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The medial preoptic area is necessary for motivated choice of pup-over cocaine-associated environments by early postpartum rats

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Abstract

Converging evidence suggests that the motivation to seek cocaine during the postpartum period is significantly impacted by the competing incentives of offspring, a stimulus unique to this life stage. In the present study, the functional role of the medial preoptic area (mPOA), a critical site involved in maternal responsiveness, on processing incentive value of pup-associated cues and influencing response allocation for pup-over cocaine-associated environments was investigated using a concurrent pup/cocaine choice conditioned place preference (CPP) paradigm. Early postpartum females with bilateral guide cannulae aimed into the mPOA or into anatomical control sites were conditioned, from postpartum days (PPD) 4 to 7, to associate different uniquely featured environments with pups or cocaine. CPP was tested on PPD8 following intra-mPOA infusions of either 2% bupivacaine or saline vehicle. In two additional experiments, the effects of intra-mPOA infusions of bupivacaine on expression of conditioned responding induced by environments associated with either pups or cocaine were examined separately. Transient inactivation of the mPOA selectively blocked the conditioned preferences for pup-associated environments, significantly contrasting the robust pup-CPP found in non-surgical and intra-mPOA vehicle-treated females. In contrast, mPOA inactivation failed to alter cocaine-CPP in postpartum females. When given a choice between environments associated with pups or cocaine, transient functional inactivation of the mPOA altered choice behavior, biasing the preference of females toward cocaine-associated environments, such that almost all preferred cocaine- and none the pup-associated option. The anatomical specificity was revealed when inactivation of adjacent regions to the mPOA did not affect CPP responses for pups. The findings support a critical role for the mPOA in mediating pup-seeking behavior, and further suggest that the competing properties of pups over alternative incentives, including drugs of abuse, rely on mPOA integrity to provide relevant pup-related information to the circuitry underlying the choice behavior between pups and alternative stimuli.

Keywords

bupivacaine neuronal inactivation; cocaine; conditioned place preference; maternal motivation; mPOA; postpartum period

Cocaine use by women during the postpartum period remains a pressing public health problem, often with a detrimental effect on mother-infant interactions and infant development, and

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lifelong-negative consequences for both the mother and child (Roland and Volpe, 1989; Dembo et al., 1990; Burns et al., 1991; Kelly et al., 1991; Murphy et al., 1991; Barabach et al., 1992; Frank et al., 1993; Ball et al., 1997; Burns et al., 1997; D'Apolito, 1998; Johnson and Leff, 1999; Sterk et al., 2000; Walton-Moss and Becker, 2000; Minnes et al., 2005). On the other hand, studies examining temporal cocaine use patterns have generally reported a decrease in cocaine use during late pregnancy and lactation (Richardson and Day, 1991; Cornelius et al., 1994), together with an increased willingness to seek help and undergo addiction recovery treatment, suggesting that this period is one in which competitive maternal motivation (to care for their child) might influence drug use (Gottwald and Thurman, 1994; Barnet et al., 1995).

Preclinical research using maternal rodents provide remarkable parallels to these human reports, demonstrating a cocaine impact on maternal care-giving (Sobrian et al., 1990; Heyser et al., 1992; Zimmerberg and Gray, 1992; Johns et al., 1994; Kinsley et al., 1994; Peeke et al., 1994; Vernotica et al., 1996, 1999) and suggesting a complex motivational picture for cocaine and pup stimuli. For instance, there is a notable reduction of cocaine self-administration around parturition and early postpartum in chronically cocaine-exposed (for a period starting prior to breeding and during gestation and lactation) female rats relative to their levels prior to pregnancy (Hecht et al., 1999). This voluntary reduction in cocaine self-administration appeared to be the result of postpartum females spending considerable amounts of the test time performing maternal caregiving behaviors toward the pups (Hecht et al., 1999). This suggests that the pups, a powerful incentive to postpartum females (Wilsoncroft, 1969; Hauser and Gandelman, 1985; Fleming et al., 1989, 1994; Lee et al., 1999; Wansaw et al., 2008), may be a competing motivation during this period.

To compare the relative incentive value of pups versus cocaine in the postpartum period, prior studies in our laboratory used a concurrent pup/cocaine choice conditioned place preference (CPP) procedure that offered postpartum maternal rats a choice between an environment associated with maternal interaction with pups versus an environment associated with cocaine administration. These experiments demonstrated that the incentive salience of pups can exceed that of cocaine during the early postpartum period (Mattson et al., 2001; Seip and Morrell, 2007; Pereira et al., 2008). While cocaine remains highly reinforcing throughout the postpartum period, readily inducing robust place preference responses across a wide range of doses, the conditioned preferences of postpartum females for environments associated with cocaine are substantially reduced when pup-associated environments became the alternative option (Mattson et al., 2001; Seip and Morrell, 2007; Pereira et al., 2008; Seip et al., 2008). Collectively, these converging lines of evidence strongly suggest that, regardless of previous history of cocaine exposure, during the unique period of early postpartum the reinforcing efficacy of cocaine might be influenced by competition from the maternal motivation to seek and interact with the pups, resulting in relatively decreased cocaine-seeking behaviors.

How the incentive properties of such diverse stimuli, as pups and cocaine, are integrated by the neurobiological substrate that mediates incentive motivational processes is not completely understood. Certainly, however, the prevailing view in the literature is that subregional and subcircuit specialization across particular brain structures selectively processes stimulus-specific information, incentive comparisons, and ultimately mediates the choice among qualitatively different stimuli including natural versus pharmacological stimuli (and conditioned stimuli associated with them) (Brown et al., 1992; Carelli et al., 2000; Carelli and Ijames, 2001; Carelli and Wondolowski, 2003; Carelli and Wightman, 2004; Di Chiara et al., 1993; Neisewander et al., 2000; Schroeder et al., 2000; Schroeder et al., 2001; Grigson, 2002; Kelley and Berridge, 2002; Mattson and Morrell, 2005). One of the best studied set of CNS components mediating motivated responses to stimuli is the mesocorticolimbic dopaminergic system, including projections from the ventral tegmental area (VTA) to the nucleus accumbens (NA) and medial prefrontal cortex (mPFC) which are particularly

important in attributing incentive value to stimuli (Robinson and Berridge, 1993; Robbins and Everitt, 1996; Ikemoto and Panksepp, 1999; Salamone and Correa, 2002), as are projections from the mPFC, hippocampus, and amygdala (BLA) to the NA (Groenewegen et al., 1996, 1999; Groenewegen and Uylings, 2000). In the case of a natural stimulus it is likely that additional specific brain regions contribute information to this circuit so that motivated responses appropriate for the natural stimulus can be generated.

In the particular state of motherhood, we posit that the medial preoptic area (mPOA), a site that integrates the hormonal and sensory inputs related to the maternal state (Numan and Insel, 2003), likely contributes pup-specific information to this motivational circuitry to influence the behavioral allocation toward offspring and related stimuli. Support for this hypothesis is provided by our previous work examining expression of Fos showing that subsets of neurons within the mPFC, NA, BLA, and mPOA were differentially activated depending on whether postpartum maternal rats expressed a conditioned place preference for pup- or cocaine-associated environments in a concurrent pup/cocaine choice CPP procedure (Mattson and Morrell, 2005). Notably, the mPOA was the only region that showed substantially greater activation (c-Fos and CART-IR) of neurons when postpartum females chose pup- over cocaine-associated environments. Data from additional studies shows that the mPOA mediates motivational processing of reproductive behaviors, including sexual (Band and Hull, 1990; Ágmo and Gómez, 1993; Paredes et al., 1993; Hoshina et al., 1994; Paredes and Baum, 1995; van Furth et al., 1995; Kindon et al., 1996; Paredes et al., 1998; Xiao et al., 2005; García-Horsman et al., 2008; Hurtazo et al., 2008; Sakuma, 2008) and maternal behavior (Lee et al., 1999; Numan and Insel, 2003; Pereira and Morrell, 2009). Furthermore, the mPOA has connections with VTA, NA, and mPFC (Numan and Callahan, 1980; Numan and Smith, 1984; Numan and Numan, 1997; Stack et al., 2002; Numan et al., 2005; Numan, 2006) that potentially allow pup-responsive mPOA neurons to interact with the motivational circuitry to influence the choice behavior for pups over alternative stimuli.

In the present study, we used the CPP procedure to investigate the hypothesis that the mPOA is a critical site mediating motivational processing of pup reinforcement, influencing maternal choice for pup- over cocaine-associated cues (interpreted as a measure of motivation, i.e. stimulus-seeking). Specifically, intra-mPOA microinfusion of bupivacaine was employed to test the hypothesis that transient neuronal inactivation of the mPOA would selectively block the conditioned preferences of postpartum females for pup-associated environments, shifting the response allocation toward cocaine-associated environment preference. To this aim, in Experiments 1 and 2, the effects of intra-mPOA administration of bupivacaine on expression of conditioned responding induced by environments associated with either pups or cocaine were examined separately. Experiment 3 examined the effects of transient inactivation of the mPOA during expression of place conditioning on the choice of postpartum females for pups-versus cocaine-associated environments in a concurrent pup/cocaine choice CPP procedure.

A methodological approach using transient site-specific neuronal inactivation by infusion of bupivacaine hydrochloride (an amide local anesthetic which blocks voltage-dependent sodium channels) was chosen to allow the analysis of the function of the mPOA in a temporally specific manner (Lomber, 1999). Specifically, this method allows the timing of the regional inactivation to be optimally chosen to be only at the time of the expression of a previously learned and established CPP, without interfering with the prior associative learning of the subject (i.e. acquisition phase), or with the memory consolidation of these learned associations, both needed to establish a CPP. Furthermore, this method preserves entirely the maternal caregiving in the conditioned environment, and in the homecage, so that interpretation of the inactivation of the mPOA is not confounded by effects other than at CPP test.

Experimental Procedures

Animals

All subjects were primiparous postpartum Sprague-Dawley female rats (original stock from Charles River Laboratories, Kingston, NY) bred in our colony at the Rutgers University Laboratory Animal Facility (accredited by the American Association for Accreditation of Laboratory Animal Care). Before giving birth, pregnant females were housed in individual cages (25.5 cm wide × 47 cm long × 23 cm high) lined with fresh woodchip bedding (Beta chip, Northeastern Products Corp., Warrensburg, NY) and containing shredded paper towels as nest-building material. Postpartum females remained with their pups for 24h after parturition, without interruption. On postpartum day 1 (birth=day 0), litters were culled to four male and four female pups per dam. All females were kept on a 12-hr light/dark cycle (light on at 0700AM) at 22 ± 1 °C, with *ad libitum* access to water, rat chow (Lab Diet 5008, PMI Nutrition International, LLC, Brentwood, MO) and sunflowers seeds.

All animal care and experimental procedures performed in this study were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996), and were reviewed and approved by the Rutgers University Animal Care and Facilities Committee. All efforts were made to minimize the number of animals used and their suffering.

Stereotaxic Surgery

Females were randomly assigned to and prepared for one of the following sets of cannula placements: (1) females stereotaxically implanted with bilateral guide cannulae aimed at the mPOA, or (2) location control groups aimed immediately adjacent to but outside the mPOA and (3) females not subjected to surgery (non-surgical control group).

On day 1 postpartum, females were anesthetized with 1mL/kg of a solution that contained ketamine HCl (75.0 mg/mL), xylazine (7.5 mg/mL) and acepromazine maleate (1.5 mg/mL). Following anesthesia, females received a subcutaneous injection of 0.5% Marcaine along the intended incision area and were placed in a Kopf stereotaxic instrument. The incisor bar on the stereotaxic apparatus was set to 3.2 mm below the interaural line such that a flat skull position was achieved (with Bregma and Lambda at the same vertical coordinate). All females received bilateral implantations of 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA). For the mPOA site, guide cannulae were implanted 2.0 mm dorsal to target at the following coordinates: AP-0.5 (from bregma), $ML \pm 0.5$ (from midline), and DV-6.5 (from the skull surface). For the dorsal control site, guide cannulae were implanted at the following coordinates: AP-0.5, $ML \pm 0.5$, and DV-5.0. The guide cannulae were secured to the skull with stainless steel screws and cranioplastic cement. To maintain patency of the cannulae prior to injection, dummy stylets were inserted. Immediately after surgery, females were reunited with their pups in their home cages. Overall condition and health were evaluated daily, particularly after surgery and during CPP conditioning and testing procedures. All females remained healthy throughout the experiment, fully exhibiting typical maternal behaviors, and their pups gained weight and developed normally.

Conditioned Place Preference Apparatus

The place preference apparatus consisted of three equal-sized chambers (21.0 cm wide × 27.5 cm long × 20.5 cm high) made of clear Plexiglas, connected by manually operated guillotine doors. Each side chamber contained unique contextual cues (that remained present throughout all conditioning and testing sessions) of wallpaper (vertical or horizontal black-and-white stripes) and tactile flooring, either small paper squares (Alpha-Dri, W. F. Fisher & Sons) or corncob pieces (Bed-o-Cobs, The Andersons, Maumee, OH) scattered on solid grey floors,

becoming a unique environment for the association learning and testing of the CPP procedure. The center chamber had white wallpaper and solid grey floor. Luminance (measured with a Konica Minolta Luminance Meter LS-100, Japan) was equal in all three chambers. Subject location within the CPP apparatus was monitored by means of infrared beam breaks, providing data on the time spent and locomotor activity in each chamber via an automated computer based interface (Med Associates Inc., St. Albans, VT, USA/MED-PC® Version IV Research Control and Data Acquisition System).

Experimental Design and Procedure

Separate groups of postpartum female rats were used for each of the CPP experiments of this study. In all three experiments, the behavioral procedure consisted of a 5-day schedule with three distinct phases, pre-conditioning, conditioning, and post-conditioning testing.

At the pre-conditioning session, females were exposed to the CPP apparatus for a single baseline session before conditioning, i.e. prior to any exposure to unconditioned stimuli in the apparatus, to identify preconditioning chamber preferences, on day 4 postpartum. Each female was placed into the center chamber and allowed free access to the entire apparatus for 60 min. The amount of time spent in each chamber was used to assess individual unconditioned chamber preferences. The majority (68%) of females did not exhibit an unconditioned preference for either conditioning side chamber, and chamber assignment was counterbalanced (on a group basis). Of those females that did exhibit a side chamber preference, relevant unconditioned stimulus was assigned to the least-preferred chamber. In the particular case of Experiment 3, females exhibiting an unconditioned side chamber preference were counterbalanced for which chamber was to be paired with either unconditioned stimulus (i.e. pups or cocaine).

Experimental groups of early postpartum females were exposed to unconditioned stimuli (pups and/or cocaine) during conditioning between postpartum days (PPD) 4–7, and then tested for their conditioned preferences on PPD8 (~12:00 PM). All parametric aspects of conditioning (i.e. number of conditioning sessions, session duration, cocaine doses and route of administration, etc) used in the present study were selected based on our previous research demonstrating that it reliably produces maximal CPP effect sizes in postpartum females for both pups and cocaine (Mattson et al., 2001; Seip and Morrell, 2007; Pereira et al., 2008; Seip et al., 2008; Wansaw et al., 2008). Specifically, the present study uses intraperitoneal (IP) administration of cocaine and hence 30-min cocaine-conditioning sessions to maximize chamber exposure during rising and peak plasma concentrations of the drug (Lau et al. 1991; Festa et al. 2004), which is important for establishing a positive incentive salience for the drug experience. On the other hand, at least 1-h pup-conditioning sessions are needed to allow postpartum females' full expression of maternal behavior, in order to elicit robust pup-associated CPP (Fleming et al. 1994; Mattson et al. 2001, 2003).

Intracranial Injection Procedure Females were gently handled once daily for approximately 5 min for 2 days before CPP testing, to habituate them to the experimenter handling associated with the intracranial drug administration as well as having their stylets removed and reinserted. On postpartum day 8, just before CPP testing, females were gently restrained while their dummy stylets were removed and 28-gauge stainless steel injectors were inserted into the guide cannulae, extending 2.0 mm beyond the tip of the guide cannulae. Injectors were connected by PE-10 tubing to 10 μ L Hamilton syringes, and bilateral infusions were driven simultaneously by a two-syringe infusion pump (Harvard 22 syringe pump; Harvard Apparatus, Holliston, MA). Infusions of bupivacaine (2% solution; Sigma, St. Louis, MO) or saline vehicle were delivered at a rate of 0.5 μ L/min. Each side received 1.0 μ L total volume, and the injectors were left in place for an additional 1 min to allow for diffusion of the drug. Immediately after, stylets were replaced. Assignment to bupivacaine or vehicle treatment was counterbalanced, so that

half of the females received site-specific bilateral infusions of bupivacaine, and the other half received site-specific bilateral infusions of saline. Five min later, postpartum females were placed in the CPP apparatus and their conditioned place preference for stimulus-associated environment was evaluated during a 60-min test. Behavioral control females did not undergo surgery but were similarly handled and tested as the cannulated groups.

Bupivacaine hydrochloride in a dose of 20mg/mL dissolved in 0.9% saline solution was used to suppress neural activity of the mPOA throughout the entire CPP test session. Bupivacaine, like the structurally similar amide-linked anesthetic lidocaine, produces an immediate neuronal inactivation by blocking voltage-dependent sodium channels, and hence action potential conductance both in neuronal cell bodies and axons (Hille, 1966 and 1977). We chose bupivacaine due to its longer duration of action over lidocaine (lidocaine approximately 10–30 min range, bupivacaine approximately 30–75 min range, Caterall and Mackie, 1986). An infusion of 1 μ L volume of 2% bupivacaine was selected on the basis of previous studies of estimates of the effective spread (i.e. the distance over which there is a physiological effect) and time course of neural inactivation caused by intracranial lidocaine infusion (Boehnke and Rasmusson, 2001; Tehovnik and Summer, 1997). Furthermore, using an infusion protocol, including infusion rate and volume, and coordinates identical to those utilized in the present study, we have previously established functional site specificity of the mPOA inactivation with respect to adjacent dorsal, rostral, lateral, and caudal anatomical control sites (Pereira and Morrell, 2009), demonstrating that bupivacaine administration resulted in selective inactivation of the mPOA only.

Specific Experiments

Experiment 1 - Effect of transient mPOA Inactivation on the expression of pup-induced CPP—This experiment examined the effect of transient inactivation of the mPOA during expression of place conditioning on the preference of postpartum females for pup-associated environments. Postpartum females were separately conditioned to associate different uniquely featured environments with their own 8-pup litter or with no specific unconditioned stimulus (empty chamber), once a day from PPD4-7. During each exposure session interval for either unconditioned stimulus, the females were confined for 1 h to the respective side chamber by closing the guillotine doors. Conditioning sessions started at 11:00 AM for the empty chamber condition. Litters were removed from their maternal cages and housed in same-litter groups in small cages. Females were returned to their home cage after the morning conditioning session for 1 h without their pups. Pup-conditioning sessions started at 13:00 PM. Immediately prior to the session, each female's 8-pup litter was placed in the corresponding conditioning chamber. Thus, postpartum females underwent the pup-conditioning session after being deprived of pups for 2h, and their pups, used for conditioning, were also deprived of maternal care for the same duration.

Conditioned place preference for the pup-associated environment was tested on PPD8, the day after the last conditioning session, by allowing females to explore all three chambers for 60 min, with no unconditioned stimuli present. Before CPP testing, females received site-specific bilateral infusions (1.0 μ L/side) of either 2% bupivacaine or saline vehicle.

Experiment 2 - Effect of transient mPOA inactivation on the expression of cocaine-induced CPP—This experiment examined the effect of transient inactivation of the mPOA during expression of place conditioning on the preference of postpartum females for cocaine-associated environments. A 5.0 mg/kg dose of cocaine was selected on the basis of previous research showing that it produces maximal CPP responses in postpartum females (Seip et al., 2008). Postpartum females received separate conditioning sessions with each one of two unconditioned stimuli, cocaine or saline, daily from PPD4-7; each stimulus for 30 min

at a time. Immediately before each conditioning session, females were intraperitoneally injected with 5mg/mL/kg cocaine or same volume of saline, and confined to their respective chamber. Cocaine hydrochloride (National Institute of Drug Abuse, Research Triangle Park, NC, USA) was freshly dissolved daily in 0.9% saline solution. The cocaine-conditioning session was always last each day, ensuring at least 12 h for residual cocaine to clear from females' circulation before subsequent conditioning sessions (Lau et al., 1991; Wansaw et al., 2005). No postpartum females developed any skin lesions or other pathology in the abdominal cavity or the mammary glands due to IP cocaine injections.

The conditioned place preferences of postpartum females for the cocaine-associated environment was tested on PPD8 following intracranial infusion (1.0 μ L/side) of either 2% bupivacaine or saline vehicle. Five minutes later, females were placed in the center chamber of the CPP apparatus and allowed free access to the entire CPP apparatus for 60 min, with no unconditioned stimuli present. Because cocaine-CPP is retained in a single retest for up to a week after initial chamber preferences (Seip et al., 2008), postpartum females were retested the following day with the alternate treatment (within-subject design).

Experiment 3 - Effect of transient mPOA inactivation on motivated choice of pup- versus cocaine-associated environments in a concurrent pup/cocaine choice CPP

—This experiment examined the effect of transient inactivation of the mPOA during expression of place conditioning on the choice of postpartum females for pup- versus cocaine-associated environments in a concurrent pup/cocaine choice CPP procedure. To allow for the analysis of female's behavioral choice following transient mPOA inactivation, it was important to choose a design that would provide preference categories represented relatively equally, and with a sufficient number of females with either a pup- or cocaine-associated CPP, so that the effect of a transient inactivation of the mPOA could be detected in relation to this baseline preference. As shown in Figures 1A and 1B, both 1.0 and 5.0 mg/kg IP cocaine similarly induced robust cocaine-CPP in postpartum females. In a concurrent pup/cocaine choice CPP procedure, the 5.0 mg/kg cocaine dose continues to establish a place preference in the majority of the females (67%), with only 10% preferring the pup-associated environment (Figure 1C and 1D). When given a choice between environments associated with pups versus 1.0 mg/kg IP cocaine, however, the desired bimodal distribution of preferences emerges, with 40% of the females preferring the cocaine- and 40% preferring the pup-associated environment (Figure 1C and 1D). On the basis of this preliminary data, a dosage of 1.0 mg/kg of intraperitoneally-administered cocaine was chosen for the concurrent pup/cocaine choice CPP study to enable us to detect statistically significant effects of transient mPOA inactivation on the choice behavior of early postpartum females.

In the current experiment, postpartum females were separately conditioned to associate different uniquely featured environments with each one of two unconditioned stimuli: their own 8-pup litter or 1mg/mL/kg IP injection of cocaine. Pup-conditioning sessions started at 11:00 AM, after females were pup deprived for 2 hours. Postpartum females were confined to the assigned pup-conditioning chamber for 1 hour with their pups. Females and pups were then returned to their home cage and left undisturbed for 2 hours before receiving the cocaine-conditioning session. Females were intraperitoneally injected with cocaine and immediately placed in the alternate conditioning chamber for 30 minutes.

On PPD8, following intracranial infusions (1.0 μ L/side) of either 2% bupivacaine or saline vehicle, CPP was assessed by allowing females free access to the entire apparatus for 60 min.

Histology and Analysis of infusion Sites

Upon completion of CPP testing, postpartum females were deeply anesthetized with Nembutal and perfused intracardially with saline followed by a 4.0% formaldehyde solution. Brains were

removed and stored in 4.0% formaldehyde for 48h. The brains were frozen using dry ice and serial cross sections of 30 μ m were cut around the implantation site on a cryostat. The sections were mounted on slides, stained with Cresyl violet, and cover slipped. The location of injection sites were documented with the aide of a Zeiss Axioplan microscope equipped with a Neurolucida imaging system, and the neuroanatomical atlas of Paxinos and Watson (1997).

Statistical Analysis

The time spent in each chamber during pre- and postconditioning sessions was used to identify subjects with preference for a particular apparatus chamber. A subject was categorized as having a preference for a particular apparatus chamber if spent at least 50% of the total session time in one chamber and that was at least 25% greater than either time in any other chamber (Mattson et al., 2001). Subjects that did not meet the preference criterion were categorized as having no preference.

CPP results are expressed as proportion of individual subjects exhibiting an environment preference and as mean \pm SEM time the group spent in each apparatus environment. Preconditioning environment preferences and times were compared to those of postconditioning, to provide evidence of conditioning. CPP was operationally defined as a statistically significant increase in conditioned preferences of individuals and group time spent in the stimulus- (pup, cocaine) associated environment postconditioning relative to preconditioning baseline. Chi-square goodness-of-fit test was used to analyze categorical data from pre- and postconditioning environment preferences of the individual females. Between-group chamber preference comparisons were examined using Chi-square test of independence and Fisher's exact test. Environment times were analyzed using two-way repeated measures ANOVA, with group as the between-subject variable and chamber (side) and session (preconditioning vs. postconditioning) as within-subject factors. Locomotor scores are expressed as mean \pm SEM and were analyzed using a one-way ANOVA. Significant main effects and interactions were further analyzed using Tukey's HSD tests. A significance level of $p < 0.05$ was used for all data.

Results

Location of the Cannulae Implantation Site

Figures 2A, 3A, and 4A represent schematic cross sections of the rat brain showing the placement of the injection cannulae tips based on the microscopic analysis of cresyl violet-stained sections. Figure 2A shows the placement of the injection cannula tips for the mPOA ($n_{\text{BUP}}=10$ and $n_{\text{VEH}}=9$) groups tested for pup-CPP, and Figure 3A shows those for the groups of postpartum females tested for cocaine-CPP ($n=12$). Figure 4A shows the location of the mPOA infusion sites for the postpartum groups tested in the concurrent pup/cocaine choice CPP procedure ($n_{\text{BUP}}=9$ and $n_{\text{VEH}}=8$). Histological analysis demonstrated that all mPOA groups had sites distributed in a similar overall pattern, with most of the bilateral injections sites located between -0.26 and -0.88 mm from bregma.

Experiment 1 - Effect of Transient mPOA Inactivation on Expression of Pup-induced CPP

In the preconditioning test, the majority of postpartum females showed no preference for either side compartments, and, on average, spent similar amounts of time in each chamber of the CPP apparatus. Importantly, unconditioned chamber preferences [$\chi^2=5.137$, $df=6$, $P=ns$] and times were similar and did not differ among groups [chamber \times group interaction: $F(3,32)=0.1649$, $P=ns$], indicating that differences in baseline responding cannot account for any selective effect of the bupivacaine-induced changes in conditioned preferences.

After conditioning, non-surgical control females exhibited a robust CPP for the pup-associated environment as demonstrated by comparison analysis of pre- and postconditioning environment preferences [χ^2 (3, N=10)=40.533, $P<0.05$]. Similarly, postpartum females receiving infusion of saline vehicle into the mPOA prior to CPP testing exhibited substantial conditioned preference for the pup-associated environment [preconditioning versus postconditioning environment preferences: χ^2 (3, N=9)=40.083, $P<0.05$]. Thus, the majority of females receiving vehicle infusion into the mPOA preferred the environment associated with the pups (78%), and this proportion was not different from the non-surgical control group (70%) (Fisher's Exact Test, $P=ns$), demonstrating that surgery, handling and intracranial drug administration procedures neither affected the acquisition nor the expression of pup-CPP of postpartum females (Figure 2C). Furthermore, the time spent in the pup-associated environment significantly increased in both the non-surgical control group and in the group receiving microinfusion of saline vehicle into the mPOA following conditioning [session \times group interaction: $F(3,32)=4.36$, $P<0.05$; chamber \times session interaction: $F(1,32)=35.4$, $P<0.001$; chamber \times group \times session interaction: $F(3,32)=5.7$, $P<0.01$]. In addition, both groups similarly spent more test time in the pup-associated environment than in the alternative one associated with no unconditioned stimuli (Tukey's HSD test: both $P_s<0.05$; Figure 2D). Thus, both environment preferences and times changed significantly as a function of conditioning, indicating a robust CPP associated with pups (see Figure 2B).

In contrast, intra-mPOA infusions of bupivacaine prior to CPP testing completely blocked the expression of the conditioned preferences for the pup-associated environment. Thus, none of the mPOA bupivacaine-treated females preferred the environment associated with pups (0%), whereas the majority of control females (both groups) preferred the pup-associated option (70–80%, Fisher's Exact Test, both $P_s<0.05$; Figure 2C), indicating that neuronal inactivation, not the mechanical or osmotic effects of drug infusion, affected expression of pup-CPP in postpartum females. Furthermore, the time spent in the pup-associated environment was significantly reduced in mPOA-inactivated females compared to notable time spent in the pup-associated environment by both control groups (Tukey's HSD test: both $P_s<0.001$; Figure 2D).

Proof of Anatomical Specificity

Regional specificity limited to mPOA of the CPP effects was demonstrated since inactivation of anatomical sites adjacent to the mPOA had no effect on CPP for pup-associated environments. Figure 2A shows the implant locations for the group of postpartum females tested for pup-associated CPP following infusion of bupivacaine into adjacent dorsal sites to the mPOA ($n=7$). In addition to this deliberately chosen dorsal control site, as a result of the normal variation in placements found with stereotaxic surgery targeting a small brain region, nine additional postpartum females treated with bupivacaine before CPP testing had infusion sites that bilaterally targeted control structures immediately outside the mPOA. Three females had an asymmetrical placement that resulted in a unilateral infusion into the lateral mPOA and a unilateral infusion into the third ventricle, 2 females had their cannulae rostral to the mPOA into the diagonal band of Broca; and 4 females had their cannulae caudal to the mPOA into the anterior hypothalamus.

Most of these control females receiving pre-testing bupivacaine infusion into dorsal sites preferred the environment associated with pups (71%). Thus, the conditioned environment preferences and times of dorsal control females were not different from those of non-surgical and vehicle-treated control females [$\chi^2=1.778$, $df=6$, $P=ns$; session \times chamber \times group interaction: $F(2, 23)=0.001$, $P=ns$; Figures 2C and 2D]. Similarly, of those postpartum females with cannulae sites that bilaterally targeted control structures either rostral or caudal to the mPOA, 78% showed robust pup-associated CPP after bupivacaine infusions (caudal to the mPOA, 3 of 4; rostral, 2 of 2; unilateral 2 of 3 preferred the environment associated with pups).

Thus, the disruptive effect on pup-associated CPP responses produced by pre-testing bupivacaine infusion into the mPOA appear unlikely to have been mediated by diffusion to these other dorsal, anterior, or posterior structures.

Experiment 2 - Effect of Transient mPOA Inactivation on Expression of Cocaine-induced CPP

Prior to conditioning, unconditioned chamber preferences [$\chi^2=3.152$, $df=3$, $P=ns$] and times [chamber \times group interaction: $F(1,22)=0.078$, $P=ns$] were similar and did not differ among groups. Following conditioning, a significant CPP to 5.0 mg/kg IP cocaine occurred in both the non-surgical and the vehicle-treated control groups, with chamber preferences [preconditioning versus postconditioning environment preferences: $\chi^2(3, N=12)=8.643$, $P<0.05$ and $\chi^2(3, N=12)=8.637$, $P<0.05$, respectively] and times [session \times chamber interaction: $F(1,22)=57.2$, $P<0.01$] differing statistically between the pre- and postconditioning sessions (see Figure 3B).

There was no significant difference in chamber preference [$\chi^2=$, $df=3$, $P=ns$] nor times [session \times chamber \times group interaction: $F(1,22)=0.61$, $P=ns$] between the non-surgical control and saline microinjection groups during the CPP test (Figure 3C–D).

Transient mPOA inactivation had no impact on the motivational responsivity of postpartum females to cocaine-related stimuli (Figure 3C–D). Thus, intra-mPOA bupivacaine infusion prior to CPP testing affected neither the number of early postpartum females preferring the cocaine-associated environment (Fisher's Exact Test, both $P_s=ns$), nor the time spent by the group in the cocaine-associated environment (Tukey's HSD test, both $P_s=ns$) compared to the non-surgical control group. Consequently, postconditioning environment preferences did not differ within and between groups, with the vast majority of postpartum females, regardless of treatment, preferring the cocaine-associated environment. All groups also similarly spent more time in the cocaine-associated environment than in the saline-associated environment (Tukey's HSD test, all $P_s=ns$; Figure 3D).

Experiment 3 - Effect of transient mPOA inactivation on motivated choice of pup- versus cocaine-associated environments in a concurrent pup/cocaine choice CPP

Prior to conditioning (at preconditioning test) the majority of postpartum females showed no preference for either side compartment, and, on average, spent similar amounts of time in each chamber of the CPP apparatus. Furthermore, there were no differences in the unconditioned chamber preferences between groups [$\chi^2=1.893$, $df=6$, $P=ns$], nor were there significant group [$F(2,24)=0.047$, $P=ns$], chamber [$F(1,24)=0.0001$, $P=ns$] and group \times chamber interaction [$F(2,24)=0.055$, $P=ns$] effects. A robust CPP effect was demonstrated, as environment preferences [behavioral control group: $\chi^2(3, N=10)=22.67$, $P<0.05$ and vehicle-treated group: $\chi^2(3, N=8)=11.2$, $P<0.05$] and times changed significantly between the pre- and postconditioning sessions (see Figure 4B).

After conditioning, most females from the two control groups developed a preference for environments associated with one of the two unconditioned stimuli, either cocaine or pups (Figure 4C). There was no significant difference in environment preferences between the non-surgical and saline microinjection control groups [$\chi^2=0.064$, $df=3$, $P=ns$; Figure 4C]. Thus, 40% of the non-surgical control females showed a preference for the cocaine-associated environment, and 40% preferred the pup-associated environment. Similarly, the vehicle-treated group showed a bimodal distribution in which postpartum females preferred either the pup- or the cocaine-associated environment after conditioning.

In contrast, intra-mPOA infusions of bupivacaine just prior to conditioned place preference testing selectively and entirely blocked the conditioned preference of postpartum females for

the pup-associated environment (Figure 4C). Furthermore, a substantial and statistically significant majority of the postpartum females infused with bupivacaine into the mPOA preferred the cocaine-associated environment (78%). Thus, the majority of early postpartum females preferred the cocaine- over the pup-associated environment following pre-testing bupivacaine infusion into the mPOA, highly contrasting the bimodal distribution of conditioned preferences of both control groups for pup- and cocaine-associated environments (Figure 4C).

As shown in Figure 4D, the analysis of environment times during the CPP test revealed a significant chamber \times group interaction effect [$F(2,24)=3.74, P<0.05$], with overall time spent in the cocaine-associated environment significantly longer in the bupivacaine-treated group than in either non-surgical or vehicle-treated control groups (Tukey's HSD test, both $P_s<0.05$). Pup- and cocaine-associated environment times during the expression test were similar and did not differ between the two control groups (Tukey's HSD test, all $P_s=ns$). However, the time spent in the cocaine-associated environment by bupivacaine-treated females was significantly longer than the time spent in the pup-associated environment (Tukey's HSD test, $P<0.05$; Figure 4D).

Five additional postpartum females treated with bupivacaine before testing had infusion sites that bilaterally targeted structures immediately rostral or caudal to the mPOA. Of these females, 3/5 preferred the pup-associated environment and 2/5 the preferred the cocaine-associated environment, resulting in a postconditioning environmental preference pattern similar to that of the control groups. This further supports the anatomical specificity of the data, showing that bilateral infusions of bupivacaine into the mPOA are needed to block the conditioned preferences of postpartum females for the pup-associated environment.

It is important to note that the CPP responses of mPOA bupivacaine-treated females for cocaine-associated environments in the concurrent pup/cocaine choice procedure are comparable in magnitude to those of postpartum females presented with a CPP choice between an environment associated with 1.0mg/kg IP cocaine and one associated with saline (78% and 75%, respectively; Figures 1 and 4). This together with the fact that transient inactivation of the mPOA completely and selectively blocked the conditioned preference of postpartum females for the pup-associated environment, regardless of which alternative option was presented (Experiments 1 and 3), suggest that the shift in the choice preference toward the cocaine-associated environments induced by mPOA inactivation in the concurrent choice procedure is most likely due to the result of a reduction in pup-associated environment preferences.

Transient inactivation of mPOA did not affect locomotor activity during CPP test

Locomotor activity during the expression test did not differ between the groups, suggesting that exploratory behaviors remained intact during CPP testing. Across mPOA-cannulated groups, locomotor scores during the 60-min CPP test were similar regardless of whether females received bupivacaine or saline treatment, and were not different from non-surgical controls [treatment \times group interaction: Experiment 1: $F(2,24)=0.06, P=ns$; Experiment 2: $F(1,22)=0.0137, P=ns$; Experiment 3: $F(2,26)=1.17, P=ns$].

Discussion

The present results indicate that the functional integrity of the mPOA is necessary for the expression of context-induced pup- but not cocaine-seeking behavior. Thus, transient inactivation of the mPOA, produced by infusion of bupivacaine, completely eliminated the ability of pup-associated environmental cues to promote pup-seeking behavior, while conditioned behavioral responses to cocaine-associated stimuli were left intact. When given a

choice between environments associated with pups or cocaine, functional inactivation of the mPOA substantially shifted the choice preference of early postpartum females toward cocaine-associated environments, demonstrating that the mPOA is necessary to promote the choice of pup- over cocaine-associated environments. Collectively, the present findings provide strong evidence that the functional integrity of the mPOA is necessary for the expression of context-induced pup-seeking behavior, suggesting that the mPOA encodes information about goal-directed behaviors for pups. Furthermore, these results demonstrate that the mPOA influences response allocation processes by providing relevant pup-related signal into the circuitry processing the choice behavior between pups and alternative stimuli.

Bupivacaine inactivation has been successfully used in behavioral studies in our laboratory and the laboratories of others to induce effective temporary neural inhibition of discrete brain areas (Waraczynski and Perkins, 2000; Ragozzino et al., 2002; Evans et al., 2003; Holahan and White, 2004; Broadbent et al., 2006; Perrin et al., 2007; Floresco et al., 2008; Hsu and Packard, 2008; Hurtazo et al., 2008; Pereira and Morrell, 2009). The effective spread of neural inactivation of the structurally similar amide-linked anesthetic lidocaine has been well characterized both in cortical and subcortical structures using electrophysiological and other indices of neural activity (Malpeli and Schiller 1979; Sandkühler and Gebhart, 1984; Sandkühler et al., 1987; Tehovnik and Sommer, 1997; Boehnke and Rasmusson, 2001; Pereira de Vasconcelos et al., 2006). Accordingly, the functional spread of intra-cerebral bupivacaine infusion for the present parameters (1 μ L, 2%) would be approximately spherical with a radius of 500–620 μ m from the tip of the injector (Tehovnik and Sommer, 1997; Boehnke and Rasmusson, 2001). Since most of the infusion sites in the present study were centrally located within the mPOA, and taking into account the approximate extent of the mPOA and the limited diffusion of bupivacaine, the vast majority of the physiological effects of bupivacaine infusions would be expected to be limited to tissue inclusive of the mPOA. Consistent with this is the fact that the disruptive effect of intra-mPOA infusion of bupivacaine on conditioned preferences for pup-associated environments was anatomically specific. Thus, infusions of bupivacaine into neighboring adjacent sites dorsal, rostral, or caudal to the mPOA were ineffective at blocking the expression of pup-associated environment preferences. As a complete blockade of the pup-CPP was observed following mPOA inactivation, the present results demonstrate that the expression of pup-seeking behavior motivated by pup-associated environmental stimuli depends upon the functional integrity of the mPOA.

Given that intra-mPOA bupivacaine infusion impaired conditioned responding in a stimulus-specific manner (i.e., selectively blocked the expression of a pup-CPP and failed to alter cocaine-CPP) and did not alter locomotor activity of females, it is unlikely that intra-mPOA bupivacaine-induced impairments in pup-seeking behavior were due to: (1) nonspecific performance deficits (i.e., sensory, motor, attentional processing and/or general memory impairment), (2) bupivacaine-induced novelty (i.e., a novel state produced by mPOA inactivation could have nonspecifically and unconditionally disrupted the expression of the CPP), (3) a state-dependent effect, such that differing states between acquisition (without bupivacaine treatment) and expression (with bupivacaine treatment) may have interfered with the retrieval of that association during the test session; Tzschenhe and Schmidt, 1997), or (4) induction of an aversive state. In other words, if any of the above-mentioned possibilities accounted for why bupivacaine infusion into the mPOA blocked pup-CPP, then it is unclear why any of these effects would have only selectively affected the expression of a pup-CPP and not of cocaine-CPP in postpartum females.

It is therefore reasonable to argue that transient mPOA inactivation impaired the ability of pup-associated stimuli to elicit pup-seeking behaviors by selectively disrupting pup-related motivational processes. Possibly the mPOA is involved in determining the incentive salience of pup-associated (environmental) conditioned stimuli and/or pups themselves. Specifically,

mPOA inactivation might have blocked the expression of a previously learned pup-induced CPP by reducing the salience of the conditioned effects of pups and/or the incentive efficacy of the pups. In this sense, conditioned preference has been shown to be sensitive to postconditioning changes in incentive value, induced by stimulus devaluation manipulation such as sensory-specific satiety or experimentally induced aversion (Adams, 1982; Yin and Knowlton, 2002). Several lines of research support the notion that the mPOA is involved in incentive motivational processes, particularly in relation to social incentives, including maternal and sexual-motivated behaviors. For instance, using an operant bar-pressing procedure, Lee and colleagues (1999) demonstrated that bar-pressing for access to pups in postpartum females is substantially reduced following permanent mPOA lesions, while food-reinforced bar-pressing was unimpaired. Similarly, several reports using both permanent mPOA lesions and pharmacological manipulations have demonstrated involvement of the mPOA in sexual incentive motivation in both male (Band and Hull, 1990; Hughes et al., 1990; Ågmo and Gómez, 1993; Paredes et al., 1993; Paredes and Baum, 1995; van Furth et al., 1995; Kindon et al., 1996; Paredes et al., 1998; García-Horsman et al., 2008; Hurtazo et al., 2008) and female rats (Hoshina et al., 1994; Xiao et al., 2005; Coria-Avila et al., 2008; Sakuma, 2008).

An alternative, although not exclusive, possibility that is somewhat more speculative, concerns the potential role of the mPOA in the storage of pup-related associations. That is, mPOA inactivation might have disrupted the ability of pup-associated stimuli to elicit pup-seeking behaviors due to unsuccessful retrieval of the association between environmental cues and the affective state produced by the unconditioned stimulus. Certainly, the role of mPOA in integrating converging pup-related sensory information coupled with previous findings indicating that plastic changes consistent with the storage of information within this structure can occur (Morgan et al., 1999; Numan and Insel, 2003) provide a basis for the speculation that the mPOA may serve as a locus for the storage of pup-context related associations. Our recent finding that transient mPOA inactivation selectively impaired the expression of active components of maternal behavior, including retrieval, licking and nest building, without affecting nursing behaviors (Pereira and Morrell, 2009), however argues against this idea. This finding demonstrates that bupivacaine-treated postpartum females do not forget about pups nor find pups aversive, as shown by their willingness to make and maintain physical contact with them, but they are just less motivated to actively interact with them (Pereira and Morrell, 2009). Thus, rather than acting as a location for storage of information, we posit that the mPOA is more likely key in the incentive motivational effects of the pup-associated environment that underlie approach behaviors during CPP testing.

The present findings make very clear that the mPOA is not necessary for the expression of cocaine-seeking behavior elicited by environmental cues repeatedly paired with cocaine. Thus we posit that the mPOA is not a neural site involved in processing the incentive motivational value of cocaine-associated stimuli.

This differential involvement of the mPOA in mediating conditioned responding to pup- but not cocaine-associated stimuli is consistent with enhanced activation of c-Fos and CART-IR neurons in the mPOA exclusively when the preference choice of pup- over cocaine-associated environments is made by postpartum females (Mattson and Morrell, 2005). The fact that the mPOA is not necessary for the expression of cocaine-CPP, but is critically involved in mediating both the unconditioned behavioral activation produced by pups (Pereira and Morrell, 2009) as well as the expression of the conditioned responses to pup-related stimuli (present results, Lee et al., 1999), raises the interesting possibility that the blunting of cocaine-seeking behaviors observed during postpartum (Hecht et al., 1999) may result from maternal state-induced mPOA activating effects on the circuitry that mediate and modulate response allocation.

Certainly, important functional changes within the mPOA occur around parturition to prepare the female rat for her maternal role and promote maternal responsiveness to pup-related stimuli (Numan and Insel, 2003). An intriguing corollary of the previous assumption is that, if indeed mPOA is responsible for the competing effects of pups on cocaine, then this capacity of the mPOA remains relatively intact, even following extensive cocaine exposure before, and across gestation and the postpartum period, suggesting that regardless of previous history of exposure, cocaine does not abolish the overall ability of the mPOA in mediating maternal incentive motivation (Hecht et al., 1999). In support of this, female rats that were treated with cocaine daily for 30 days (20 days of pregnancy and 10 days of the postpartum period) showed impaired maternal behaviors only while cocaine was in their circulatory system, a behavioral effect that was remarkably similar to that of a single acute systemic cocaine injection on maternal responsiveness (Sobrian et al., 1990; Heyser et al., 1992; Zimmerberg and Gray, 1992; Johns et al., 1994; Kinsley et al., 1994; Peeke et al., 1994; Vernotica et al., 1996). These same cocaine-exposed females, when drug free exhibited typical maternal behaviors, regardless of the number of days they had been previously exposed to cocaine (Vernotica et al., 1996).

Collectively, the present results suggest that the mPOA, a site that integrates the hormonal and sensory inputs related to the maternal state, is a key component in motivational processing of pup-related incentive stimuli. These data further suggest that the competing effects of pups over alternative incentives, including drugs of abuse, rely on mPOA integrity to provide important pup-related information to the choice circuitry and hence influence the process through which goal-directed action-outcome associations influence behavior. Thus, functional inactivation via bupivacaine effectively eliminated mPOA-mediated input to the circuitry, silencing the influence of pup-related cues and thereby allowing the conditioned effects of cocaine, which are mediated by other areas, to exert relatively greater influence on the choice process. We posit that the mPOA influences the integration of the stimulus value of pup-related information at several points within the motivational circuitry. Much data shows that the salience and the activational effects of pups and related stimuli depends on mPOA interactions with two major components of the mesolimbic dopamine (DA) system, the VTA and NA (Numan and Callahan 1980; Numan and Smith, 1984; Hansen et al., 1991a,b; Numan and Numan, 1997; Numan and Insel, 2003; Champagne et al., 2004; Numan et al., 2005; Numan, 2006; Pereira and Ferreira, 2006; Pereira and Morrell, 2009), allowing pup-responsive mPOA neurons to influence, via NA, the motor systems required for goal-directed behavior. Accordingly, transient inactivation of the VTA has been shown to block the expression of pup-CPP (Seip and Morrell, 2009). The nucleus accumbens also receives convergent synaptic inputs from cortical and limbic structures such as the mPFC, the hippocampus and amygdala (Groenewegen et al., 1996; Groenewegen et al., 1999; Groenewegen and Uylings, 2000; Saper, 2000; Eichenbaum and Cohen, 2001). It has been suggested that the NA mediates goal-directed behavior by integrating hippocampus-dependent contextual information and amygdala-dependent affective information with mPFC executive functions, including attentional processes and response allocation to select appropriate behavioral responses (Mogenson et al., 1980; Groenewegen et al., 1999). We posit that during the postpartum period, the NA also integrates mPOA-dependent pup-related information. The mPOA might also influence the response allocation process through direct reciprocal interactions with the mPFC (Groenewegen and Uylings, 2000; Saper, 2000). Specifically, the mPFC is thought to modulate the salience and motivational significance of stimuli (Tzschentke, 2000; Dalley et al., 2004) and regulate goal-directed behaviors by regulating attention to the sensory input that enters the BLA and hippocampus (Kolb, 1984; Rosenkranz and Grace, 2001) and by mediating BLA input to the NA (Jackson and Moghaddam, 2001), respectively. Ongoing research is examining the functional role of these other brain areas to further refine the understanding of the neural circuitry underlying response selection among the competing alternatives: pup- and cocaine-associated environments, during the postpartum period.

In conclusion, the present study has demonstrated that motivation for pups and cocaine can be differentially modulated. A better understanding of the neural substrate underlying response selection among competing alternatives might importantly contribute to developing strategies for combating maternal drug abuse during the postpartum period. The present results strongly suggest that interventions that build upon promoting and/or restoring early mother-infant bonding might be more successful in terms of facilitating maintenance of maternal abstinence. In this sense, several studies have shown a more positive outcome in those practice-based and home-based intervention programs for postpartum substance using women that had special emphasis on enhancing parenting effectiveness (i.e., maternal-infant bonding), particularly in terms of reducing parenting stress and maternal depression, as well as maintaining greater levels of abstinence from illicit drug use (Ashley et al., 2003; McComish et al., 2003; Nair et al., 2003; Mullins et al., 2004; Porowski et al., 2004; Porter and Porter, 2004).

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List of Abbreviations

BLA	Basolateral Amygdala
CPP	Conditioned Place Preference
mPFC	Medial Prefrontal Cortex
mPOA	Medial Preoptic Area
NA	Nucleus Accumbens
PPD	Postpartum Day
VTA	Ventral Tegmental Area

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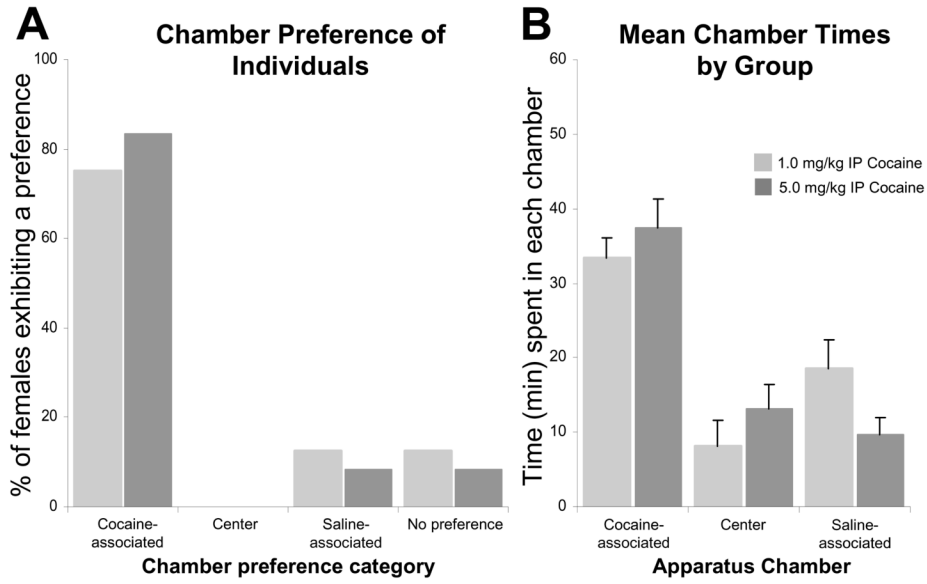
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COCAINE-CPP



PUP/COCAINE-CPP

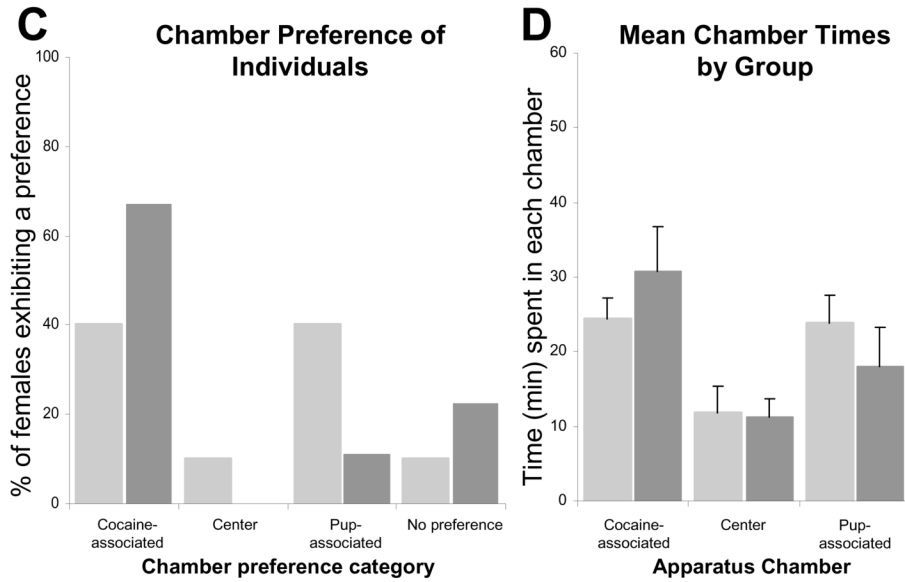


Figure 1. Comparison of the conditioned response of postpartum females to an environment associated with either 1.0 or 5.0 mg/kg IP cocaine versus one associated with saline or pups. (A) Conditioned chamber preferences and (B) mean time spent in each chamber of the apparatus during the cocaine-CPP test session, after conditioning with IP injections of either 1.0 or 5.0 mg/kg cocaine and saline. (C) Conditioned chamber preferences and (D) mean time spent in each chamber of the apparatus during the concurrent pup/cocaine-CPP test session, after conditioning with IP cocaine injections (either 1.0 or 5.0 mg/kg) and maternal interaction with pups.

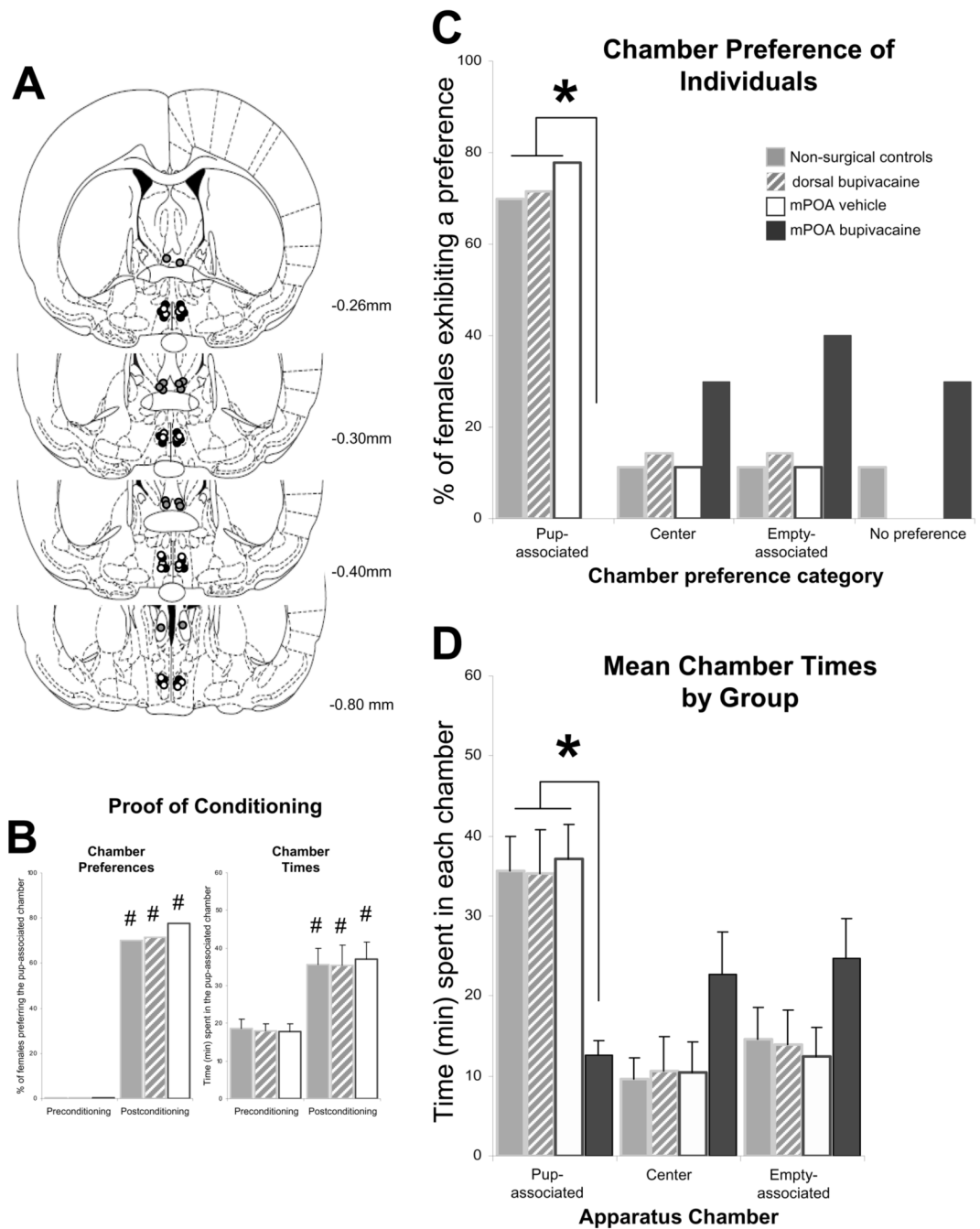


Figure 2. Effect of transient inactivation of the mPOA on expression of pup-induced CPP. (A) Schematic representation, based on the microscopic analysis of cresyl violet-stained sections, of mPOA and dorsal control sites for all rats receiving infusion treatments. Circles represent the most ventral extent of the injector tracks in the brain. Plates were taken from the atlas of Paxinos and Watson (1997). Numbers beside each plate indicate the distance caudal to bregma in millimeters. (B) Pup-associated chamber preferences and times during the pre- and postconditioning sessions. (C) Conditioned chamber preferences and (D) mean time spent in each chamber of the CPP apparatus during the test session by non-surgical control (n=10), dorsal bupivacaine-treated (n=7), mPOA bupivacaine-treated (n=10), and mPOA vehicle-

treated (n=9) postpartum females. * denotes significant differences between groups, and # indicates significant within-group differences in pre- vs. postconditioning responding; all $P < 0.05$.

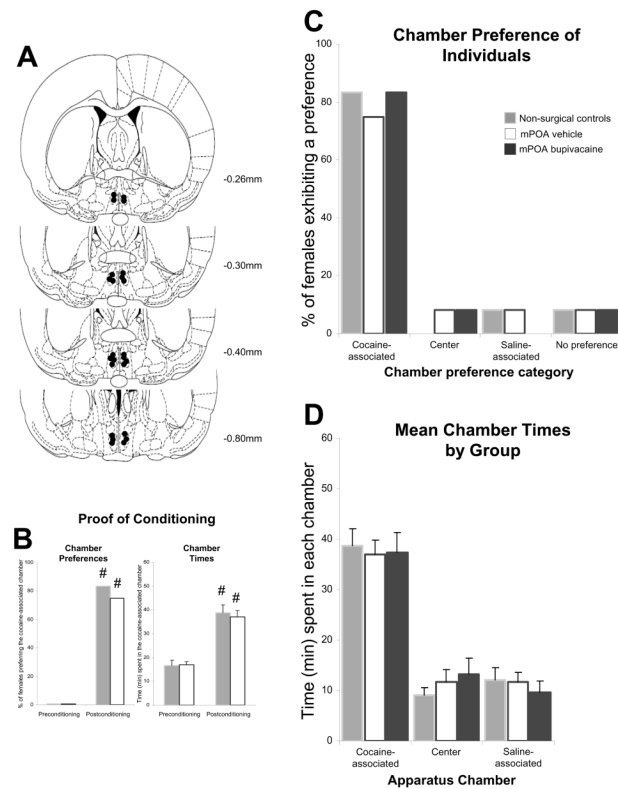


Figure 3. Effect of transient inactivation of the mPOA on expression of cocaine-induced CPP. (A) Schematic representation, based on the microscopic analysis of cresyl violet-stained sections, of injection sites for all rats receiving infusion treatments. (B) Cocaine-associated chamber preferences and times during the pre- and postconditioning sessions. (C) Conditioned chamber preferences and (D) mean time spent in each chamber of the CPP apparatus by non-surgical control females (n=12), and mPOA cannulated females (n=12) following either vehicle or inactivation treatment with 2% bupivacaine. * denotes significant differences between groups, and # indicates significant within-group differences in pre- vs. postconditioning responding; all $P < 0.05$.

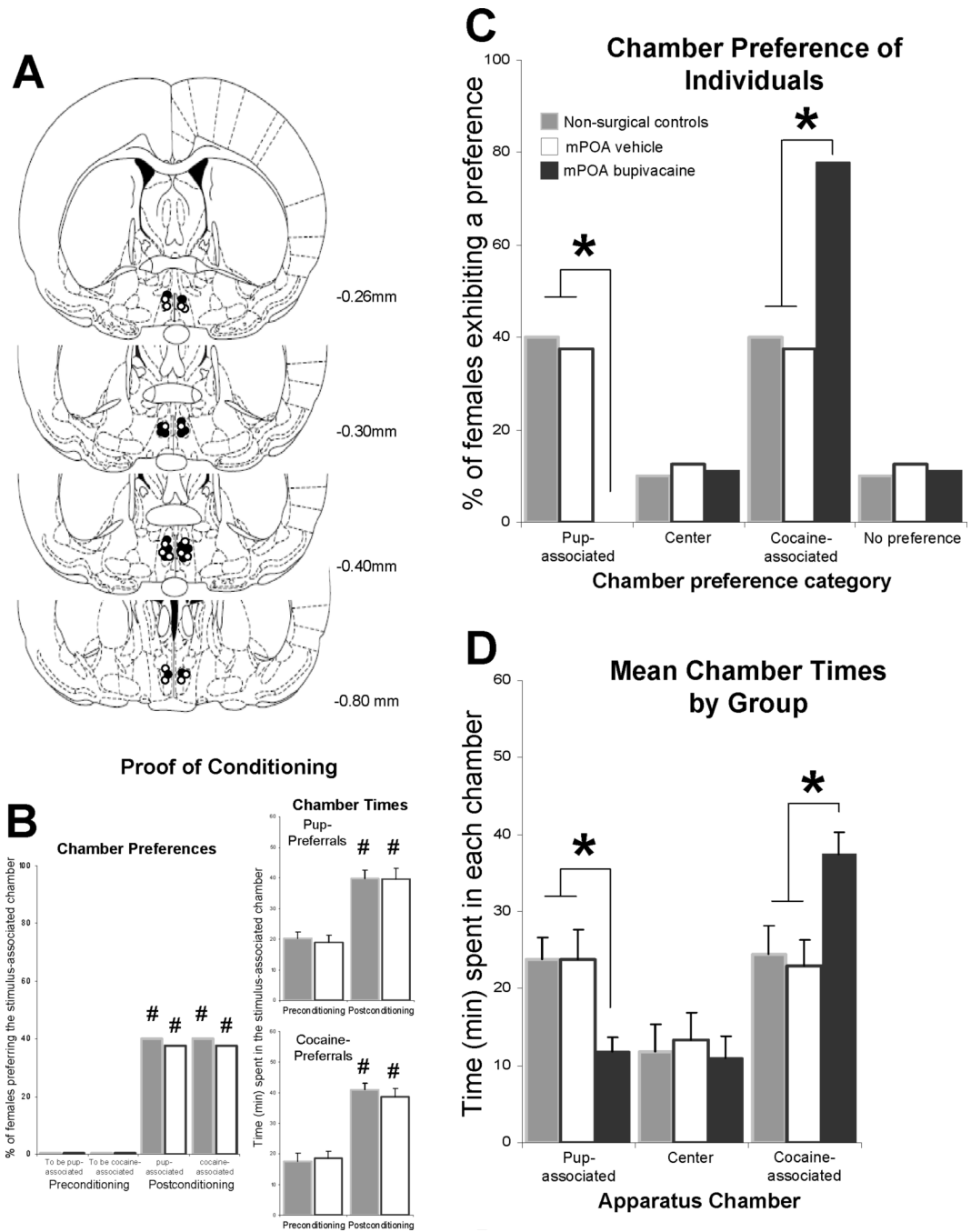


Figure 4. Effect of transient mPOA inactivation on motivated choice of pup- versus cocaine-associated environments in a concurrent pup/cocaine-CPP. (A) Schematic representation, based on the microscopic analysis of cresyl violet-stained sections, of injection sites for all rats receiving infusion treatments. (B) Chamber preferences and times during the pre- and postconditioning sessions for the subset of females that exhibited a conditioned preference for either pup- or cocaine-associated environments. (C) Conditioned chamber preferences and (D) mean time spent in each chamber of the CPP apparatus during the test session by non-surgical control (n=10), mPOA bupivacaine-treated (n=9), and mPOA vehicle-treated (n=8) postpartum

females. *denotes significant differences between groups, and # indicates significant within-group differences in pre- vs. postconditioning responding; all $P < 0.05$.