17 β-Estradiol Exacerbates Methamphetamine-Induced Anxiety-Like Behavior in Female Mice

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Highlights

- The open-field task assessed anxiety-like behavior in estradiol-treated mice
- Estradiol-treated mice were more anxious compared to oil-treated female mice
- Methamphetamine enhanced anxiety-like behavior in estradiol-treated female mice

Abstract

The present experiment investigated the effect of 17 β-estradiol (E_2) on anxiety-like behavior following methamphetamine administration in female, Swiss-Webster mice. Mice underwent bilateral ovariectomy (OVX) followed by a subcutaneous implantation of a Silastic capsule containing either sesame oil (OVX+Oil) or E_2 (36 µg/ml; OVX+E_2). One week later, mice were placed in an open-field chamber for an 8-hour session. During the first 3 hours of the session, mice were permitted to run in the absence of any drug (baseline). Then, mice were injected intraperitoneally with methamphetamine (0.25, 0.5 or 1.0 mg/kg) or vehicle (physiological saline) and returned to the open-field chamber for the remaining five hours of the session. Mice were injected with vehicle or a different methamphetamine dose once a week for 4 weeks. Four measures of anxiety were assessed: distanced traveled, vertical counts, time in the center, and time resting in the perimeter of the chamber. OVX+E_2 were less active and spent less time in the center than OVX+Oil mice during Hour 1 at certain doses, but not during remaining baseline hours (Hours 2-3). Furthermore, group differences were not observed during the Stimulant Phase (Hour 4) following injection of any methamphetamine dose (0.25, 0.5 or 1.0 mg/kg) or the vehicle. However, OVX+E_2 mice were less active, spent less time in the center, and spent more time resting in the perimeter of the chamber compared to OVX+Oil mice during certain hours of the Clearance Phase (Hours 5-8) following injection of the high (1.0 mg/kg), but not the low (0.25 mg/kg) or moderate (0.5 mg/kg), methamphetamine doses. These results suggest that E_2 exacerbates anxiety-like behavior during acute clearance from a high methamphetamine dose in OVX female mice, perhaps indicating that E_2 contributes to drug relapse in women by worsening anxiety-related withdrawal symptoms.

Keywords: Anxiety; Estradiol; Female; Methamphetamine; Mouse; Open Field

1. Introduction

Women compared to men have been found to initiate methamphetamine use earlier, escalate to regular use in a shorter time, and show signs of greater methamphetamine dependency [5,10]. Methamphetamine use disorder also is more comorbid with other psychiatric disorders (e.g., depression) for women compared to men [28]. These sex differences may be due to the ovarian hormone, estradiol (E_2; see [1] for a review of sex differences in drug addiction). In fact, when E_2 levels are highest during a woman's menstrual cycle (i.e., late follicular phase), women report unpleasant responses (e.g., anxiety) to acute amphetamine administration [15]. Furthermore, the interaction of methamphetamine with the body's stress system [i.e., hypothalamic-pituitary-adrenal (HPA) axis] may predispose women to methamphetamine use disorder. Indeed, drug-induced dysregulation of anti-reward neural circuits (e.g., HPA) theoretically has been suggested to produce negative-affective states (i.e., anxiety) that underlie drug abuse in people [17].

Preclinical rodent studies have shown that methamphetamine activates the HPA axis and this HPA activation is sexually dimorphic (see [29] for a recent review). For example, Zuloaga et al. (2014) injected male and female mice with methamphetamine (1.0 mg/kg) and then assayed corticosterone 30, 70 or 120 minutes later [29]. They found that methamphetamine augmented corticosterone levels 70 and 120 minutes after the injection in female mice more so than male mice. Furthermore, these authors found sex differences in c*Fos* expression (females < males) in the CA3 region of the hippocampus and the cingulate cortex. E_2 may contribute to these sex differences, as E_2 has been shown to interact with the HPA axis. For example, research by Burgess and Handa (1992) examined the effect of E_2 on HPA functioning [8]. In this experiment, corticosterone levels were compared between E_2-treated and control female rats

following exposure to a stressor (e.g., footshock). These authors found that footshock stress increased corticosterone levels more so in E_2-treated compared to control rats. In addition, in female rats E_2 has been found to facilitate release of corticotrophin-releasing hormone [21] and enhance activity of the HPA axis via an increase in adrenocorticotropic hormone and corticosterone [8], perhaps inducing anxiety-like behavior in rodents.

To date, considerable research has shown that chronic methamphetamine exposure induces anxiety-like behavior in rodents, particularly associated with withdrawal (see [16] for a review). Less research has examined the effects of *acute* methamphetamine exposure on inducing anxiety-like behavior in rodents and the results have been equivocal. On the one hand, acute methamphetamine exposure has been shown to produce anxiolytic effects in male rats, as assessed in the open-field test [14]. For example, Herbert and Hughes (2009) found that methamphetamine (2.0 mg/kg) exposure increased rearing, ambulation and frequency in the center area [14]. On the other hand, studies have found that low methamphetamine doses (1 – 4 mg/kg) have anxiogenic effects in male rats [22] and mice [13,26]. For example, a single injection of a higher methamphetamine dose (4.0 mg/kg) has been found to induce anxiety-like behavior in male, adolescent mice, as assessed by the open-field test [26].

To our knowledge, no research has examined the anxiogenic effects of methamphetamine in female mice, let alone specifically assessing the role of E_2. Previous work has shown that female mice are more anxious compared to male mice [3]. Moreover, these sex differences appeared to be mediated by E_2, as E_2 has been shown to be anxiogenic in females [18]. Therefore, the present experiment examined the interaction of E_2 and methamphetamine on anxiety-like behavior in female mice. We hypothesized that E_2-treated female mice would be more anxious compared to oil-treated female mice following acute methamphetamine exposure.

To test this hypothesis, the open-field task was used (see [9] for a review). In this task, rodents that are "anxious" will display characteristic patterns of behavior (e.g., less general locomotion, less time in the center of the chamber, less rearing and more time immobile (see [2] for a review). Similar measures [distance traveled, rearing, time in center and time resting in the perimeter] were adopted in the present experiment. We assessed these behaviors for an 8-hour period. The 8-hour period was divided into 3 phases: baseline, stimulant and clearance. Mice were placed in the open-field chambers for a 3-hour period in the absence of any methamphetamine (baseline). After 3 hours, mice were injected with vehicle or a given methamphetamine dose and their behaviors recorded for an additional 5-hour period. Because the half-life of methamphetamine in mice is approximately 50 minutes [6], the additional 5-hour period of time permitted us to examine group differences with respect to the onset (Stimulant Phase; Hour 4) and offset (Clearance Phase; Hours 5-8) of methamphetamine. The use of an 8-hour session is an unusually-long behavioral-sampling period for the open-field test. However, the long sampling-period was intended to 1) examine possible baseline groups differences, 2) eliminate any potential group differences prior to methamphetamine administration, and 3) examine group differences in methamphetamine response over a protracted period of time (i.e., onset and offset of methamphetamine). Finally, mice were injected with vehicle or a different methamphetamine dose once a week for 4 weeks. Previous work has shown that a repeated behavioral-testing procedure at 1-week intervals does little to diminish anxiety-like behavior as assessed by the open-field task in mice and is an efficient within-subjects' procedure for examining dose-response curves [4].

Methods and Materials

2.1. Subjects

Female, Swiss-Webster mice (*N* = 30) were obtained from Charles River Laboratories. The mice were approximately 55-60 days of age on arrival. Upon arrival, mice were group-housed (4-5 mice/tub) in translucent Polysulfone tubs measuring 157.3 x 71 x 210.4 cm (length x width x height), lined with paper bedding (Care-free Ultra), and contained within a ventilated-caging system (FA72-UD-WB, Alternative Design Mfg, Siloam Springs, AR) which cycled 30 air changes/hour into the tubs. Following surgery and for the remaining duration of the experiment, mice were singly housed in order to protect the surgical site. Food (Purina Fortified Rodent Chow) and water were made available *ad libitum*. The room was kept at ~ 21 degrees (Celsius) and the lights cycled on a 12:12 light/dark cycle in which the light turned on at 0900 h. Mice were run during the light phase. Mice were acclimated to the colony for a 7-day period prior to the start of the experiment. The experiments conform to the guidelines established by the *NIH Guide for the Care and Use of Laboratory Animals* [19]. The Institutional Animal Care and Use Committee at Dickinson College approved (IACUC Protocol # SP14MMN01) the experiment described.

2.2. Apparatus

Fifteen, open-field chambers (MED-OFA-510; Med-associates, VT) were used for all locomotor activity sessions. Each chamber was placed in a sound-attenuated cubicle (MED-OFA-022, Med-Associates, VT) containing a fan [M = 78.8 (dB), SD = 1.01] and dim lights [M = 25.92 (Lux), SD = 11.19]. The walls of the activity chambers were made of Plexiglas with the interior dimensions measuring 27.9 cm x 27.9 cm. Measures of anxiety-like behavior (distance traveled, vertical counts, time in center and time resting in the perimeter) were determined by three, 16-beam I/R arrays (X, Y and Z axes) and photo beam breaks recorded by a personal computer (Activity Monitor software; Med-associates, VT) located in the same room as the

chambers. A computer-generated square was placed in the center of the chamber and was used to calculate the time in center and time resting in the perimeter measures. The perimeter area was defined as 3.0 cm from each wall and the center as the remaining area. The chambers were cleaned with a disinfectant solution (Precise QTB/Caltech, Midland, MI) following each 8-hour locomotor activity session.

2.3. Drugs

Methamphetamine HCl (St. Louis, MO) was dissolved into physiological saline and injected intraperitoneally (i.p.) in a volume of 10 ml/kg (body weight) based on its salt weight. 17 β-Estradiol (36 µg/ml) was dissolved in a Silastic capsule (2 cm in length) containing sesame oil.

2.4. Procedure

After the acclimation period, the mice underwent a 1-day surgery. During surgery, mice were anesthetized using a cocktail of 100 mg xylazine + 10 mg ketamine. The cocktail was injected at a volume of 10 ml/kg. Mice underwent bilateral ovariectomy (OVX) followed by a subcutaneous implantation of a Silastic capsule containing either sesame oil (OVX+Oil; n = 15) or E_2 (OVX+E_2; n = 15). This E_2-exposure protocol has been shown to produce physiologically-relevant levels of circulating E_2 for at least 5 weeks [27]. One week after surgery, mice were placed in open-field chambers for an 8-hour session. During the first 3 hours of the session, mice were permitted to run in the absence of any drug (baseline). Then, mice were injected with methamphetamine (0.25, 0.5 or 1.0 mg/kg) or vehicle (physiological saline) and returned to the open-field chamber for the remaining five hours of the session. These methamphetamine doses previously have been used in the laboratory and been shown to have a stimulatory effect in male mice [23]. In order to establish a within-subjects dose response curve, mice were injected with

vehicle or a different methamphetamine dose once a week for 4 weeks. In order to control methamphetamine-dose order effects, half of the mice received an escalating methamphetamine-dose regimen (vehicle → 0.25 → 0.5 → 1.0 mg/kg) and the other half received a de-escalating methamphetamine-dose regimen (1.0 → 0.5 → 0.25 → vehicle). At the end of the experiment, mice were killed and their uteri weighed in order to verify the integrity of the E_2 capsules.

2.5. Data Analysis

SPSS (version 24) and Graph Pad Prism were used to analyze and plot the data, respectively. Four dependent measures of anxiety-like behavior were recorded: distance traveled (cm), rearing (vertical counts), time in center (minutes) and time resting in the perimeter (minutes). Previous research found that E_2 mice are more anxious compared to OVX+Oil mice, as assessed by the open-field test [18]. In order to demonstrate the validity of our open-field testing procedure, we first compared OVX+E_2 and OVX+Oil mice during Hour 1 on their *first* exposure to the open-field chamber. These data were subjected to separate independent-samples *t* tests for each dependent measure of anxiety-like behavior. In addition, to explore group differences with respect to methamphetamine dose, the data next were subjected to a Hormone Treatment (Oil vs. E_2) x Session Hour (1-8) x Methamphetamine Dose (Vehicle vs. 0.25 vs. 0.5 vs. 1.0 mg/kg) ANOVA. Separate ANOVAs were conducted for each dependent measure of anxiety. Only statistically significant main effects and interactions were reported. Hormone Treatment was a between-groups factor, Session Hour and Methamphetamine Dose were within-groups factors. *Post hoc* contrasts involved independent-samples *t* tests. For all statistical decisions α was set at .05 (two, tailed).

2. Results

3.1. Hour 1 of the First Exposure to the Open-Field Chamber

Separate independent *t* tests revealed that OVX+E_2 mice were less active, spent less time in the center, and spent more time resting in the perimeter than OVX+Oil mice, *t*s (28) > 2.21, *p*s ≤ .035 (Figure 1A-C). Groups differences were not detected on the rearing measure (data not shown).

3.2. Methamphetamine Dose Response

3.2.1. Distance Traveled

A Hormone Treatment x Session Hour x Methamphetamine Dose ANOVA revealed statistically significant main effects of Methamphetamine Dose and Session Hour , *Fs* ≥ 8.71, *ps* < .001 (Figure 2 A-D). The Hormone Treatment x Methamphetamine Dose, Hormone Treatment x Session Hour, and Methamphetamine Dose x Session Hour interactions were statistically significant, *Fs* ≥ 2.24, *ps* ≤ .033. OVX+E_2 mice were more active than OVX+Oil during Hour 8 of the vehicle dose session, *t* (28) = 2.70, *p* = .012. OVX+E_2 mice also were more active than OVX+Oil during Hour 3 of the moderate dose session, and Hours 1, 6 and 7 of the high dose session, *t*s (28) ≥ 2.15, *p*s ≤ .04.

3.3.2. Time in Center

A Hormone Treatment x Session Hour x Methamphetamine Dose ANOVA revealed statistically significant main effect of Session Hour, *F* (7, 196) = 58.82, *p* < .001, and Methamphetamine Dose x Session Hour interaction, *F* (21, 588) = 4.33, *p* < .001 (Figure 2E-H). The Hormone Treatment x Session Hour interaction approached statistical significance, *F* (7, 196) = 2.01, *p* = .055. OVX+E_2 mice spent less time in the center during Hour 1, but more time

in the center during Hour 8, compared to OVX+Oil mice on the vehicle session. OVX+E_2 mice also spent less time in the center during Hour 1, and Hours 5 and 7, on the moderate and high dose session, respectively, *t*s (28) ≥ 2.13, *p*s ≤ .042.

3.3.3. Time Resting in the Perimeter

A Hormone Treatment x Session Hour x Methamphetamine Dose ANOVA revealed statistically significant main effects of Session Hour and Methamphetamine Dose, *F*s ≥ 2.74, *p*s ≤ .048 (Figure 2I-L). The Methamphetamine Dose x Session Hour, and Hormone Treatment x Session Hour, interactions were statistically significant, *F*s ≥ 3.33, *p* ≤ .002. The Hormone Treatment x Methamphetamine Dose x Session Hour interaction approached statistical significance, *F* (21, 588) = 1.53, *p* = .063. OVX+E_2 mice spent more time resting in the perimeter compared to OVX+Oil during Session Hour 8 on the vehicle session, *t* (28) = 2.34, *p* = .027. Furthermore, OVX+E_2, mice spent more time resting in the perimeter than OVX+Oil during Hours 5 - 7 on the high dose session, *t*s (28) > 2.07, *p*s ≤ .047.

3.2.4. Vertical Counts

A Hormone Treatment x Session Hour x Methamphetamine Dose ANOVA revealed only statistically significant main effects of Methamphetamine Dose and Session Hour, *F* (3, 84) ≥ 4.02, *ps* ≤ .01 (Data Not Shown). The Hormone Treatment x Methamphetamine Dose approached statistical significance, *F* (3, 84) = 2.43, *p* = .071; however, the Hormone Treatment x Session Hour, and Methamphetamine Dose x Session Hour, interactions were statistically significant *F*s ≥ 3.25, *p*s ≤ .003. OVX+E_2 mice reared more compared to OVX+Oil during Session Hour 8 of the vehicle session, *t* (28) = 2.28, *p* =. 031, and Session Hour 3 on the moderate dose session, *t* (28) = 2.72 = .011. OVX+E_2 mice reared less than OVX+Oil during

Session Hours 5 and 7 of the high dose session, but these differences only approached statistical significance, *t*s (28) ≤ 1.98, *p*s ≤ .058.

3.3. Post hoc exploratory correlations.

We were interested in whether or not a mouse's initial behavior during the first hour on the *first* exposure to the open-field chamber was related to its response to the high methamphetamine dose during the clearance phase. To this end, we performed several Pearson correlations examining the relationship between the total activity (distance traveled, vertical counts, time in center and time resting in the perimeter) during the first hour on the first exposure day and their total activity (distance traveled, vertical counts, time in center and time resting in the perimeter) during Hours 5-8 (Clearance Phase) following an injection with the high methamphetamine dose. These correlations found only a statistically significant positive correlation between the total distance traveled during the first hour on the first day and the total distance traveled during the methamphetamine clearance phase, r (30) = .558, p = .001. The other behaviors were positively correlated, but not statistically significant, *r*s (30) ≤ .315, *p*s ≥ .09.

3.4. Uterine and Body Weights

Uterine weights of OVX+E_2 mice were heavier than the uterine weights of the OVX+Oil mice, $p < 0.05$ (Figure 3A), indicating that the integrity of the E_2 capsules were maintained. A Hormone Treatment x Week (1-4) ANOVA, conducted on the body weights, revealed statistically significant main effects of Week, F (3, 84) = 77.99, $p < .001$, and Hormone Treatment, F (1, 28) = 4.96, p = .034, as well a statistically significant interaction, F (3, 84) =

15.12, $p < .001$. OVX+E_2 mice weighed less than OVX+Oil mice during the last two weeks of the experiment (Weeks 3 and 4), ts $(28) \geq 3.24$, ps $\leq .003$ (Figure 3B).

3. Discussion

During the first hour of the *first* exposure to the open-field chambers, OVX+ E_2 mice traveled less, spent less time in the center, and spent more time in the perimeter of the open field compared to OVX+Oil mice, suggesting that E_2 itself induces anxiety consistent with previous work [18]. Moreover, this finding suggests that our experimental protocol is sensitive to detecting anxiogenic effects. Moreover, while initial group differences during Hour 1 may have been detected for the distance traveled measure on the high methamphetamine dose session (see Figure 2D), these group differences dissipated by the end of Hour 3 of the baseline period for the distance traveled and time resting in the perimeter measures. Thus, subsequent group differences were not complicated by baseline group differences on these measures. However, OVX+E_2 mice spent less time in the center compared to OVX+Oil mice, though not statistically significant, during the baseline phase on the high methamphetamine dose session (see Figure 2H); thus, potentially complicating the interpretation of group differences observed during the methamphetamine clearance phase. While baseline group differences may have existed for the time in center measure, the observed group differences on this measure during the high methamphetamine dose clearance phase mirror those group differences for the other dependent measures (distance traveled and time resting in the perimeter) during this phase. The lack of baseline group differences for the distance traveled and time in center measures, combined with the consistency between all three measures, provide confidence that the observed group differences during the high methamphetamine dose clearance phase is *not* due to baseline differences.

Differences between OVX+E_2 and OVX+Oil mice were *not* detected during the Stimulant Phase (Hour 4) at any methamphetamine dose. This result is not consistent with other studies showing that E_2 enhances the locomotor-activating effects of methamphetamine in rats [20]. Indeed, female rats have been shown to acquire methamphetamine self-administration more quickly, and respond more vigorously for methamphetamine, compared to male rats [24,25]. Taken together, these latter results imply that female rats are *more* sensitive to the reinforcing effects of methamphetamine and this increased sensitivity is related to E_2. As no work previously examined this in Swiss-Webster mice, species differences perhaps account for the discrepancy between studies. Alternately, the repeated-testing procedure used in the present experiment may have led to habituation to the open-field and obscured group differences.

During clearance from the high methamphetamine dose (1.0 mg/kg), OVX+E_2 mice traveled less, spent less time in the center, reared less (though not significantly), and spent more time resting in the perimeter of the chamber. Pharmacokinetic differences between OVX+E_2 and OVX+Oil mice cannot be excluded. OVX+E_2 mice weighed less than OVX+Oil mice by Weeks 3 and 4 of the present experiment (see Figure 3B), perhaps contributing to group differences in metabolism following an injection with the high methamphetamine dose. Estrogen has been found to decrease food intake, promote weight loss and redistribute adipose tissue in female mice [7]. However, it is unlikely that pharmacokinetic factors solely explain the observed group differences. Given that the methamphetamine-dose regimen was counterbalanced, with half the mice receiving an escalating methamphetamine-dose regimen (vehicle → 0.25 → 0.5 → 1.0 mg/kg) and the other half receiving a de-escalating methamphetamine-dose regimen (1.0 → 0.5 → 0.25 → vehicle), group differences following the high methamphetamine dose did not

completely correspond to group differences in weight (i.e., Week 4). Finally, the methamphetamine dose was based on the mouse's body weight.

The findings that OVX+E_2 mice compared to OVX+Oil mice traveled less, spent less time in the center, and spent more time resting in the perimeter of the chamber, following an injection with the high methamphetamine dose, is consistent with our hypothesis that E_2 exacerbated anxiety-like behavior during acute methamphetamine exposure. While previous studies have shown that a 1.0 mg/kg methamphetamine dose induces anxiety-like behavior in male rats [22] and mice [13,26], our results show that this methamphetamine dose also induces anxiety-like behavior in E_2-treated female mice. Moreover, our results showed that group differences were detected during the methamphetamine clearance phase. Other authors have provided evidence that a drug-clearance period is accompanied by a negative affective state (e.g., anhedonia). For example, Ettenberg and his colleagues showed that rats receiving cocaine immediately prior to placement in conditioned place preference chambers displayed a conditioned place preference whereas rats receiving the same dose of cocaine, and placed in the chambers 15 minutes later, displayed a conditioned place aversion [11]. Thus, it is possible that E_2 exacerbated a negative affective state (e.g., anxiety) associated with methamphetamine clearance. Moreover, this exacerbated negative affective state by E_2 during methamphetamine clearance most likely involves its interaction with the neuronal stress system (i.e., the HPA axis), as dysregulation of the neuronal stress system, and associated changes in negative affective states, has been suggested theoretically to underlie drug abuse in people [17].

Activation of the HPA axis by E_2 may account for the observed differences in anxiety-like behavior during methamphetamine clearance, as E_2 has been shown to enhance HPA activity related to stressors [8] and exacerbate anxiety-like in female mice [18]. Methamphetamine too

has been shown to enhance HPA activity, facilitating the effects of corticosterone, and this enhancement is sexually dimorphic [29]. Indeed, Zuloaga et al. (2014) found that a single methamphetamine injection (1.0 mg/kg) elevated corticosterone levels 70 – 120 minutes after the injection in females more so than male mice. The effects of a single methamphetamine injection on corticosterone levels in female mice observed in the Zuloaga et al. (2014) study temporally mirror the anxiety-like behavior observed in the E_2 mice during the methamphetamine clearance phase (e.g., 2 hours after the injection) in the present experiment. A limitation of the current experiment is that corticosterone was not assessed. A future experiment should determine if the E_2 exacerbation of anxiety-like behavior during methamphetamine clearance is associated with elevated levels of corticosterone. However, while corticosterone was not measured, the reported failure of E_2-treated mice to gain weight similarly to oil-treated mice in the present experiment may be an *indirect* indictor that these mice were physiologically stressed (see [12] for a recent review).

4. Conclusions

A *post hoc* analysis in the present experiment found that the distance traveled during the first hour on the first exposure to the open-field chamber, when OVX+E_2 mice were less active than OVX+Oil mice (i.e., more anxious), was moderately correlated (r = .558) with subsequent distance traveled during the methamphetamine clearance phase. This result implies that E_2 predisposes female mice to the anxiogenic effects of methamphetamine, especially during a "withdrawal-like" period (i.e., drug clearance). This finding is consistent with the observation that female rats compared to male rats have shown a greater propensity to seek methamphetamine following a withdrawal period [24,25]. Taken together, these preclinical rodent studies *may* imply that E_2 exacerbates withdrawal-induced anxiety, contributing to greater

drug relapse propensity in women compared to men [1]. Indeed, it has been found that women report unpleasant responses (e.g., anxiety) to acute amphetamine administration during the late follicular phase when E_2 levels are the highest [15].

Figure Captions

Figure 1. Distance traveled (A), time in the center, (B), and (C) time resting in the perimeter for OVX+E_2 and OVX+Oil mice during the first hour on the first exposure to the chamber. Asterisks indicate a significance difference, $p < .05$. Error bars represent + 1 SEM.

Figure 2. Distance traveled (Left Column), time in the center (Middle Colum), and time resting in the perimeter (Right Column) following an injection of vehicle, low, moderate or high methamphetamine dose for OVX+E_2 and OVX+Oil mice. The arrow denotes the injection was given at the end of Hour 3. Asterisks indicate a significance difference, $p < .05$. Error bars represent ± 1 SEM.

Figure 3. Uterine (A) and body (B) weights for OVX+E_2 and OVX+Oil mice. Asterisks indicate a significance difference, $p < .05$. Error bars represent +/± 1 SEM.

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