Published in final edited form as:

Behav Pharmacol. 2017 September ; 28(6): 489–492. doi:10.1097/FBP.0000000000000317.

The Effect of Litter Separation on Methamphetamine Conditioned Place Preference in Post-Partum Dams

C. R. Lewis^a, A.N. Baker^a, P. Fennig^a, P. Conrad^a, L. Hess^a, R.M. Bastle^b, and M. F. Olive^a

^aDepartment of Psychology, Arizona State University, Tempe, AZ 85287 USA

^bSchool of Life Sciences, Arizona State University, Tempe, AZ 85287 USA

Abstract

Methamphetamine (METH) abuse among women has recently increased to levels comparable to those observed in men. While studies using animal models of addiction have begun to include more female subjects, examination of the effect of drugs of abuse on post-partum females is currently lacking. This is especially important in light of the significant hormonal and neurobiological changes that accompany pregnancy and rearing experiences. Furthermore, stress is a known factor in addiction vulnerability and the post-partum experience in the clinical population can be highly stressful. Here, we utilized the conditioned place preference (CPP) paradigm to investigate the conditioned rewarding effects of METH either in virgin rats or in dams exposed to brief (15 min, BS) or long (180 min, LS) separation from the litter. We found that females in the BS group exhibited significantly greater METH CPP compared to both the LS and virgin groups. No differences were found in locomotor activity during the conditioning sessions. These findings suggest that peripartum experience and brief litter separation may enhance the rewarding effects of METH.

Keywords

addiction; reward; post-partum; female virgin; pregnant rodent; methamphetamine; rat

Introduction

Although methamphetamine (METH) use has historically been most common among males (Vearrier et al., 2012), use among women has undergone a sharp increase in recent years and is currently equivalent to that observed in men (Courtney and Ray, 2014; Venios and Kelly, 2010). Women also initiate METH use at an earlier age and are more susceptible to METH dependence, as compared to men (Dluzen and Lui, 2008; Smith, 2014). Furthermore, admissions into rehabilitation programs for pregnant women abusing METH had a three-fold increase from 1994 to 2006 (Terplan et al., 2009). Indeed, women who used METH while

Corresponding Author: Candace Renee Lewis, Ph.D., Arizona State University, 950 S McAllister Ave, Tempe, AZ 85281, Candace.lewis@asu.edu.

The authors have no conflicts of interest to declare.

Statement of Interest

None.

pregnant were 12% more likely to develop a substance use disorder (SUD) than non-pregnant METH-using women (Derauf et al., 2007). This population of METH-abusing mothers typically has a lower socioeconomic status (SES), delayed prenatal care, and increased rates of single parenting (Wouldes et al., 2013). SUDs typically impede parenting and mothers often struggle in recognizing and satisfying their children's needs (Barnard and McKeganey, 2004). Furthermore, mothers abusing drugs typically provide an at-risk environment for their children and the drug use has negative influences on the mother-child bond (Barnard and McKeganey, 2004; Stocco et al., 2012). Therefore, understanding how METH dependence manifests in both pregnant and non-pregnant women is necessary to develop effective and specific treatments.

All major classes of abused drugs interact with gonadal steroid hormones, and although males are more likely to abuse drugs, females tend to show increased sensitivity, especially to psychostimulants (Wood, 2010). Sex differences in psychostimulant-induced behavioral effects are well established in rodents, where female rats display enhanced METH-induced locomotor activity, greater sensitivity to cocaine conditioned place preference (CPP), and facilitated acquisition of METH self-administration (Roth and Carroll, 2004; Russo et al., 2003; Schindler et al., 2002). However, these studies are typically carried out with virgin females, with few studies investigating how the peripartum experience may affect addiction-like behavior. Pregnancy, lactation, pup exposure, and rearing behavior alter monoamine, oxytocin, and GABAergic systems in various regions of the female brain that overlap with the reward circuit (Macbeth and Luine, 2010), suggesting these regions may respond differently to drugs after reproductive experiences. For instance, during pregnancy and the early peripartum period, dams demonstrate decreased cocaine reward and reinforcement and exhibit brain changes that promote both resiliency and risk towards anxiety and depression (Lonstein et al., 2014), factors which are known to influence addiction vulnerability. Therefore, we sought to examine METH reward after litter weaning in dams using CPP.

Moreover, preclinical and clinical research demonstrate that stress is a risk factor for SUD development and relapse vulnerability (Sinha, 2009), and clinical work suggests that women may be more sensitive to the effects of stress on addiction vulnerability (Fox and Sinha, 2009). Despite this, preclinical investigation into the effect of stress on drug abuse vulnerability in post-partum females is sparse (Moffett et al., 2006; Kalinichev et al., 2000). Therefore, we sought to examine: 1) the effect of reproductive and rearing experience on conditioned reward to METH, and 2) the effect of chronic stress, in the form of long litter separation, on METH CPP. Dams were stressed by using repeated long separations (LS) from their litters, a well-established stress paradigm (Maniam and Morris, 2010; Boccia et al., 2007), and compared them to female virgins and dams with brief separations (BS). We predicted that post-weaning dams would show increased CPP to METH and that LS would exacerbate this effect.

Methods

Subjects, Litter Separation, and Stress Procedures

Pregnant dams and age-matched virgins (Charles River Laboratories, San Diego, CA) arrived on gestational day 12 or ~post-natal day (PND) 85. Rats were housed individually in

standard polycarbonate cages in a temperature- and humidity-controlled room with food and water freely available. Upon discovery, litters were culled to a maximum of 12. Day of birth was considered postnatal day 0 (PND0). Dams were randomly assigned to one of two conditions: LS (180 min) or BS (15 min) from PND2–14. We chose a brief separation group over a no-separation group to control for experimenter intrusion and handling, and since this is more representative of naturalistic maternal care (Nylander and Roman, 2013). Separation procedures commenced at 08:00 h (reverse light cycle, lights off at 07:00 h). After the corresponding separation period, pups were returned to the home cage followed by the dam's return. During PND15–20, dams and litters were left undisturbed with the exception of once/week cage cleaning. Litters were weaned on PND21. In order to enhance the effect of METH on CPP, all animals received one 30-min restraint stress session 24 hr prior to conditioning (Capriles and Cancela, 1999).

Estrous Cycle

A subset of rats received vaginal smears in order to assess if the estrous cycle affected METH CPP. To verify phase, vaginal smears were taken prior to behavioral testing and divided into either estrous or di-estrous phase based on microscope visual analysis of cells (Goldman et al., 2007).

Conditioned Place Preference

An unbiased CPP chamber was used (Med Associates, St. Albans, VT), consisting of two compartments with visually distinct walls and textured floors. Each compartment contained 4 sets of infrared photobeams for detection of animal location and assessment of general locomotor activity. On PND22, rats were habituated to the apparatus during a single 30-min session with free access to both compartments. On the following day, animals underwent a 30-min preconditioning test to determine any initial preference for one of the compartments. For the next five consecutive days, animals received twice-daily 30-min conditioning sessions (4 h between daily sessions) where they first received saline (1 ml/kg, i.p.) in their initially preferred compartment and METH (0.5 mg/kg i.p.) in their initially non-preferred compartment. All animals received saline first to avoid carry-over effects of METH. On the day following conditioning, animals were tested for place preference by allowing free access to both compartments for 30 min.

Data Analysis

For each rat, a CPP score was calculated as time spent in the METH-paired chamber during the preference test minus the pre-conditioning test. CPP scores were analyzed by one-way ANOVA followed by post hoc Tukey tests. Locomotor activity (beam breaks during saline and METH conditioning) was analyzed by a mixed-factor ANOVA (Group X Drug X Day), where significant interactions were further analyzed using tests for simple effects. Statistical significance was considered $p < 0.05$.

Results

CPP Magnitude

For estrus cycle, no group differences were found in either percent time spent in METH chamber or CPP score. A significant ANOVA for CPP score was observed ($F(2,24) = 4.30$, $p < 0.05$), where post hoc analyses revealed that the dams in the BS group exhibited significantly higher CPP scores as compared to dams from the LS (Tukey, $p = 0.05$) and virgin (Tukey, $p = 0.042$) groups (Figure 1). No differences in CPP scores between the dams in the LS and virgins groups were observed.

Locomotor Activity

A mixed-factor ANOVA revealed a significant main effect of drug ($F(2,15) = 65.01$, $p < 0.001$) and day ($F(2,15) = 17.11$, $p < 0.001$), but no significant group effect on locomotor activity (Figure 2). There was a trend towards an interaction between day and group ($F(2,15) = 3.42$, $p = 0.059$), and a significant interaction between drug and day ($F(2,15) = 11.11$, $p < 0.005$). No other significant interactions were found. One-sample t -tests revealed more locomotor activity during the METH condition compared to the saline condition on all five conditioning days (all p 's < 0.001).

Discussion

Here, we found that postweaning dams displayed a higher magnitude of conditioned METH reward as compared to virgins and that stress in the form of long litter separation possibly negated that effect. Although the mechanisms driving the increased conditioned reward to METH in postweaning dams is unclear, peripartum and pup-related cues alter the maternal brain in a variety of ways that may alter drug response (Lonstein et al., 2014). For example, GABAergic systems play a major role in the development of drug dependence and undergo alterations during the postpartum experience (Addolorato et al., 2012; Macbeth and Luine, 2010). Furthermore, the peripartum experience is associated with altered risk and resilience towards anxiety- and depression-like behaviors (Lonstein et al., 2014), which may play a role in the rewarding properties of METH. Finally, the act of weaning may have led to abrupt hormonal changes capable of affecting the dam's response to METH. These changes could alter drug sensitivity to other doses of METH, a possibility that clearly warrants further study.

Most litter separation research has focused on the development of the offspring, but litter separation also serves as a stressor for the dam (Boccia et al., 2007; Eklund et al., 2009; Von Poser Toigo et al., 2012). Therefore, this procedure may be used to investigate the effects of separation on vulnerability to drug abuse in mothers. Surprisingly, we found that long litter separation negated the increased CPP in postweaning dams. Litter separation modulates various neurophysiological systems that influence the rewarding and reinforcing properties of drugs of abuse, including hypothalamo-pituitary adrenal axis function and glucocorticoid receptor levels in the hippocampus (Maniam and Morris, 2010). Litter separation decreases expression of both serotonin and tryptophan hydroxylase in the dorsal raphe nucleus of dams (Sung et al., 2010) and can influence both anxiety and depressive-like behavioral phenotypes

in dams long after litter weaning (Boccia et al., 2007; Kalinichev et al., 2000; Maniam and Morris, 2010). Interestingly, Moffett and colleagues (2006) did not find a difference in cocaine self-administration between dams exposed to litter separation and those not separated, whereas Kalinichev and colleagues (2000) found that prolonged litter separations reduced the dam's sensitivity to morphine. While it is unclear why dams exposed to brief separation displayed higher METH CPP, it is possible that long litter separation may have increased or decreased sensitivity to the rewarding effects of METH, thus making the tested dose either too low or high. However, we found no differences in locomotor activity during the conditioning sessions, suggesting drug sensitivity was equivalent across groups. Alternatively, it is possible that dams exposed to long litter separations may develop anhedonia (Von Poser Toigo et al., 2012), which may reduce the rewarding effects of METH. Future studies will test these hypotheses.

We advocate the importance of investigating maternal stress during and after the postpartum experience, as this is translationally relevant for mothers struggling with a SUD. The brain undergoes vast alterations in response to pregnancy, lactation, and pup rearing that may influence the behavioral and physiological response to drugs (Frankfurt et al., 2011; Seip et al., 2008). In order to design adequate treatment strategies for women suffering from a SUD during the postpartum period, there is a need to further evaluate vulnerability to drug abuse in post-partum females.

Acknowledgments

Funding

This work was supported by National Institutes of Health (DA025606 to MFO, DA035069 to RMB).

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Figure 1.

BS (n=8) female rats displayed a significantly larger CPP magnitude compared to LS (n=9) dams and virgins (n=8). CPP score was calculated by seconds spent in METH-paired chamber pre-conditioning subtracted from time spent in METH-paired chamber post-conditioning. Data points represent group mean + SEM. * $p < 0.05$ vs. LS and % $p < 0.05$ Virgins.

Figure 2.

Locomotor response to saline or METH (0.5 mg/kg, i.p.) across five conditioning sessions in BS, LS, and Virgin female rats. Dashed line separates activity following METH or saline priming. Data points represent group mean $\pm$ SEM. No significant differences between groups were observed.