Acute ovarian hormone treatment in freely cycling female rats regulates distinct aspects of heroin seeking

Maribel Vazquez, Jessica H. Frazier, Carmela M. Reichel, and Jamie Peters

Department of Neuroscience, Medical University of South Carolina, Charleston, South Carolina 29425, USA

Females are at higher risk for certain opioid addictive behaviors, but the influence of ovarian hormones is unknown. In our rat model of heroin self-administration, females exhibited higher relapse rates that correlated with rates of heroin seeking on the first extinction session. We administered estradiol alone, or in combination with progesterone, 30 min prior to the first extinction session in freely cycling, heroin-seeking female rats. Although neither treatment produced long-term effects on relapse, each treatment regulated distinct aspects of heroin seeking. Estradiol treatment enhanced extinction memory retention, whereas the combination treatment acutely reduced expression of heroin seeking.

[Supplemental material is available for this article.]
time-out period followed each lever press (house light off, heroin unavailable). These conditioned cues are effective at triggering cued reinstatement after extinction.

Extinction training, wherein responding on both levers was without consequence (no heroin, no cues), commenced 5 d after the last heroin self-administration session. Notably, the first extinction training provides an opportunity to measure heroin seeking when heroin is no longer available. Responding on this session can thus be viewed as the first “lapse” in drug seeking, reflecting motivation to seek heroin. Females had significantly higher rates of heroin seeking on extinction day 1 than males (Fig. 1C). Lever discrimination disappeared by extinction day 3, and the sexes exhibited similar rates of extinction over the remaining sessions (10 total). We also examined the extinction index, as a measure of extinction memory retention, using the formula: 1 − [labeled lesion press rate + unlabeled lesion press rate + non-schedule lever press rate]. This resulted in a value between 0 and 1, where higher values indicate better extinction memory. This within-subject measure of the decrease in heroin seeking from extinction day 1 to extinction day 2 was analyzed using an unpaired, two-tailed t-test and was similar between the sexes (Fig. 1D). Lever discrimination reemerged on the cued reinstatement test, wherein the heroin light-tone cue (FR2, 20 sec time-out) became available again on the active lever, to trigger a relapse. Females relapsed at a higher rate than males on this test (Fig. 1E). Interestingly, extinction day 1 response rates on the active lever correlated with relapse rates only in females (Pearson’s $r = 0.88$, $P = 0.009$) (Fig. 1F).

With regards to extinction, female rats in the low-estrogen phase of metestrus exhibit poor fear extinction memory, similar to women in the early follicular phase, also marked by low estrogen. However, extinction is more likely to be successful if extinction training is conducted during proestrus (in female rats) or the late follicular/mid-luteal phase (in women), when estradiol is high (Milad et al. 2009; Zeidan et al. 2011; Graham and Daher 2016). We were unable to assess the role of the estrous cycle phase on extinction success in this experiment, as all but one female rat was in metestrus or diestrus on extinction day 1; one was in estrus. Interestingly, Graham and Scott (2018) recently assessed the interaction between estradiol dose and estrous cycle phase in freely cycling female rats undergoing fear conditioning and extinction procedures. They found that a low dose of β-estradiol (15 µg/kg, s.c.) improved extinction memory retrieval across all estrous cycle phases except proestrus, where it had no effect. Thus, if females are in a low-estrogen state, this low dose of β-estradiol enhances extinction memory, but if they are already in a high-estrogen state, they do not reap additional benefits. In fact, a higher dose of β-estradiol (100 µg/kg, s.c.) impaired extinction memory retrieval in rats that were in proestrus (but not other phases) at the time of administration (on extinction day 1). These results are consistent with a proposed inverted U-shaped dose-response curve for estradiol on drug seeking (Hu and Becker 2008).

We next assessed the role of acute, low-dose estradiol administration in freely cycling, heroin-seeking female rats on extinction memory retention, as well as any long-term effects this treatment might have on relapse. Estradiol (15 µg/kg, s.c.) or vehicle (sesame oil) was administered 30 min prior to extinction day 1 training. This dose and treatment interval has been shown to enhance fear extinction memory retention (Milad et al. 2009; Zeidan et al. 2011; Graham and Daher 2016; Maeng et al. 2017; Graham and Scott 2018) and increase activity acutely in extinction neural circuits in both female rats and humans (Zeidan et al. 2011; Maeng et al. 2017). Notably, these rapid effects of estradiol are consistent with nongenomic actions of estradiol (Luine and Frankfurt 2012; Srivastava et al. 2013; Kow and Pfaff 2018). For this experiment (and the next), where treatments were administered prior to extinction day 1, we conducted two-tailed, unplanned pairwise comparison t-tests for key variables. These included active and inactive lever responding on extinction day 1, extinction day 2, and the cued reinstatement test. Lever pressures over the entire course of each experiment were also analyzed using three-way ANOVAs, and two-way ANOVAs where appropriate (Supplemental Table 1). Rats acquired heroin self-administration, indicated by successful lever discrimination at the end of self-administration (Fig. 2A), and there were no group differences in the number of heroin infusions self-administered (two-way ANOVA: Time $F_{(11,143)} = 11.26, P < 0.0001$) (Fig. 2B), indicating that the groups were appropriately balanced before treatments were administered.

No effects of treatment were observed acutely on the first extinction session (Fig. 2C). However, the estradiol group had a higher extinction index than the vehicle group ($F_{(13,13)} = 2.729, P = 0.0172$) (Fig. 2D) and had lower rates of heroin seeking on extinction day 2 ($F_{(13,13)} = 3.324, P = 0.0098$) (Fig. 2E), indicating that estradiol treatment selectively enhanced extinction memory retention. Lever discrimination disappeared by extinction day 3 in the vehicle group, and extinction day 2 in the estradiol group. The groups responded similarly over the remaining nine daily extinction

![Figure 1](https://example.com/figure1.png)
sessions, and they were then subjected to the cued reinstatement test. No effects of treatment were observed on this test (Fig. 2F), and lever discrimination reemerged in both groups (Fig. 3A). Thus, combination treatment with estradiol + progesterone selectively reduced acute heroin seeking on extinction day 1, but produced no additional therapeutic benefit on extinction retention, and produced no long-term effects on relapse.

Because we rarely sampled proestrus (the high-estrogen phase) in our female rats, groups were underpowered for cycle phase analyses. However, pooling the estradiol groups from the last two experiments provided sufficient power to compare the low-estrogen phases of metestrus/diestrus versus estrus (Supplemental Fig. 1). Since no studies have assessed the role of exogenously administered ovarian hormones on heroin-seeking in freely cycling females, this study was designed to address this gap in knowledge by examining the effects of acutely administered exogenous estradiol and progesterone on the expression of heroin-seeking behavior versus extinction memory success. The hormones were administered 30 min prior to the first extinction training session to simulate conditions under which they might be used clinically to enhance extinction retention, and to maintain consistency with prior studies (Milad et al. 2009; Zeidan et al. 2011; Graham and Daher 2016; Maeng et al. 2017; Graham and Scott 2018). We chose this approach, as opposed to using ovariectomized (O VX) females, to preserve the clinical relevance of our findings and because ovariectomy alters heroin self-administration (Roth et al. 2002), which we wanted to avoid.

Consistent with a growing literature indicating that estradiol has beneficial effects on extinction memory (Wegerer et al. 2014; Graham and Daher 2016; Maeng et al. 2017; Graham and Scott 2018), we found that β-estradiol enhanced extinction memory retention in heroin-seeking female rats, measured by the extinction index. A proposed mechanism by which estradiol may enhance extinction is through increased production of endogenous brain-derived neurotrophic factor (BDNF) (Liu et al. 2001; Scharfman et al. 2003; Barker et al. 2015), as well as more rapid, synergistic signaling interactions that increase hippocampal spine density (Srivastava et al. 2013). Exogenously applied hippocampal BDNF induces extinction memory for conditioned fear (Peters et al. 2010). We have observed similar extinction-like reductions in heroin seeking with exogenous-hippocampal BDNF; however, these effects did not reach statistical significance (Barker et al. 2015). The extent to which such known mechanisms of fear extinction memory are shared for heroin seeking is an area that warrants further investigation (Peters et al. 2009, 2015). Additional studies are also needed to examine other acute doses of β-estradiol, as well as endogenous estradiol levels (e.g., in blood plasma), which may correlate with extinction memory success.

Because our final experiment compared the combination treatment of estradiol + progesterone to an estradiol-only
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One mechanism by which progesterone may decrease drug seeking is through its known anxiolytic effects mediated by its metabolite, allopregnanolone (Bitran et al. 1995). This nongenomic effect of progesterone occurs via allopregnanolone’s ability to rapidly enhance GABA<sub>A</sub>-receptor mediated currents (Bitran et al. 1993, 1995). Opioid withdrawal is characterized by an aversive emotional and physical state entailing increased anxiety and hyperalgesia (Le Roy et al. 2013; Koob 2019). This withdrawal-associated anxiety may be more pronounced in female opiate abusers compared to males (Kosten et al. 1985), and may account, at least in part, for the additional therapeutic benefit conferred by gabapentin in conjunction with naltrexone to manage opiate withdrawal symptoms (Martínez-Raga et al. 2004). Interestingly, women with posttraumatic stress disorder (PTSD) exhibit a blunted metabolism of progesterone to allopregnanolone compared to trauma-exposed women without PTSD (Pinelès et al. 2018), suggesting that this metabolic pathway may provide endogenous protection against multiple forms of anxiety, and perhaps anxiety-precipitated relapse.

We found that females had higher rates of heroin seeking than males on the first day of extinction, a form of postabstinence relapse (Fuchs et al. 2006; Reichel and Bevins 2009; Giannotti et al. 2018). Females also had higher rates of cued reinstatement of heroin seeking after extinction. Because both extinction day 1 responding and cued reinstatement responding have been shown to correlate with the variable $\alpha$ (a measure of demand elasticity) in behavioral economics models (Bentzley et al. 2014; Cox et al. 2017), both forms of relapse likely relate to the motivational drive to seek heroin (or inelastic demand). Interestingly, we observed a correlation between heroin seeking on extinction day 1 and cued relapse in females only. However, the latter studies, which were conducted using psychostimulants, indicate that $\alpha$ correlates with relapse rates in both sexes (Bentzley et al. 2014; Cox et al. 2017). The present study is the first to report a correlation between extinction day 1 and reinstatement for opioid seeking; thus the apparent lack of correlation in males may be unique to opioids. Since our hormone treatments were restricted to early extinction, and no long-term effects of this acute treatment on relapse were noted, future studies should examine the effects of ovarian hormone treatment on cued relapse rates in freely cycling females.

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