pickel, 1995), prefrontal cortex (PFC) (Carr and Sesack, 2000), amygdala (Rosenkrantz and Grace, 2002), hypothalamus and MPOA (Swanson, 1982; Miller and Lonstein, 2006), ventral pallidum (Fields et al., 2007) and lateral habenula (Swanson, 1982). These projections may contribute importantly to different aspects of reward-related and goal-directed behaviors, including decision-making and affective processing (McBride et al., 1999; Tzschentke, 2000; Schultz, 2002; Fields et al., 2007; Matsumoto and Hikosaka, 2007, 2009). Importantly, unlike blocking specific classes of receptors located exclusively on specific subpopulations or projections of VTA neurons, transient inactivation via bupivacaine depressed the activity of all neurochemical subpopulations of VTA neurons, effectively eliminating VTA-mediated input to a broadly distributed neural circuitry participating in motivated behavior.

As voltage-gated Na+ channels are also located at the nodes of Ranvier on axonal fibers, bupivacaine also blocked the propagation of action potentials in fibers of passage. One such fiber bundle, the fasciculus retroflexus (FR), projects from the habenula to the interpeduncular and raphé nuclei through the midbrain, slightly dorsal to the VTA. Though the habenula and its projections can signal the motivational value of stimuli and their predictive cues (Matsumoto and Hikosaka, 2007, 2009), it is unlikely that intra-VTA bupivacaine-induced blockade of FR affected CPP, as our injection controls

expressed normal CPP despite being given rostral and/or dorsal bupivacaine microinfusions that contained the FR.

Given the injected volume and expected diffusion of bupivacaine, we posit that physiological inactivation induced by bupivacaine microinjection was limited to the VTA. The VTA extends approximately 1,000 microns (μm) mediolaterally, 800-1,000μm dorsoventrally, and 600-800μm rostrocaudally. Bupivacaine is structurally similar to the amide anesthetic lidocaine, which diffuses in an estimated sphere of 1000μm from the tip of the injector cannulae, per 1μl microinfusion (Sandkühler et al., 1987; Martin and Ghez, 1999; Pereira de Vasconcelos et al., 2006). Elegant visualization and electrophysiological studies suggest that this sphere of diffusion coincides closely with a spherical area of significant depression in physiological activity (Boehnke and Rasmusson, 2001) and local glucose uptake (Martin and Ghez, 1999), with little inactivation extending beyond that perimeter (Tehovnik and Sommer, 1997). As expected for bupivacaine, lidocaine-induced neuronal inactivation is greatest at the site of microinjection, where drug concentration is greatest, and decreased gradually with increasing distance from injection site (Martin, 1991; Martin and Ghez, 1999). Given its smaller molecular mass (Tucker and Mather, 1998), bupivacaine is presumed to spread to equal or lesser distances than an identical volume of lidocaine. Thus, we posit that our bupivacaine microinjections of 0.5μl/side diffused about 500μm from all directions of the injector cannula and, given the accuracy of cannula tip placement, inactivated the majority of the VTA without diffusing into neighboring structures.

The site specificity of microinfusions was also verified behaviorally in our locomotor analyses. In VTA females, locomotor habituation emerged consistently in

153

each chamber, mean locomotor rates did not differ, females moved easily between CPP chambers and postpartum females carried and manipulated pups normally after bupivacaine and saline microinfusions. However, unilateral bupivacaine microinjection into the adjacent substantia nigra immediately produced profound locomotor impairment, verifying that bupivacaine effectively inactivated neurons at the infusion site to produce measurable behavioral deficits. As VTA females were motorically normal after bupivacaine microinfusion, we posit that bupivacaine did not diffuse into substantia nigra and functional inactivation was spatially restricted to the VTA.

Region-specific mesocorticolimbic lesions have been used frequently to modify the acquisition of CPP (for review, see Tzschentke, 1998, 2007) but very rarely to alter the expression of CPP. Interventions targeting the acquisition of CPP disrupt the ability of a subject to encode the valence of the primary stimulus (e.g., pups) and/or attribute incentive value to the contextual cues (i.e., chamber) paired with that stimulus via associative learning. In contrast, interventions directed at the expression of CPP target subjects' motivation to seek out and spend time in the chamber attributed with certain incentive value. While this motivation also involves a subject's contextual memory regarding the conditioned value of each CPP chamber, it is unlikely that mesolimbic manipulations dramatically modulate the retrieval of associative learning that has already been consolidated (Dalley et al., 2005; Berridge, 2007), as in CPP expression. Thus, we posit that the abolished expression of pup CPP following VTA inactivation primarily reflects a selective disruption of goal-directed behavior toward cues paired with the salient pup stimulus. While previous studies have explored the expression of CPP for morphine-paired cues after VTA inactivation (Moaddab et al., 2008) or amphetamine-

paired cues after lesion of mesolimbic DA projections (Sellings and Clarke, 2003), this study is the first to describe the expression of CPP for cocaine- or pup-paired cues after VTA disruption of any kind.

The disruption of pup CPP by VTA inactivation extends growing evidence that the incentive value of pups depends upon connections between the maternally responsive medial preoptic area (MPOA) and the mesolimbic system. The MPOA is necessary for the integration of polysensory pup-related input, the performance of maternal behavior (Lee et al., 2000; Numan et al., 2007) and, most recently, the expression of maternal motivation to seek out and interact with offspring: MPOA inactivation reduces a maternal female's motivation to reunite with offspring (Perrin et al., 2007), bar-press for access to offspring (Lee et al., 2000) and prefer a pup-paired chamber (Pereira and Morrell, in preparation). Maternally responsive neurons in the MPOA and ventral bed nucleus of the stria terminalis (vBNST) project directly to VTA (Numan and Smith, 1984; Numan and Numan, 1997; Numan and Insel, 2003) and, at least in the case of vBNST projections, strongly excite DA neurons in the VTA (Georges and Aston-Jones, 2002). It has been proposed that these maternally activated projections to VTA mediate the unique incentive value attributed to pups during the performance of motivated pup-directed behavior (Numan, 2007; Numan and Stolzenberg, 2008, 2009). We posit that, in the present study, inactivating the VTA blocked MPOA/vBNST access to the mesolimbic system to disrupt females' motivation to seek out pups in their absence. This localized disruption was sufficient to abolish females' preference for the pup-paired chamber.

While this study is the first to assess the role of the VTA in maternal motivation, discrete lesions to MPOA-VTA circuitry impair the performance of maternal behavior. Permanent (Lee et al., 2000) or transient (Perrin et al., 2007) lesions of the MPOA disrupt motivated maternal behaviors in postpartum females, likely by eliminating pup-specific input to VTA (Numan, 2007; Numan and Stolzenberg, 2008, 2009). Lesion of the entire VTA (Gaffori and LeMoal, 1979) or blockade of oxytocin (Pedersen et al., 1994), opioid (Thompson and Kristal, 1996), or GABAergic receptors in the VTA (Numan and Stolzenberg, 2009) also impairs maternal behavior. Specifically, damage to ascending DAergic projections from the VTA (Hansen et al., 1991) or their termination sites (PFC: Afonso et al., 2007; NAc: Lee et al., 2000) impairs pup retrieval. In the present study, VTA inactivation also impaired pup retrieval and anogenital licking, though females were still fully capable of carrying and transporting pups with their mouths, grouping pups in the nest, and licking pups (see corporal licking data) following bupivacaine microinfusions. Impaired pup retrieval also extended females' latency to hover or crouch over pups: our observations revealed that, once initiated, these nursing-like positions were assumed normally. As similarly specific deficits in pup retrieval and licking behaviors were recently reported following permanent PFC lesions (Afonso et al., 2007), these behaviors may rely on intact VTA-PFC projections, which are functionally disabled by intra-VTA bupivacaine microinjections. This is also the first study to reveal a relatively selective disruption in motivated maternal caretaking behavior and motivated seeking of pup-related stimuli (pup CPP) after VTA inactivation in the same females.

While the expression of preference for chambers paired with a natural stimulus required intact VTA function, preference for the cocaine-paired chamber remained intact

despite widespread VTA inactivation. Extensive research suggests that the mesolimbic circuitry, including the VTA and NAc, contribute importantly to an animal's response to cocaine and cocaine-paired stimuli and cocaine-seeking behavior (Carelli et al., 2000; Carelli and Ijames, 2001; Carelli, 2002, 2004; Phillips et al., 2003a; Roitman et al., 2004; Yun et al., 2004; Sombers et al., 2009). In the present study, however, the expression of cocaine CPP remained unaffected by inactivation of the VTA and its ascending projections. Further, the only other study to manipulate VTA function to affect the expression of CPP (Tzschentke, 1998, 2007) revealed that the expression of morphine CPP does require intact VTA function (Moaddab et al., 2008).

Based on these findings, it is possible that the resilience of motivated cocaine-seeking behavior during VTA inactivation may be attributable to relatively rapid cocaine-induced changes in the females' extended motivational circuitry, such as altered neuronal activity (Einhorn et al., 1988; Mercuri et al., 1992; Bonci et al., 2003; Febo et al., 2004) and/or synaptic plasticity (Ungless et al., 2001). As similar changes can correspond to cocaine-induced locomotion (Borgland et al., 2004; Wanat and Bonci, 2008) and persistent cocaine-seeking behavior (Engblom et al., 2008), these changes may also facilitate the recruitment of compensatory motivational mechanisms by cocaine, but not natural, stimuli. Though natural and drug stimuli activate overlapping but distinct components of motivation-related brain circuitry (Carelli et al., 2000; Carelli, 2002; Grigson, 2002), cocaine can induce cellular and/or molecular changes within this circuitry more rapidly and with greater magnitude than natural stimuli. Conditioned preference for a cocaine-paired chamber evokes significantly stronger cFos expression in the PFC, basolateral amygdala (BLA), NAc, and cingulate than does preference for

chambers paired with natural stimuli (Mattson and Morrell, 2005; Zombeck et al., 2008). Self-administration of cocaine but not natural food reward can also induce synaptic plasticity (Chen et al., 2008). Further, limited cocaine exposure can induce functional long-lasting changes to the mesolimbic system, such as increased basal DA levels in the NAc (e.g., Chefer and Shippenberg, 2002), whereas extensive pup exposure is needed to intiate similar changes (Afonso et al., 2008). We now reveal that, compared to pup CPP, the expression of cocaine CPP is also more resilient to disruption by VTA inactivation.

Given the diffuse network of motivation-related circuitry, a number of alternative, non-VTA dependent mechanisms may exist to maintain cocaine-seeking behavior during the expression of CPP. The activity of the basolateral amygdala (BLA) provides one possible compensatory mechanism, as BLA is strongly activated by preference for a cocaine-paired chamber (Mattson and Morrell, 2005) and cocaine-paired cues (Carelli et al., 2003). The BLA projects strongly to NAc and, through these projections, can facilitate DA efflux in NAc directly through presynaptic mechanisms and regardless of VTA inactivation (Floresco et al., 1998; Phillips et al., 2003a). As the BLA is not activated as strongly by preference for a pup-paired chamber (Mattson and Morrell, 2005), the BLA may be able to sustain CPP during VTA inactivation if the primary stimulus is a highly potent drug like cocaine.

The prefrontal cortex (PFC) may also contribute to the expression of cocaine CPP during VTA inactivation. The PFC helps mediate the reinforcing properties of cocaine (Isaac et al., 1989) and participates in goal-directed behavior (Tzschentke, 2000; Hitchcott et al., 2007). As prefrontal disregulation can increase responsivity to drugs and drug-related stimuli (Jentsch and Taylor, 1999), particularly cocaine (Tzschentke, 2000),

inactivating the VTA and its ascending DA projections to the PFC may actually facilitate responses to cocaine-paired cues and thus maintain cocaine CPP. Indeed, high-frequency PFC stimulation reduces bar-pressing to obtain cocaine but not food (Levy et al., 2007), suggesting that PFC disregulation may raise the incentive value of drugs and drug-paired stimuli, but not less potent natural stimuli.

We conclude that the role of the VTA in goal-directed behavior depends on the identity of the target stimulus. As virgin and postpartum females express identical CPP for intraperitoneal cocaine (Seip et al., 2008) and, if sensitized to behave maternally, for a pup-paired chamber (Seip and Morrell, 2008), we fully expect that, following VTA inactivation, cocaine CPP would remain intact in postpartum females and pup CPP would be abolished in maternal virgin females. Together, these findings emphasize the importance of characterizing females' motivated behavior toward various salient stimuli in her environment. The choice to invest time with a specific stimulus over available alternatives can be evolutionarily advantageous, such as when a female seeks out stimuli that will directly benefit her progeny, e.g., food or maternal care, or maladaptive, such as drug-seeking behavior. The present work now reveals that goal-directed behavior toward these natural and highly adaptive stimuli may be strikingly vulnerable to loss of VTA function compared to drug-seeking behavior.

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Figure captions.

Figure 1. Histology. The placement of the injector tips of bilateral internal cannula for individual virgin (a) and postpartum (b) ventral tegmental area (VTA) females, and pooled substantia nigra controls (c), injection controls (d), and surgical controls (e). Approximate stereotaxic coordinates, represented as millimeters posterior to Bregma, are listed to the right of each section.

Figure 2. Conditioned chamber preferences (a) and times (b) following bupivacaine (*striped bars*) and saline microinfusions (*solid bars*) in injection controls, in which cannulae terminated dorsally and/or rostrally to the ventral tegmental area.

Figure 3. Mean time (min) spent in each chamber of the place preference apparatus during the pre-conditioning session by virgin (a) and postpartum (b) females. *P*<0.05 for all within-groups (*) comparisons.

Figure 4. Individual chamber preferences (a) and mean time (min) spent in each cue-paired chamber (b) during the post-conditioning test session(s) by virgin females. Immediately prior to the start of the test session, VTA females were given a microinjection containing 2% bupivacaine *(striped bars)* or saline solution *(solid bars)*; all control females were handled but no microinjections were administered (*open bars*).

Figure 5. Individual chamber preferences (a) and mean time (min) spent in each cue-paired chamber (b) during the post-conditioning test session(s) by postpartum females. Immediately prior to the start of the test session, VTA females were given a microinjection containing 2% bupivacaine *(striped bars)* or saline solution *(solid bars)*;

all control females were handled but no microinjections were administered (*open bars*). All between-groups (#) and within-groups (*) comparisons, *P*<0.05.

Figure 6. Mean locomotor rate (beam breaks per second) of VTA females across the post-conditioning test session, immediately following intra-VTA microinfusions of 2% bupivacaine *(open circles)* or saline *(filled circles)*. Locomotor rates of postpartum females in the pup-paired (a) and empty (b) chambers and of virgin females in the cocaine-paired (c) and saline-paired (d) chambers are shown. *Inset:* Mean locomotor rate in each CPP chamber pooled across entire 60min session following intra-VTA microinfusions of bupivacaine (*striped bars*) or saline (*solid bars*).

Figure 7. Mean latency to perform (a) and number of observations of (b) various maternal behaviors by postpartum females, immediately following intra-VTA microinfusions of 2% bupivacaine *(striped bars)* or saline *(solid bars)*. Intact females were handled but no microinjections were administered (*open bars*). All between-groups (#) and within-groups (*) comparisons, *P*<0.05.

170

Figure 1.

VTA Females - Virgin (a.); VTA Females - Postpartum (b.); Substantia Nigra Controls (c.); Injection Controls (d.); Surgical Controls (e.)

● Microinfusions into VTA
△ Microinfusions outside of VTA
◇ No Microinfusions

Figure 2.

Figure 3.

a. **Virgin Females**

b. **Postpartum Females**

Figure 4.

Figure 5.

a.

Chamber Preferences

b.

Chamber Times

Figure 6.

Figure 7.

GENERAL DISCUSSION

The experiments in this dissertation were designed to characterize the motivational state of the postpartum female rat. A combination of conditioned place preference (CPP) experiments, behavioral observations, and neurobiological studies were used to examine the motivational state of the female rat across a unique and biologically critical time in her lifecycle.

The motivational state of the postpartum female has remained virtually unexplored in both animal and human literature. In the rat, the postpartum females' drive to seek out her pups (i.e., maternal motivation) is extremely strong, particularly during early postpartum. However, existing studies have only characterized postpartum females' maternal motivation in contrast with motivation for alternative, non-pup stimuli such as cocaine (Mattson et al., 2001) or during a very limited portion of the pos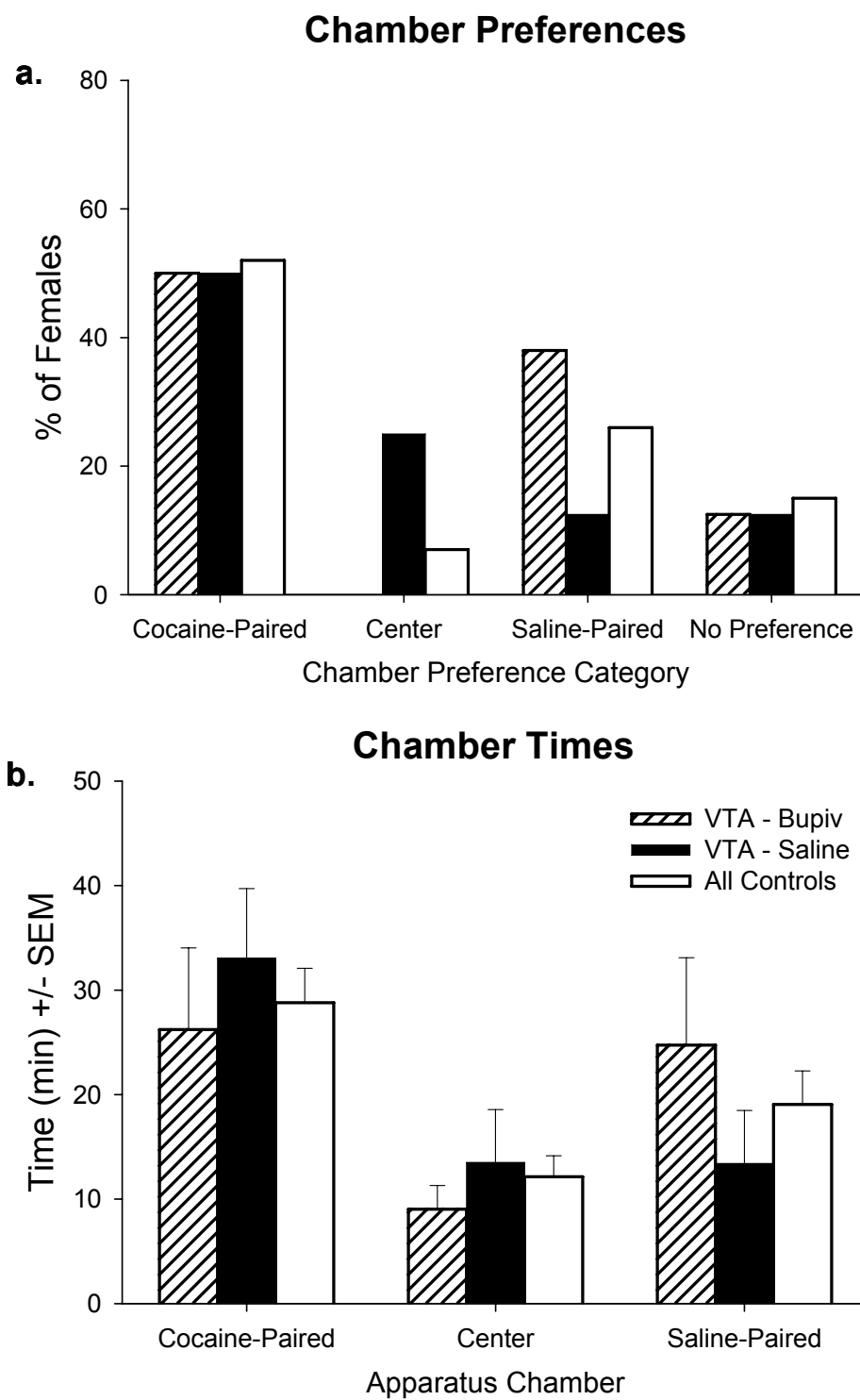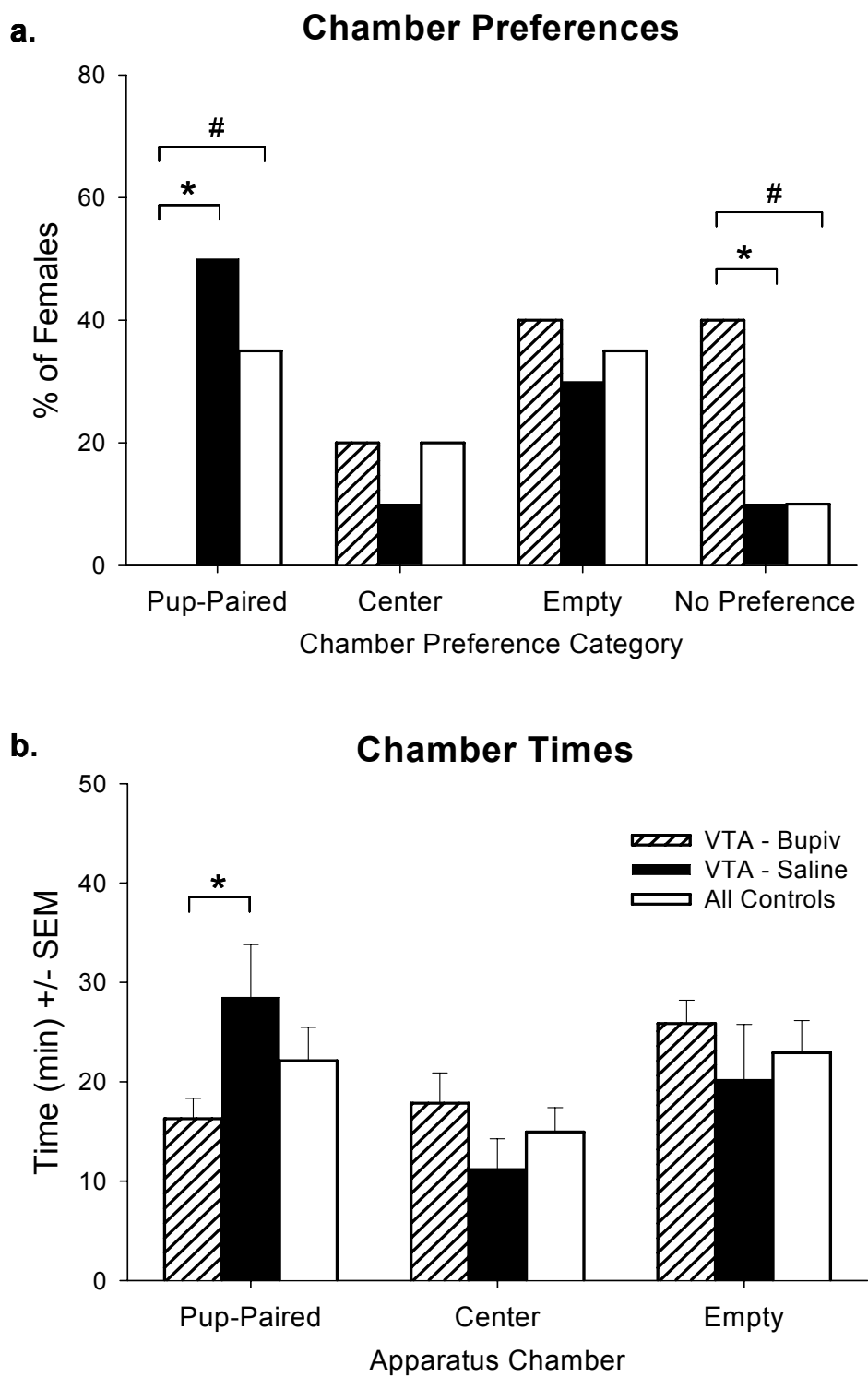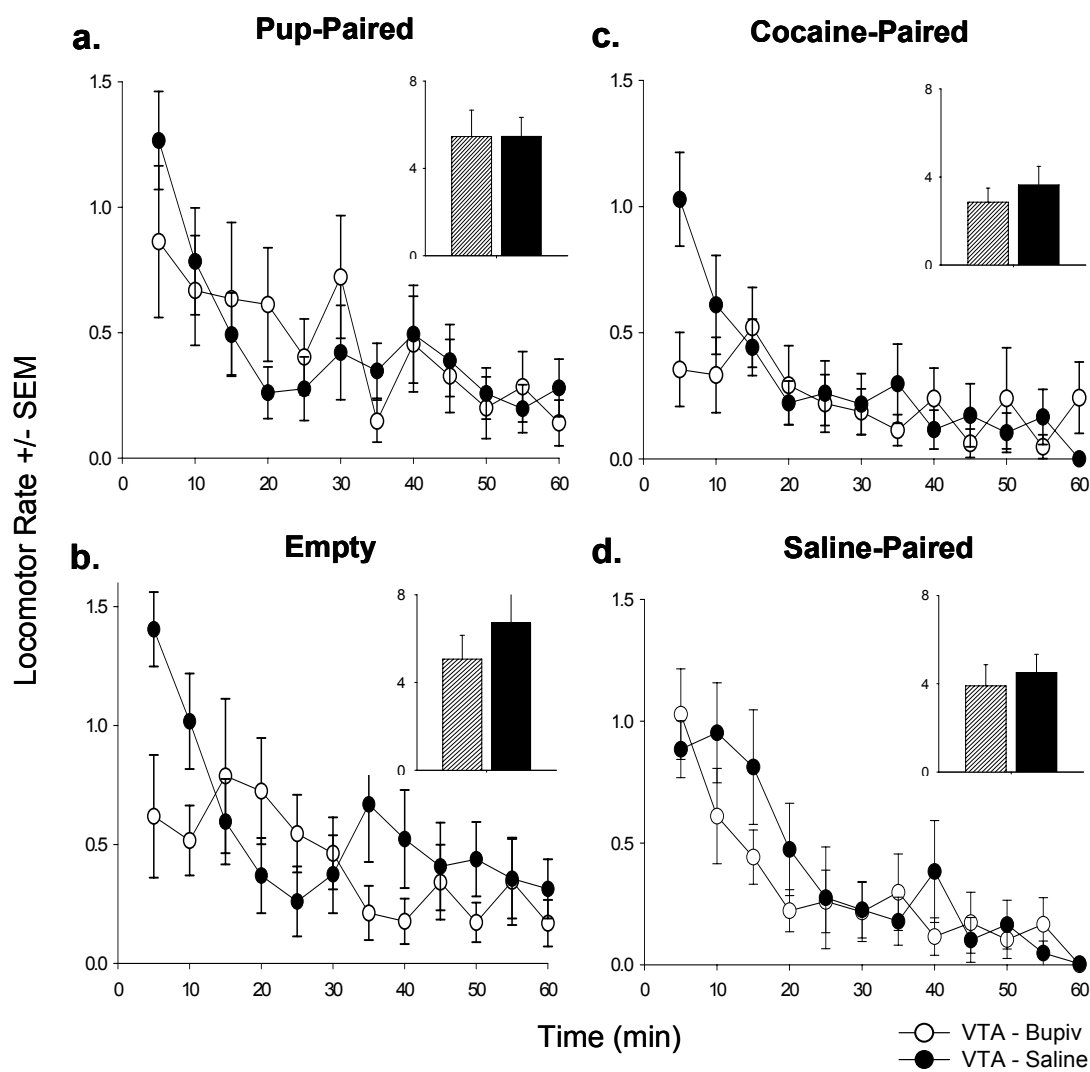tpartum period (Fleming et al., 1994). Postpartum motivation for non-pup stimuli, such as cocaine, has not been studied across the full postpartum period; the only existing study utilized an operant paradigm (Hecht et al., 1999) that included drug deliveries, confounding interpretations regarding cocaine's motivational effects during this period. Additionally, critical corollary measures of locomotion and stimulus-directed consummatory behavior are often omitted from motivational analyses.

To investigate the strength of maternal motivation across the postpartum period, the first goal of this dissertation was to challenge females' preference for a pup-paired chamber when presented with an alternative choice of a maximally motivating cocaine stimulus, as described in Chapter 1. A few studies have used a dual-choice CPP

paradigm to assess simultaneously the relative incentive-motivational value of two distinct stimuli (Mattson et al., 2001). However, the dual-choice CPP paradigm offers the opportunity to seek out cues associated with various stimuli of different reward value and biological relevance, more closely approximating the natural and complex environment of an exploratory, maternal rodent. While previous studies revealed that most females prefer a pup-paired chamber over a chamber paired with moderately salient cocaine in early postpartum but strongly prefer the cocaine-paired chamber by late postpartum (Mattson et al., 2001), it was unclear whether early postpartum females' pup-preference was attributable to the high incentive value of pups, a blunted responsivity to the cocaine stimulus, or a combination of factors. As described in Chapter 1, maximizing the incentive-motivational value of the cocaine stimulus paired with the alternately available chamber increased early postpartum females' preference for the cocaine-paired chamber. Thus, a proportion of both early and late postpartum females found cocaine highly rewarding. Many early postpartum females, however, retained strong preference for the pup-paired chamber, indicating that the strength of maternal motivation could trump cocaine-seeking exclusively during early postpartum (Seip and Morrell, 2007).

While this highly salient cocaine stimulus significantly increased the proportion of early postpartum females preferring the cocaine-paired chamber from previously reported levels (Mattson et al., 2001), there were still significantly more late postpartum females that preferred cocaine. These data raised an intriguing question: might the complex endocrinological, physiological and behavioral components of the early postpartum period blunt the incentive value of cocaine? The second goal of this dissertation was to determine whether early and late postpartum females expressed

similar preference for a cocaine-paired chamber when pups were not presented as an alternative choice. As described in Chapter 2, females consistently preferred a cocaine-paired chamber over a neutral saline-paired chamber across a broad range of drug administration parameters (Seip et al., 2008). Across all administration routes and doses, preference for the cocaine-paired chamber was strikingly similar between early and late postpartum, indicating that the attribution of incentive value to cocaine remained unchanged across the entire postpartum period. Further, the systematic dose-preference curve revealed the sensitivity of the CPP paradigm in complex drug administration studies. Importantly, preference for the cocaine-paired chamber was significantly stronger in postpartum females than in virgin females and males, representing the first systematic analysis of cocaine CPP across sexes, reproductive states, and postpartum status. That females' locomotor patterns could predict their eventual chamber preference confirmed the usefulness of corollary measures in predicting an individual's vulnerability to drug-seeking behavior.

The striking similarity between early and late postpartum females' motivation to seek out cocaine, as described in Chapter 2, revealed that postpartum females attributed identical incentive value to a pharmacological stimulus with well-established rewarding properties when contrasted with a neutral stimulus. Thus, the distinct interaction between postpartum state and preference for a pup- versus cocaine-paired chamber, as revealed in Chapter 1, was probably not mediated by females' response to cocaine. Two intriguing hypotheses emerged from this conclusion. First, the experiences of parity (including gestation, parturition, and lactation) may prime the females' motivational circuitry to respond similarly to highly potent pharmacological stimuli (e.g., cocaine) but

differentially to subtle natural stimuli. In the case of biologically relevant pup stimuli, this incentive value is posited to be highest with proximity to parturition and to decrease as the postpartum period progresses. Second, the stimulus qualities specific to young pups may alter the motivational state of the early postpartum female to promote pup-directed caregiving behavior and maternal motivation exclusively during this period. As the pups' stimulus qualities change considerably as they develop, older pups may be unable to elicit similarly strong motivation by late postpartum. Since the publication of this dissertation research, work from this laboratory has explored the second hypothesis (Wansaw et al., 2008) and will be described later.

To examine how parity influences maternal motivation, preference for a pup-paired chamber was examined after systematically manipulating females' exposure to young pups and the nature of female-pup interactions. A third goal of this dissertation was to develop a behavioral paradigm, as described in Chapter 3, that would delineate the relative contributions of parity and pup exposure to maternal motivation while keeping pup stimuli constant. Accordingly, nulliparous (virgin) females were exposed to pups for various lengths of time to induce various levels of maternal responsivity, while young pups were used at their maximally salient developmental time point (i.e., during early postpartum) and continuously replaced to minimize the impact of ongoing development. Longer pup exposures not only elicited higher quality maternal behavior from these nulliparous females but also prompted females to attribute stronger incentive value to pups. Strikingly, even brief pup exposure elicited notable preference for the pup-paired chamber in non-maternal females that received some physical contact from pups. That young pups can alter motivated behavior toward maternally relevant stimuli is

striking, given that pups have little biological relevance to the virgin female. Whether

pup exposure alters motivated behavior directed toward other non-pup stimuli, like

drugs, remains an open question.

Together, these behavioral experiments reveal that the pup stimulus is a salient,

natural stimulus capable of altering the motivational state of the female rat, regardless of

parity. The final goal of this thesis was to identify critical neural components of circuitry

supporting maternal motivation. As described in Chapter 4, transient inactivation of the

ventral tegmental area (VTA) completely abolished early postpartum females' preference

for a pup-paired chamber and impaired motivated maternal behaviors. Pup preference

was fully restored following VTA recovery, indicating that intact VTA function is

critical to the expression of maternal motivation. Importantly, virgin females' preference

for a cocaine-paired chamber remained undisrupted after VTA inactivation. These data

suggest a key role for the VTA in motivated behavior directed toward natural,

biologically relevant stimuli.

It is important to note that the motivational impact of this site-specific

inactivation can be expected to apply to a broad spectrum of subjects, regardless of

reproductive or postpartum status. As described in Chapters 2 and 3, virgin and

postpartum females express identical preference for a cocaine-paired chamber (if

administered intraperitoneally) and, if sensitized to behave maternally, for a pup-paired

chamber. It can be expected that VTA inactivation would also abolish preference for a

pup-paired chamber in maternally responsive virgin females while leaving preference for

a cocaine-paired chamber fully intact in postpartum females. The role of the VTA in

goal-directed behavior, therefore, seems to depend on the identity of the target stimulus.

Together, these findings emphasize the importance of characterizing subjects' motivated behavior toward the wide variety of salient stimuli in her environment.

Offspring characteristics influence motivation

In the natural environment, the progressive development of pups across the postpartum period has emerged as a critical factor sculpting the maternal responsiveness of the female rat. During early postpartum (days 1-8), young pups are unable to thermoregulate or express waste and are entirely reliant upon the female for warmth and sustenance (Numan and Insel, 2003). Early postpartum females consequently spend the vast majority of their time in contact with pups and performing vigorous pup caretaking activities (Grota and Ader 1969, 1974; Pereira et al., 2008). By late postpartum (days 12-16), pups' eyes and ears have opened and pups have gained a significant amount of weight (Russell, 1980; Seip et al., 2008). In response to these changes, late postpartum females invest less energy and time in pup-directed caretaking behaviors and allow pups to venture outside of her nest (Grota and Ader 1969, 1974; Reisbeck et al., 1975; Pereira et al., 2008). By day 18, pups have developed adult-like motor capacities (Smith and Morrell, 2007), sample solid foods, and are able to survive in the absence of their mother. At this point, the postpartum female has removed the barricade formed by the female with nest-material that confines her pups to her nest (Pereira et al., 2008), allowing pups to explore their environment; the female's physiology also shifts away from supporting lactation and toward resumed estrus cyclicity and sexual responsiveness. Thus, the pups' developmental capacities critically shape the motivational state of the postpartum female, with young pups facilitating motivated caregiving behavior most dramatically.

Given these findings, it can be inferred that maximal incentive value is attributed to young pups and the polysensory stimuli (e.g., odors, vocalizations) associated with them. Recent work revealed that late postpartum females strongly prefer a chamber paired with young pups over a chamber paired with older pups aged-matched to her postpartum state (Wansaw et al., 2008), confirming that young pups are maximally motivating to the maternally responsive female. Full and vigorous maternal behavior can also be fully restored in late postpartum by replacing females' older pups with young pups (Pereira et al., 2008), suggesting that the dramatic decline in maternal behavior and pup contact across postpartum (Grota and Ader 1969, 1974; Reisbeck et al., 1975) may be mediated by the incentive value of the developing pup stimulus and not the unique hormonal and physiological state of late postpartum. Young pups elicit proactive pup-seeking behaviors most rapidly in non-maternal females, as well, as young pups induce maternal behavior in virgin females more rapidly than do older pups (Stern and Mackinnon, 1978). I posit that the strong maternal motivation expressed by early postpartum and virgin females exposed to young pups, as described in Chapters 1 and 3, was critically facilitated by pup age, confirming that positive valence is consistently attributed to young pups regardless of parity or postpartum status.

Identifying neurons activated by maternal motivation

An emerging body of research has identified discrete components of the neural circuitry that participates in maternal motivation. While permanent and reversible site-specific lesions have been used to disrupt motivated pup-seeking behavior (Lee et al., 2000; see Chapter 4), examining the neuronal activity of intact brains provides critical insight into the brain circuitry supporting this complex behavior.

One well-established means of identifying neurons activated in response to a particular stimulus (e.g., pups) or sensory-behavioral event (e.g., preference for pup-paired cues) is by analyzing the expression of immediate-early genes (IEGs) and their protein products. A neuron receives stimulus- or event-specific input via multiple mechanisms, including NMDA-receptor activation (Cole et al., 1989) and cell surface signaling (Hoffman et al., 1993), which initiate a series of intracellular signal transduction pathways. Select molecular components of these pathways enter the cell nucleus and alter the expression of target genes and transcription factors, including IEGs. Located in the nucleus (Sagar et al., 1988; Hoffman et al., 1993), the IEG family consists of *fos*, *arg3.1*, *zif,* and *jun*, each of which encodes specific protein products (i.e., cFos, Arc, zif/268, and Jun-B, respectively). While the intracellular actions of IEG proteins may differ slightly, most IEG family members rapidly alter gene transcription, affecting the expression of a variety of target genes and subsequent protein synthesis. Thus, the rapid and transient expression of IEG mRNA or associated protein products following a sensory-behavioral event can be quantified to reflect patterns of neuronal activity across anatomically discrete brain structures.

Previous work in postpartum females revealed that the expression of preference for a pup-paired chamber was associated with increased expression of cFos protein in neurons located in the medial preoptic area (MPOA), prefrontal cortex (PFC) and basolateral amygdala (BLA; Mattson and Morrell, 2005). Notably, the MPOA contained more cFos-positive neurons in postpartum females preferring a pup-paired chamber than those preferring a cocaine-paired chamber (Mattson and Morrell, 2005), suggesting that the MPOA is a particularly critical component of the circuitry supporting motivation

directed at natural, biologically relevant stimuli such as pups. The number of cFos-positive MPOA neurons was also significantly higher in early versus late postpartum (Pereira et al., 2008), suggesting that strong MPOA activity during early postpartum may mediate maternal motivation during this period (Seip and Morrell, 2007, 2008; Wansaw et al., 2008). Further analysis revealed an increase in the number of these cFos-positive neurons across the rostrocaudal axis of the MPOA, with the most cFos-positive neurons located in the caudal MPOA and medial preoptic nucleus (Seip et al., 2007; Pereira et al., 2008). Subregional analyses did not reveal increased numbers of cFos-positive neurons localized in any discrete subnuclei of the MPOA or BNST (Seip et al., 2007; Pereira et al., 2008). Together, these findings suggest that the integrity of the entire MPOA and BNST, particularly the caudal MPOA, participate importantly in maternal motivation.

Neurochemical identity of maternal motivation-activated neurons

To understand how these MPOA neurons participate in the neural circuitry supporting maternal motivation, it is crucial to explore the neurochemical identity of MPOA and BNST neurons activated by maternal motivation. Few MPOA/BNST neurons release neuromodulators: the preoptic area contains relatively few catecholaminergic or acetylcholinergic somata (Ruggiero et al., 1984; Woolf et al., 1984), aside from scattered DAergic somata in the periventricular nucleus (A14 cell group; Chan-Palay et al., 1984). Virtually no serotonergic or noradregenergic somata exist in the MPOA. Most MPOA and BNST neurons, however, synthesize and release either glutamate or gamma-aminobutyric acid (GABA). While *in situ* work strongly suggests that MPOA contains some glutamatergic cells (Lin et al., 2003), between 50-90% of MPOA cells express glutamate decarboxylase (GAD), the synthesizing enzyme

for GABA (Mugnaini and Oertel, 1985; Martin and Rimvall, 1993), suggesting the majority of MPOA neurons are GABAergic. In fact, prior work revealed that 50-60% of MPOA neurons activated during the expression of maternal behavior contain GAD (Lonstein and de Vries, 2000). It is plausible that these GABA-synthesizing neurons are similarly activated by preference for a pup-paired chamber and thus also participate in maternal motivation.

To examine this issue, I assessed the extent of co-localized immunoreactivity for cFos protein associated with maternal motivation (i.e., preference for a pup-paired chamber) and for a number of neurochemical markers (Seip et al., 2007; Pereira et al., 2008). Antibodies targeting GABA and two different isoforms of GAD were used to directly identify GABAergic neurons. These antibodies produced non-specific labeling (GABA) and weak, diffuse labeling in MPOA/BNST (GAD), indicating a non-specific and/or weak affinity for their antigens. Antibodies targeting calcium (Ca^{2+})-binding proteins known to co-localize with GABA were then used to indirectly identify GABAergic neurons. Three Ca^{2+}-binding proteins – parvalbumin, calretinin, and calbindin – are abundantly expressed in the nervous system (Celio, 1990; Résibois and Rogers, 1992; Rogers and Résibois, 1992) and in a proportion of hypothalamic and/or MPOA neurons (Baimbridge et al., 1992; Arai et al., 1993; Lephart et al., 1997; Lephart and Watson, 1999; Stuart and Lephart, 1999; Brager et al., 2000); importantly, parvalbumin is often expressed in GABAergic neurons (Celio, 1986). However, few MPOA/BNST neurons expressed parvalbumin or calretinin. While a moderate number of MPOA/BNST neurons expressed calbindin, few of these neurons expressed cFos associated with maternal motivation (Seip et al., 2007; Pereira et al., 2008). As calbindin

can be colocalized in either glutamatergic or GABAergic neurons, depending on discrete brain region, it is difficult to make any conclusions about the neurochemical identity of these maternal motivation-activated neurons. This question remains unanswered.

Dopamine and the medial preoptic area

An interesting neurochemical feature of the MPOA is its significant concentration of DA (Versteeg et al., 1976; Hull et al., 1995; Lonstein et al., 2003; Olazabal et al., 2004). As the MPOA does not contain DAergic cell bodies (e.g., Chan-Palay et al., 1984), DA concentrations in the MPOA can be almost exclusively attributed to post-synaptic release from afferent dopaminergic fibers terminating in the MPOA. Elegant neuroanatomical studies have revealed that most of these DAergic projections to the MPOA originate in the periventricular nucleus of the rostral hypothalamus (Björklund et al., 1975; Day et al., 1980), where the A14 cluster of DAergic somata are located (Chan-Palay et al., 1984). Though the majority of the projections from the VTA to the MPOA are non-DAergic (Day et al., 1980; Oades and Halliday, 1987), recent work identified an additional subpopulation of DAergic projections extending from the VTA to the MPOA (Miller and Lonstein, 2006). That the MPOA is exposed to multiple sources of DA is suggestive of a subtle yet important role for dopaminergic action within the MPOA in both males and females.

While no simple correlation between DA content in the MPOA and maternal responsiveness exists in the female rat (Lonstein et al., 2003; Olazabal et al., 2004), DA levels in the MPOA drop dramatically toward the end of gestation, rebound immediately after parturition and stay elevated for the duration of the early postpartum period

(Lonstein et al., 2003). These DA changes coincide closely with the onset of maternal behavior in the female rat, and therefore may contribute to the relatively rapid development of maternal responsivity. It is possible that the concentrations of steroid hormones during gestation may prime the MPOA of pregnant females to respond maternally to pups just prior to parturition (Numan and Stolzenberg, 2009). However, basal DA levels are also chronically elevated in the MPOA of maternally responsive virgin females (Olazabal et al., 2004), suggesting that, while gestational hormones may contribute to maternal responsiveness, DA action in the MPOA may be sufficient to support the onset of maternal behavior in even nulliparous (virgin) females. In accord with this hypothesis, recent work revealed that, in female rats for which pregnancy was terminated during mid-gestation, injection of a dopaminergic agonist (SKF-38393) into the MPOA facilitated the onset of maternal behavior expressed by these females toward pups; in fact, this rapid onset matched that females that continued gestation and gave birth normally (Stolzenberg et al., 2007). Thus, DAergic action in the MPOA may provide an adaptive mechanism to promote proactive pup-directed responses in the female rat, regardless of parity.

Manipulating DAergic action within the MPOA does produce substantial changes in motivated maternal responses. Early work revealed that microinjections of cocaine directly into the MPOA produce dramatic and specific impairments in maternal behavior (Vernotica et al., 1999). As cocaine dramatically increases extracellular monoamine levels by blocking presynaptic monoamine transporters for DA, serotonin, and norepinephrine, local microinfusion of cocaine into the MPOA produced dramatic deficits in maternal care that were likely attributable to DA dysregulation in the MPOA.

Later, site-specific blockade of D1-type and D2- type DA receptors located in the MPOA was revealed to produce distinct deficits in maternal behavior (Numan et al., 2005; Miller and Lonstein, 2005). In the MPOA, blocking D1-type receptors by microinjecting the DA antagonist SCH-23390 impaired motivated pup retrieval and licking behaviors, while blocking D2-type receptors via raclopride microinfusions solely affected nursing behavior (Numan et al., 2005; Miller and Lonstein, 2005). As recent work revealed that systemic stimulation of D1-type DA receptors facilitates the performance of active maternal behaviors and promotes maternal motivation (Pereira et al., 2008), DAergic action on D1-type receptors within the MPOA seem to be a key contributor to goal-directed behavior toward pups. Thus, I posit that stimulating D1-type receptors located within the MPOA may facilitate maternal motivation.

Indeed, well-established research in the male rat has confirmed that DA release in the MPOA plays an important role in the performance of goal-directed behavior toward biologically relevant stimuli (Giuliano and Allard, 2001; Hull and Dominguez, 2006). In males, extracellular DA concentrations increased in the MPOA during pre-copulatory behaviors considered to reflect sexual motivation (Hull et al., 1995; Giuliano and Allard, 2001). These changes were not attributable to changes in locomotor activity and, further, did not require gonadal hormones (Hull et al., 1995). Thus, in male rats, DA action in the MPOA contributes to motivated behavior toward a sexually receptive female, regardless of the hormonal state of the males and associated effects of steroid hormones in the MPOA. I posit that elevated DA in the MPOA may similarly facilitate pup-directed behavior and maternal motivation in the maternally responsive female. Further, it is

plausible that the DA concentrations within the MPOA may contribute to the individual differences in maternal motivation in otherwise homogenous populations of females.

Mesolimbic dopamine participates in maternal motivation

Outside of the MPOA, dopamine (DA) released from ascending mesolimbic projections from the VTA to the NAc also plays a critical role in maternal motivation and the attribution of incentive value to pups and pup-related stimuli. Maternal and pup-directed behaviors activate the VTA and NAc (Febo et al., 2005; Hernández-González et al., 2005a, 2005b) and transiently increase DA release in NAc during acute bouts of maternal behavior, such as pup licking (Hansen et al., 1993; Champagne et al., 2004). Recently, an elegant study extended these findings to reveal that, following brief exposure to pups, DA release in NAc was elevated in non-maternal females that had previous maternal experience but did not presently express maternal behavior (Afonso et al., 2008). Thus, in actively maternal females, elevated accumbal DA release during the performance of acute caretaking behaviors may prime the mesolimbic system to respond more rapidly and proactively toward pups. The concept of mesolimbic priming also has interesting implications for the re-establishment of females' maternal responsivity to future pup exposures, after she has weaned her own litter and resumed a normal cycling state (Afonso et al., 2008).

In this way, consistent interactions with young pups, which elicit the most vigorous caregiving and maternal motivation from females (e.g., during early postpartum), would be expected to contribute to mesolimbic DAergic alterations most readily. These alterations, in turn, may provide a potential neurochemical mechanism

contributing to the high incentive value attributed exclusively to young pups. For example, early postpartum females spend the majority of their time in contact with their young pups (Numan and Insel, 2003; Pereira et al., 2008), which would presumably have a substantial impact on females' mesolimbic DAergic tone; these females are also the most strongly maternally motivated (Mattson et al., 2001; Seip and Morrell, 2007, 2008; Wansaw et al., 2008). Virgin females exposed to young pups express chronically elevated DA levels in the NAc (Afonso et al., 2008); it is possible that DA levels measured after increasingly long exposures to young pups may parallel the stepwise increases in maternal behavior and motivation that emerge with increasing exposure to young pups, as described in Chapter 3. In contrast, decreased pup contact during late postpartum may shift the DA tone in the NAc away from acutely elevated levels seen in highly interactive maternal females (Hansen et al., 1993; Champagne et al., 2004) to a state that responds differently to future pup presentations (Afonso et al., 2008) but that cannot maintain robust maternal motivation.

A constellation of recent work has also revealed that DA may participate in females' attribution of incentive value to pup-paired cues (e.g., acquisition of pup CPP; Fleming et al., 1994) as well as the expression of motivated pup-seeking behavior in the absence of pups (e.g., expression of pup CPP; see Chapter 4). First, learning a conditioned association between pups and pup-paired stimuli, such as that acquired during CPP conditioning, requires a positive, motivated response toward pups presented in a specific context as well as associative learning during which the pup-paired context is attributed with incentive value that matches that of the pups. It is important to note that accumbal DA responds to the presentation of the pup stimulus (Hansen et al., 1993;

Champagne et al., 2004) and contributes to associative learning (Ikemoto and Panksepp, 1999; Berridge and Robinson, 2003; Salamone and Correa, 2002; Salamone, 1996; Salamone et al., 2005, 2007). Further work must be performed to delineate these dual functions of DA during CPP conditioning.

Second, evidence suggests that the expression of motivated pup-seeking behavior in the absence of pups, such as during the expression of CPP, also involves accumbal DA release. DA release is known to mediate the seeking out of desired stimuli (Salamone, 1996; Ikemoto and Panksepp, 1999; Carelli et al., 2002, 2004; Roitman et al., 2004; Berridge, 2007), such as pup-paired contextual stimuli within a CPP apparatus, but not, however, the retrieval of consolidated learning, including the associative learning involved in the expression of CPP (Dalley et al., 2005; Berridge, 2007). Thus, DAergic manipulations that affect the expression of CPP can be attributed to motivation-related but not learning-related behaviors. Recent data suggest this is the case: preference for a pup-paired chamber is boosted by systemic stimulation of D1-type receptors (Pereira et al., 2008) and requires intact function of the VTA and its ascending DA projections, as described in Chapter 4. As DA may contribute to the expression of goal-directed behavior toward pup-paired stimuli, it is possible that the individual differences in maternal motivation by maternal females may reflect differences in endogenous and/or pup-initiated DA release and receptor binding. To date, no work has assessed this possibility.

Endogenous opioids contribute to motivation and reward

While a great deal of literature has characterized the roles of DA in the brain (Ikemoto and Panksepp, 1999; Salamone and Correa, 2002; Salamone et al., 2005, 2007; Berridge, 2007), the endogenous opioid peptide system also contributes to motivated and reward-related behavior (Bardo, 1998; Van Ree et al., 2000; Grigson, 2002; Barbano and Cador, 2006, 2007; Smith and Berridge, 2007; Peciña, 2008). Both endogenous opioid peptides and opiate drugs can elicit conditioned place preference on their own (Bals-Kubik et al., 1993; Bardo, 1998) and may even increase the incentive value of drugs of abuse, such as cocaine (e.g., Mello et al., 1989).

Based on these findings, an extensive body of research has focused on exploring the action of opioids within the mesolimbic system. The mesolimbic system contains mu (μ) and kappa (κ) opioid receptors and responds strongly to opioid administration (Johnson and North, 1992; Thompson et al., 2000; Svingos et al., 2001a; Margolis et al., 2003). Mesolimbic regions therefore seem to be ideally situated to integrate opioidergic and DAergic contributions to motivated behavior. It is possible that, within the VTA, individual differences in concentrations of endogenous ligands and/or in the expression of opioid receptors could mediate the motivated behavior expressed by an individual toward stimuli.

In the VTA, μ receptors are located primarily on GABAergic interneurons and, when stimulated, hyperpolarize these interneurons (Johnson and North, 1992). As GABAergic interneurons project primarily to DA projection neurons, μ receptor stimulation reduces the inhibitory input to these DA neurons, resulting in increased DA neuronal firing (Johnson and North, 1992) and accumbal DA release (Di Chiara and Imperato, 1988; Spanagel et al., 1992). Many μ-containing interneurons in the VTA also

contact projections to the prefrontal cortex (PFC; Svingos et al., 2001b) and possibly the basolateral amygdala (BLA; Ford et al., 2006), suggesting that μ receptor stimulation can affect prefrontal and affective processes as well. As μ agonism within the VTA elicits strong conditioned place preference (CPP) (Bals-Kubik et al., 1993), it is likely that the action of endogenous μ-receptor ligands, such as enkephalin, within the VTA may contribute to the positive incentive value of stimuli. I posit that high levels of endogenous μ-agonists (e.g., enkephalin) and/or increased μ-receptor expression within the VTA may promote the attribution of positive incentive value to various stimuli and subsequent stimulus-seeking behavior directed toward those stimuli, such as during the expression of CPP.

In contrast, kappa (κ) opioid receptors in the VTA are located exclusively on DAergic neurons that project to the PFC (Margolis et al., 2003, 2006). Stimulation of κ receptors, such as by the endogenous ligand dynorphin, hyperpolarizes these DA neurons (Margolis et al., 2003) and elicits strong conditioned place aversion (CPA) (Bals-Kubik et al., 1993). Thus, elevated concentrations of κ-agonists (e.g., dynorphin) and/or increased κ-receptor expression within the VTA may facilitate motivated behavior directed away from a target stimulus, i.e., avoidant behavior. It is additionally possible that κ-mediated dysregulation of prefrontal processes may drive non-adaptive choice behavior, such as the preference for the cocaine- over pup-paired chamber that emerged in the small population of early postpartum females described in Chapter 1. As mesocortical projections exert a strongly inhibitory influence over PFC (Yokofujita et al., 2008), κ agonism within the VTA would presumably disinhibit the PFC. By disregulating decision-making, choice behavior, and other prefrontal processes, PFC

disinhibition can facilitate motivated responses to reward-paired cues (Jentsch and Taylor, 1999). Thus, κ-mediated activity within the VTA may contribute to maladaptive, non-maternal choice behavior by disinhibiting prefrontal decision-making processes and/or mediating the aversive qualities of pup stimuli.

An additional point of evidence in support of these contributions comes from accumbal κ activity, which may also contribute to motivated behavior toward aversive stimuli. κ receptors are located pre-synaptically on the terminals of mesolimbic DA projections within the NAc (Svingos et al., 2001a), and action at these accumbal κ receptors has been proposed to reduce extracellular DA (Di Chiara and Imperato, 1988; Spanagel et al., 1992), even that induced by cocaine (Thompson et al., 2000). Not surprisingly, local stimulation of these κ receptors also elicits strong conditioned place aversion (CPA) (Bals-Kubik et al., 1993). Further evidence supporting κ-mediated aversive processes comes from studies on chronic drug exposure, which has been demonstrated to upregulate endogenous dynorphin and κ receptor activity (Shippenberg et al., 2001; Shippenberg et al., 2007). It has been proposed that dysregulated κ-mediated activity helps to maintain drug-seeking behavior seen in drugs addicts, despite their aversive schedule of drugs administration (Shippenberg et al., 2007). While the experiments described in Chapters 1 and 2 of this dissertation only used a limited number of acute exposures to drug, it is possible that an emerging upregulation of κ receptor activity may have contributed to individuals' attribution of added incentive value to drug stimuli and preference for the drug-paired chamber.

In addition to contributing to the incentive value of stimuli via the mesolimbic system, opioids also participate in the reward value of stimuli (Grigson, 2002; Yeomans

and Gray, 2002). While few objective measures of pleasure and hedonia exist in the literature, most of which are limited to measuring food palatability (Berridge and Robinson, 1998; Berridge, 2007; Berridge et al., 2009), human self-report has provided key insights into the effect of opioidergic modification of a number of complex rewarding stimuli. In humans, blocking μ- and κ-receptors via naltrexone reduces ratings of pleasure associated with highly palatable food (Fantino et al., 1986, Yeomans and Gray, 1996), and systemic administration of naloxone, a high affinity μ-receptor antagonist, reduces feelings of pleasure and reward during a gambling task (Petrovic et al., 2008). Together, these studies provide critical evidence that endogenous opioids may also contribute to the hedonic value of complex, non-food stimuli like pups.

An extended network of regions, including the anterior cingulate, anterior insula and NAc, has been associated with the perception of a stimulus as rewarding or aversive (Barbano and Cador, 2007; Smith and Berridge, 2007; Petrovic et al., 2008). Specifically, recent neuroanatomical work has identified so-called hedonic "hotspots" located in the medial shell of the NAc that affect reward-related responses (Peciña and Berridge, 2005); critically, stimulation of these regions of the medial NAc does not affect motivated and goal-directed behavior (Peciña, 2008). Thus, opioid release within the medial NAc may increase the hedonic perception of stimuli, such as the palatability of food (Barbano and Cador, 2006, 2007; Smith and Berridge, 2007). Future research should be directed toward identifying whether hedonic hotspots in the NAc also respond to pup stimuli.

Together, these data suggest that the endogenous opioid system can contribute importantly to the incentive value and reward value of stimuli. Further, individual

differences in opioid action may affect the reward value attributed to a particular stimulus and subsequently sculpt the motivated behavior directed toward attaining that stimulus. Variations in μ receptor genes have already been linked to interstrain and individual differences in opioid-induced analgesia (Han et al., 2004), and emerging evidence links polymorphisms in the prodynorphin gene (coding for the dynorphin peptide) with vulnerability to cocaine dependence (Chen et al., 2002; Dahl et al., 2005; Shippenberg et al., 2007). It is likely that these or similar variations involving opioid-related genes may contribute to the naturally occurring differences in opioid receptor binding and/or endogenous ligands. I posit that such variations within otherwise homogenous populations of subjects (e.g., early postpartum females) may contribute to individual differences in motivation- and reward-related processing of both drug and natural stimuli.

Potential role of neuroactive peptides in mediating maternal motivation

While identifying neural substrates contributing to general motivation- and reward-related processing informs our understanding of individual differences in motivated behavior, it is also important to examine potential factors contributing to individual differences in motivation directed toward highly specific stimuli, such as pups. A large body of research indicates that oxytocin and vasopressin may contribute to natural variations in maternal motivation.

Oxytocin. Oxytocin (OTA) is a neuroactive peptide released centrally by magnocellular neurons in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus and peripherally by the posterior lobe of the pituitary and by intrauterine and ovarian

tissues in the female. While peripheral OTA binds to receptors located on local and distal peripheral tissues but cannot easily cross the blood-brain barrier (Gimpl and Fahrenholz, 2001), OTA released by PVN neurons binds to membrane-bound receptors on neurons located throughout the brain, including the amygdala, BNST, MPOA, NAc, VTA, lateral septum, striatum, hippocampus, and throughout the extended amygdala (Tribollet et al., 1988; Insel, 1990; Krémarik et al., 1993; Pedersen et al., 1994; Veinante and Freund-Mercier 1997; Olazábal and Young 2006a). It is important to note that steroid hormones can regulate OTA gene expression and receptor binding, particularly in maternally responsive brain regions, including the BNST (Insel, 1990), MPOA and lateral septum (Francis et al., 2000; Champagne et al., 2001). Thus, central OTA is well-positioned to participate in the expression of reproductive and maternal behaviors.

OTA mediates a wide variety of social behaviors, including social attachments and proactive interactions, stress- and anxiety-related behaviors, pair bonding with a mate, sexual receptivity and copulation, and maternal care, including bonding between a mother and her offspring (Kendrick et al., 1997; Kendrick, 2000; Insel and Young, 2000; Gimpl and Fahrenholz, 2001; Young and Wang, 2004). In fact, region-specific levels of OTA receptor binding have been shown to correlate with the acquisition of partner preference (Ross et al., 2009), social bonding (Young and Wang, 2004), and the performance of parental caregiving behaviors (Olazábal and Young 2006a, 2006b). As OTA mediates caregiving interactions between a maternal female and her offspring and influences OTA receptors located throughout an extended neural circuitry involved in maternal motivation (Lee et al., 2000; Mattson and Morrell, 2005; Pereira et al., 2008), I

posit that OTA action in the MPOA and related motivational circuitry may facilitate the attribution of strong incentive value to pups in the postpartum female.

A well-established role for central OTA in mediating maternal responsiveness and mother-infant attachments has already been identified (Kendrick, 2000; Numan and Insel, 2003). Blocking central OTA throughout the brain (Champagne et al., 2001; Pedersen and Boccia, 2004) or exclusively in the MPOA or VTA (Pedersen et al., 1994) produces severe deficits in maternal behavior, as does the deletion of the entire OTA gene (Russell and Lang, 1998; Pedersen et al., 2006). Importantly, while OTA also facilitates the onset of maternal behavior in both postpartum and virgin females (Kendrick 2000; Gimpl and Fahrenholz 2001; Numan and Insel, 2003), even firmly established maternal behavior performed by maternal females is also impaired by central OTA blockade (Champagne et al., 2001). Though OTA receptor binding is not affected by exposure to pups and/or time in contact with pups (Insel, 1990; Champagne et al., 2001), central OTA blockade reduces pup licking and grooming (Champagne et al., 2001), suggesting that OTA may mediate select, motivated aspects of the full spectrum of maternal behavior.

Intriguingly, recent work revealed that OTA also mediates naturally occurring differences in maternal behavior expressed by individuals. OTA receptor binding is higher in the MPOA, BNST, central nucleus of the amygdala (CeA) and lateral septum of early postpartum females that express high levels of pup licking and grooming behavior compared to those that express low licking/grooming (Francis et al., 2000; Champagne et al., 2001). These differences within an otherwise homogenous population of postpartum females suggest that OTA participates in the quality of select, motivated

maternal behaviors. It is possible that a similar substrate supports individual differences in maternal motivation.

It is important to note that increased OTA action in the BNST and CeA emerges not only in postpartum females but in the virgin female offspring of highly maternal females, as well (Francis et al., 2000). As these offspring express reduced anxiety (Francis et al., 2000) and are rapidly maternal following repeated pup exposure (Champagne et al., 2001), it is likely that OTA action in the CeA and extended amygdala contributes to the anxiolysis required later in life for full maternal responsivity (Francis et al., 2000). Reduced anxiety is also necessary for pup-initiated contact in non-maternal females, which recent work (Seip and Morrell, 2008) suggests may precede emergent maternal motivation in virgin rats.

Vasopressin. The neuroactive peptide arginine vasopressin (VP) is similar in structure and function to OTA. VP is located on the same chromosomal locus as OTA, differs in OTA molecular structure by only one amino acid, and acts as a partial agonist at OTA receptors (Gimpl and Fahrenholz, 2001). VP also plays a critical role in social behavior (Young and Wang, 2004), pair bonding (Cho et al., 1999; Young and Wang, 2004), and even parental care (Wang et al., 2000; Parker et al., 2001). While OTA and VP can facilitate social contact in both sexes (Cho et al., 1999), VP facilitates social behaviors most effectively in males (Insel and Young, 2000; Young and Wang, 2004). Parentally responsive males that care for offspring also express decreased VP receptor binding in the lateral septum compared to non-parental males (Parker et al., 2001), and VP gene expression increases in parentally responsive males and females following parturition (Wang et al., 2000). Intriguingly, male offspring that received high levels of maternal

care during early development express increased levels of VP receptor binding in the CeA (Francis et al., 2002). Together, these data may suggest that a bidirectional relationship between the parent and offspring exists in which the quality of parental care and parent-offspring interactions produces relatively long-lasting changes in the brains of both parties.

It is important to note that changes in VP action are not mediated by steroid hormones but can be regulated by stress hormones such as glucocorticoids (Watters et al., 1996, 1998). While VP modulates females' behavior less readily than OTA, it is possible that steroid-independent changes in VP may also contribute to the strength of mother-offspring bonding.

Conclusions

The experiments in this dissertation were designed to characterize the motivational state of the postpartum female rat during a biologically critical period in her lifecycle. The motivated choices that a mother makes during this period can uniquely impact a child's social, emotional and physical development. By characterizing the biological and environmental factors that drive a female's choice behavior, it will be possible to identify vulnerable populations of females that are more likely to participate in ineffective caregiving behaviors. Factors contributing to proactive, nurturing behavior and maternal motivation, on the other hand, can importantly inform public health programming in critical areas such as effective childcare education. Further research should be directed toward exploring the cellular and molecular correlates of individual differences in motivation involved in effective parental care. By broadening the scope of

this research to encompass molecular to clinical levels of analysis, we will importantly

expand our understanding of motivational processes during this critical period in the

lifecycle of the female, during which a female's motivated behavior has long-lasting

implications for a healthy and productive society.

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211

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Poster Presentations (16)