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Methamphetamine-Induced Conditioned Place Preference In Adolescent Male and Female Mice of Two Strains

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by

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Abstract

Men and women differ in their use and response to methamphetamine. Compared to men, women initiate use of methamphetamine at an earlier age, become regular users faster, and use it for a different purpose, such as weight loss. While numerous studies have used rodent models to understand the cellular basis of addiction, the majority of these studies have been conducted in adult male animals. We used a conditioned place preference paradigm to investigate the rewarding effects of methamphetamine (1 mg/kg) in male and female mice of two different strains, C57Bl/6 and 129/SvEv. Given that substance abuse is often initiated in humans during adolescence, our experiments began during this developmental time period (postnatal day 41). We found that methamphetamine induced conditioned place preference in adolescent female C57Bl/6 mice but not adolescent female 129/SvEv mice. Conversely, methamphetamine induced conditioned place preference in adolescent male 129/SvEv mice, but not adolescent male C57Bl/6 mice. Methamphetamine significantly enhanced locomotor activity in all sexes and strains tested, but only female and male 129/SvEv mice showed sensitization. These results indicate both strain and sex differences in the rewarding effects of methamphetamine in adolescent mice.

Keywords: Conditioned place preference; locomotor activity; methamphetamine; C57Bl/6; 129/SvEv; mice; adolescence; sex difference

Methamphetamine-Induced Conditioned Place Preference In Adolescent Male and Female Mice of Two Strains

Addiction to methamphetamine is a serious public health issue, (Gonzales, Mooney & Rawson, 2010; Maxwell & Rutkowski, 2008), with approximately 133,000 new users of this drug each year, resulting in an annual cost of $23.4 billion to the United States (Gonzales, Mooney & Rawson 2010; Zuloaga, et al., 2014). Interestingly, there are several differences between men and women in their use of and response to methamphetamine (reviewed in Dluzen & Liu, 2008). Women begin using methamphetamine earlier than men, are younger when they first enter treatment programs (Hser et al., 2005; Lin et al., 2004), and have a shorter transition from initial use to regular use (Rawson, Gonzales, Obert, McCann, & Brethen, 2005). While both men and women often use more than one substance of abuse, more women than men report methamphetamine as their main drug of choice (Cretzmeyer, Sarrazin, Huber, Block, & Hall, 2003; Polcin, Buscemi, Nayak, Korcha, & Galloway, 2012).

Despite this sex difference in drug taking behavior, research involving animal models has primarily used male rodents to understand the genetic and cellular basis of addiction (Buck & Siegel 2015; for review, see Fattore, Altea, & Fratta, 2008; Zakharaova, Wade, & Izenwasser, 2009). In addition, these studies are often conducted in adult animals, even though substance abuse is most frequently initiated during adolescence (Schramm-Sapyta, Walker, Caster, Levin, & Kuhn, 2009). In the present study, we investigated the rewarding effects of methamphetamine in adolescent male and female mice of two strains, C57Bl/6 and 129/SvEv, using the conditioned

place preference paradigm. Conditioned place preference is a commonly used test for investigating the rewarding effects of drugs of abuse in rodents (for review, see Tzschentke, 1998) and involves training animals to associate the drug-induced state with one side of the conditioning chamber. Given that both strains are commonly used background strains for creating transgenic mice, the goal of this work is to provide insight into which strain would be appropriate for future work involving selective manipulation of proteins in the brain.

Method

Animals

Male and female C57Bl/6 and 129/SvEv mice (Taconic Biosciences, Germantown, NY) were shipped to the Hunter College Animal Facility at postnatal day (PND) 21. Mice were group-housed 4 per cage and kept on a 5am/5pm, 12-h light/dark cycle with food and water available *ad libitum*. Experimental procedures began on PND 38 (middle adolescence) and all testing occurred during a portion of the light cycle (10am - 4pm). Each sex was tested separately. Experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of Hunter College, CUNY and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Drug

Methamphetamine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in sterile saline (0.9%). On each day of drug administration, methamphetamine was made fresh prior to being injected intraperatoneally (i.p.) at a dose of 1mg/kg.

Apparatus

Conditioned place preference was conducted in a three-compartment apparatus (Columbus Instruments, Columbus, OH). One compartment contained white Plexiglas walls and floors and was scented with orange Clorox wipes (light side). The other compartment had black walls and a red floor that was textured with strips of white tape and was scented with 100% ethanol (dark side). A removable divider was used to separate the light and dark compartments. The third compartment was a clear holding chamber that provided access to both the light and dark compartments when the adjoining door was opened. Prior to each trial, the holding chamber was wiped down with a wet paper towel and the light and dark chambers were wiped down with their respective scents. The light and dark compartments were each 8.25" long, 12" high and 12" wide. The holding chamber was 3.5" long x 3.5" wide x 5" deep.

Procedure

At the start of each session, all mice were weighed and given a distinct tail marking with a Sharpie pen for identification purposes. There were four phases of the conditioned place preference procedure: handling (days 1 and 2), preconditioning (day 3), conditioning (days 4-11), and postconditioning (day 12). Handling occurred on PNDs 38 and 39 and involved gently holding the mouse by the tail for 2 minutes while it walked freely on the experimenter's gloved hand and sleeve. During preconditioning (PND 40), each mouse was allowed to freely explore the light and dark compartments for 30 minutes. Place conditioning took place on the subsequent 8 days (PND 41-48). On days 4, 6, 8, and 10, all mice received either methamphetamine (drug group) or saline (saline group) before being confined to the light compartment for 30 minutes. On days 5, 7, 9, and 11, all mice in both groups were injected with

saline prior to being confined to the dark compartment for 30 minutes. During the postconditioning test (PND 49), all mice had free access to the light and dark compartments for 30 minutes and preference was tested. Animals were not injected with drug or saline during the postconditioning test.

Statistical Analysis

Cameras mounted above the conditioned place preference apparatus recorded behavior during preconditioning, the first and last days drug was administered (conditioning sessions 1 and 7), and postconditioning. Videos were analyzed using ANY-maze software (Stoelting, Wood Dale, IL) to determine time spent on each side of the conditioned place preference box and locomotor activity in each compartment. Preference was determined by calculating the difference between the amount of time spent in the drug-paired compartment (light chamber) during postconditioning minus the amount of time spent in that compartment (light chamber) during preconditioning (CPP score). A two-way ANOVA was used to analyze behavior during preconditioning and to compare methamphetamine-induced locomotor activity across strains. Tukey HSD was used for post-hoc analyses. For each sex and strain, a repeated-measures ANOVA was used to evaluate the effects of drug and saline on locomotor activity following the first (first injection) and last (fourth injection) exposure to methamphetamine. Student's *t* test was used to compare CPP scores of methamphetamine-treated and saline-treated groups.

Results

Conditioned Placed Preference in Female Mice

For time spent in each side of the CPP box during preconditioning, the two-way ANOVA revealed a significant main effect of compartment ($F_{(1,157)} = 366.19$, $p < 0.01$) and strain × compartment interaction ($F_{(1,57)} = 139.7176$, $p < 0.01$). Post-hoc tests showed that female C57Bl/6 mice spent significantly more time in the dark compartment ($\overline{x}$= 1094.18 seconds) than the light compartment ($\overline{x}$= 697.52 second) ($p < 0.05$). Similarly, female 129/SvEv mice spent more time in the dark compartment ($\overline{x}$= 1739.17 seconds) than the light compartment ($\overline{x}$= 59.58 seconds) prior to treatment with drug or saline ($p < 0.05$). There was no main effect of strain ($F_{(1,157)} = 0.0030$ $p = 0.9567$), indicating that both C57Bl/6 and 129/SvEv female showed an innate preference for the dark compartment. Therefore, drug was paired with the light compartment, so that increases in time spent on the drug-paired side could be detected (Figure 1).

For female C57Bl/6 mice, methamphetamine significantly increased locomotor activity. The repeated-measures ANOVA revealed a significant main effect of treatment ($F_{(1,43)} = 49.380$, $p < 0.01$), but no significant main effect of injection day ($F_{(1,43)} = 3.3649$, $p = 0.0735$) and no significant treatment × injection day interaction ($F_{(1,43)} = .4353$, $p = 0.5129$). These results indicate that methamphetamine increased locomotor activity at both time points and there was no drug sensitization effect (Figure 2).

Methamphetamine also significantly increased locomotor activity in female 129/SvEv mice. The repeated-measures ANOVA revealed a significant main effect of treatment ($F_{(1,30)} = 15.885$, $p < 0.01$), but no significant main effect of injection day ($F_{(1,30)} =.0123$, $p < 0.912$) and no treatment × injection day interaction ($F_{(1,30)} = 1.1766$, $p = 1.1766$). These results indicate that methamphetamine increased locomotor activity at both time points (Figure 3).

To compare the effects of methamphetamine on locomotor activity across strains, movement of each drug-treated female was calculated as a percentage of the respective saline-treated control group and averaged. The repeated-measures ANOVA revealed a significant main effect of strain ($F_{(1,35)} = 13.341$, $p < 0.001$), injection day ($F_{(1,35)} = 6.1440$, $p < 0.01$), and strain × injection day interaction ($F_{(1,35)} = 4.488$, $p = 0.041$). A two-way ANOVA confirmed these results. Post-hoc tests revealed no significant difference between 129/SvEv and C57Bl/6 mice in locomotor activity in response to the first drug injection ($p > 0.05$). However, 129/SvEv mice did exhibit significantly more locomotor activity than C57Bl/6 mice in response to the fourth drug injection ($p < 0.05$). Furthermore, within the 129/SvEv strain, mice showed significantly more locomotor activity in response to the fourth than the first injection of drug, suggestive of drug sensitization (Figure 4).

A CPP score was calculated to determine the rewarding effects of methamphetamine during the postconditioning session. We found that the CPP score for drug-treated and saline-treated C57Bl/6 mice was significantly different ($t(29) = 2.51$, $p < .001$) (Figure 5), indicating methamphetamine-induced conditioned place preference in that strain. In contrast, there was no significant difference between drug-treated and saline-treated 129/SvEv mice ($t(29) = 1.77$, $p = 0.08$), indicating that this strain did not demonstrate methamphetamine-induced conditioned place preference (Figure 6).

Conditioned Placed Preference in Male Mice

A two-way ANOVA was used to compare time spent in each side of the CPP box prior to any treatment and revealed a significant main effect of compartment ($F_{(1,90)} = 3174.336$, $p < 0.0001$), and strain × compartment interaction ($F_{(1,90)} = 188.6308$, $p < 0.05$). Post-hoc tests

revealed that C57Bl/6 mice spent significantly more time in the dark compartment ($\overline{x}$=1411.50 seconds) than the light compartment ($\overline{x}$= 388.49 seconds) ($p < 0.05$). Similarly, 129/SvEv mice spent more time in the dark compartment ($\overline{x}$= 1743.50 seconds) than the light compartment ($\overline{x}$= 56.49 seconds) during preconditioning ($p < 0.05$). There was no significant main effect of strain ($F_{(1,90)} = 0$, $p = 1.00$), indicating that both male C57Bl/6 and 129/SvEv mice had an innate preference for the dark compartment. Drug was therefore paired on the light side, so that increases in time spent on the drug-paired side could be detected and testing conditions were the same as those used in the females (Figure 7).

For male C57Bl/6 mice, methamphetamine increased locomotor activity following the first and fourth injection of drug. The repeated-measures ANOVA revealed a significant main effect of treatment ($F_{(1,22)} = 13.65$, $p < 0.001$), but no significant main effect of injection day ($F_{(1,22)} = 2.4941$, $p = 0.1285$) and no treatment × injection day interaction ($F_{(1,22)} = .0005$, $p = 0.9831$), indicating lack of drug sensitization (Figure 8).

Methamphetamine also increased locomotor activity in male 129/SvEv mice during the first and fourth injection of meth. A repeated-measures ANOVA revealed a significant main effect of treatment ($F_{(1,22)} = 21.725$, $p < 0.0001$)and a treatment × injection day interaction ($F_{(1,22)} = 5.1308$, $p = .0337$), but no main effect of injection day ($F_{(1,22)} = 2.0788$, $p = 0.1634$). These results were confirmed with a two-way ANOVA. Post-hoc tests indicated that compared to saline-treated controls, methamphetamine significantly increased locomotor activity following the fourth injection ($p < 0.05$), but not the first injection of drug, indicating drug sensitization. However, locomotor activity following the first and fourth injection of methamphetamine was not significantly different (Figure 9).

We compared the effects of methamphetamine on locomotor activity across strains using a repeated-measures ANOVA. We found a significant main effect of strain ($F_{(1,22)} = 14.9370$, $p < 0.001$), injection day ($F_{(1,22)} = 8.8459$, $p < 0.001$), and strain × injection day interaction ($F_{(1,22)} = 9.3134$, $p < 0.01$). A two-way ANOVA confirmed these results. Post-hoc tests revealed that methamphetamine-induced locomotor activity was significantly higher in 129/SvEv mice following the fourth injection than the first injection ($p < 0.05$). Furthermore, methamphetamine increased locomotor activity significantly more in 129/SvEv mice than C57Bl/6 mice following the fourth ($p < 0.05$) but not the first injection (Figure 10).

During postconditioning, the CPP score for drug-treated and saline-treated C57Bl/6 male mice was not significantly different ($t(22) = 0.23$, $p = 0.81$), indicating a lack of meth-induced conditioned place preference (Figure 11). In contrast, the CPP score for drug-treated and saline-treated 129/SvEv mice were significantly different ($t(21) = 2.74$, $p < 0.01$) indicating that methamphetamine-induced conditioned place preference in that strain (Figure 12).

Discussion

Despite known sex differences in the use of methamphetamine, the vast majority of animal studies investigating the behavioral effects of this drug have used male rodents (Buck & Siegel 2015). Furthermore, this line of research is typically conducted in adult animals, even though drug use is most often initiated during adolescence (Schramm-Sapyta, Walker, Caster, Levin, & Kuhn, 2009). To the best of our knowledge, this is the first study to investigate the rewarding effects of methamphetamine in both male and female mice of two strains during adolescence. Our results reveal that methamphetamine (1mg/kg) induced conditioned place preference in adolescent female C57Bl/6 mice but not female 129/SvEv mice. Conversely,

methamphetamine induced conditioned place preference in adolescent male 129/SvEv mice, but not male C57Bl/6 mice. In contrast to our findings, previous studies report that male C57Bl/6 mice tested during late adolescence display conditioned place preference to amphetamine (2 mg/kg) and cocaine (2.5, 5 and 10 mg/kg) (Belzung, & Barreau, 2000) and adult male 129SvJ mice do not exhibit conditioned place preference to cocaine (5 and 10 mg/kg) (Miner, 1997). This discrepancy might be explained by differences in the drugs of abuse that were tested, the exact substrain that was used (129/SvJ vs. 129/SvEv) and the age of the animals used. Although Belzung, & Barreau also tested mice during adolescence, their mice were at least one week older than the mice used in our study at the time of drug exposure. Adolescence is a developmental time period characterized by ongoing brain development, which in humans, may contribute to novelty seeking and high-risk adolescent behavior (Steinberg, 2007). It is therefore possible that the adolescent mice used in the current study respond differently to drugs of abuse than the older mice used in previous work. It is also possible that our results are dependent on the dose of methamphetamine used and a higher dose might lead to different behavioral results.

There are several potential mechanisms that may account for the sex and strain differences that we report. One possibility is that methamphetamine triggered a differential stress response in each group of animals tested, leading to a stronger drug-associated memory in some groups than others. Indeed, it has been shown that the same dose of methamphetamine used in our study leads to a longer lasting increase in the stress hormone corticosterone in adult C57Bl/6 females than males (Zuloaga et al., 2014). Following release by the adrenal glands, corticosterone is known to activate glucocorticoid receptors located throughout the brain, where it can function to enhance memory consolidation (Aubry, Serrano, & Burghardt, 2016). It is therefore possible that the C57Bl/6 females and the 129Sv/Ev males in our study had a relatively

stronger corticosterone response to methamphetamine than the129/SvEv females and C57Bl/6 males, respectively, and as a result consolidated the drug-associated memory better. This, in turn, would lead to better retrieval of the drug-associated memory during the postconditioning session, leading animals to spend more time in the drug-paired compartment. Additional studies are currently underway to test whether the behavioral responses reported in our study correlate with differences in methamphetamine-induced plasma corticosterone.

Before any treatment was given, we found that C57Bl/6 and 129/SvEv mice of both sexes spent more time in the dark compartment than the light compartment. These results are in line with an earlier report that male C57Bl/6 mice spend most of their time exploring the dark compartment in the light/dark task, in spite of their tendency to be highly active (Bourin & Hascoët, 2003; HascoëT, & Bourin,1998). Similarly, mid-adolescent male 129/SvEv mice have also been reported to spend more time exploring the dark compartment of the light/dark task (Rodgers, Boullier, Chatzimichalaki, Cooper, & Shorten, 2002). Avoidance of the light compartment in the light/dark test has been used as a measure of anxiety. Therefore, an increase in time spent in the light compartment of the CPP box after being associated with methamphetamine would not only suggest that the drug was rewarding but it may have led to an attenuation of the initial anxiety-like response.

We found that regardless of sex, 129/SvEv mice but not C57Bl/6 mice display methamphetamine-induced sensitization, demonstrating a strain difference in the locomotor-inducing effects of this drug. These results are similar to a previous study that tested the effects of repeated administration of cocaine (20 mg/kg) on locomotor activity. They found that both male 129/J and C57Bl6 mice showed an increase in activity over successive trials, but this increase was significantly greater in 129/J mice (Zhang, Mantsch, Schlussman, Ho, & Kreek,

2002), indicating enhanced sensitization in this strain. Behavioral sensitization to methamphetamine has been attributed to an increase in the extracellular levels of dopamine in the nucleus accumbens and caudate-putamen (He & Shippenberg, 2000; Zhang, Mantsch, Schlussman, Ho, & Kreek, 2002), leading to activation of D1 and D2 receptors (Kalivas, & Stewart, 1991). Therefore, it is possible that the 129/SvEv strain is more sensitive to the locomotor enhancing effects of methamphetamine because they respond to repeated administration of this drug with a larger increase in extrasynaptic levels of dopamine, leading to more binding of the D1 and D2 receptors. Another possibility is that repeated exposure to methamphetamine alters D1 and D2 receptor density and/or function in this strain. While such changes could account for the sensitization effect exhibited by both male and female 129/SvEv mice, they do not account for the sex differences in methamphetamine-induced CPP that we report. Specifically, our results showing that methamphetamine induced behavioral sensitization but not CPP in female 129/SvEv mice indicates the existence of nonoverlapping mechanisms mediating these two behaviors.

Given that the two strains tested in our study are commonly used background strains for creating transgenic mice, our findings provide insight into which sex and strain might be most appropriate for future work testing the role of specific proteins in methamphetamine-induced CPP. For example, if the goal of a study is to test the hypothesis that removal of a specific protein blocks methamphetamine-induced conditioned place preference, then the females should be tested if the knockout mouse is on C57Bl/6 background and males should be tested if a 129/SvEv background is used. Alternatively, if the hypothesis is that upregulation of a protein will enhance conditioned place preference, then it might be easiest to detect this enhancement if overexpression occurs in animals that do not normally exhibit methamphetamine CPP (i.e.

females on 129/SvEv background or males on a C57Bl/6 background). Finally, if the goal of a study is to address mechanisms underlying sex differences in the rewarding effects of methamphetamine, the C57Bl/6 strain is appropriate, since we found that the females are more sensitive than the males. Indeed enhanced sensitivity in females mirrors what has been described in humans. It is hoped that our work will provide the basis of future studies using genetic techniques to both identify the neural basis of sex differences in drug abuse and investigate factors that might enhance vulnerability to addiction within a species.

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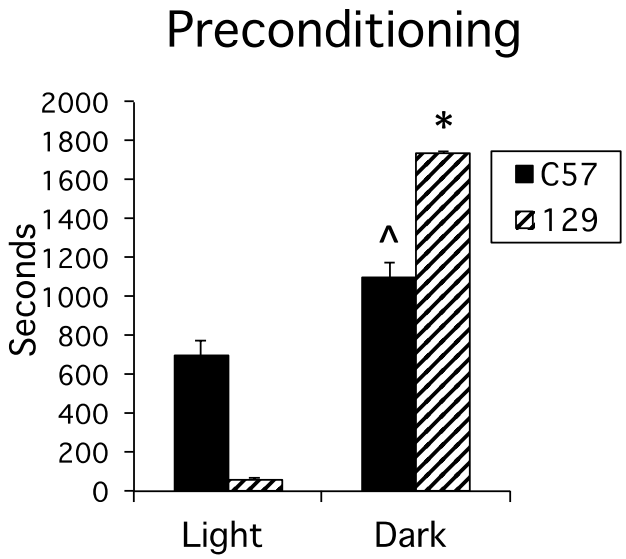

Figure 1. Mean time (seconds) that female C57Bl/6 (n= 40) and 129/SvEv (n= 40) mice spent in the light and dark compartments during preconditioning; ^p < 0.01 versus C57Bl/6 on light side; *p < 0.01 versus 129/SvEv on light side.

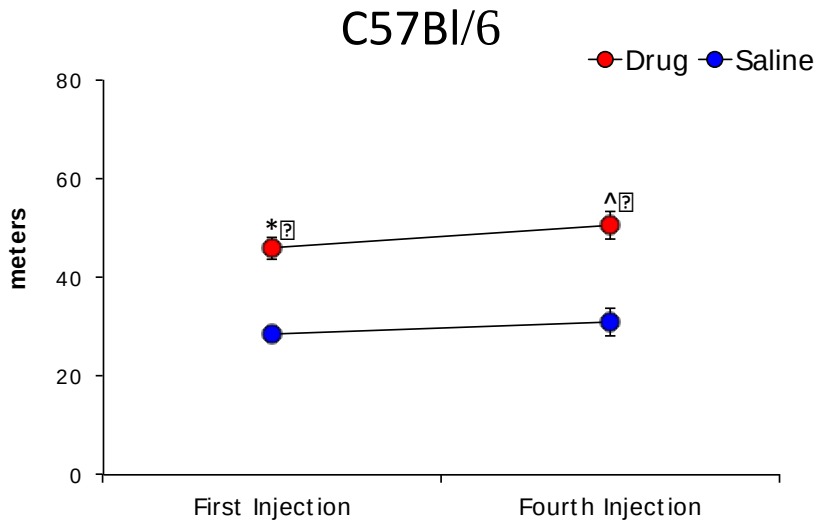

Figure 2. Locomotor activity of methamphetamine-treated (n=22) and saline-treated (n=23) adolescent female C57Bl/6 mice on the days that mice received their first (first injection) and last (fourth injection) exposure to methamphetamine. *p < 0.05 versus saline control after the first injection; ^p < 0.05 versus saline control after the fourth injection.

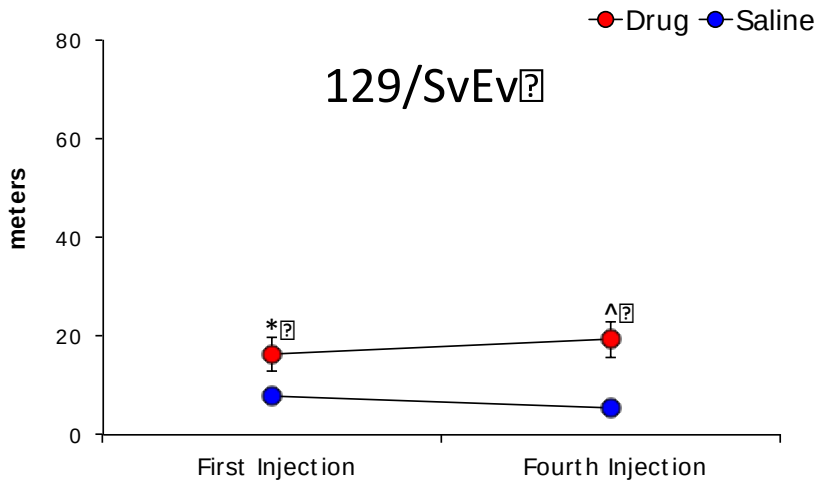

Figure 3. Locomotor activity of methamphetamine-treated (n= 16) and saline-treated (n= 16) adolescent female 129/SvEv mice on the days that mice received their first (first injection) and last (fourth injection) exposure to methamphetamine. *p < 0.05 versus saline control after the first injection; ^p < 0.01 versus saline control after the fourth injection.

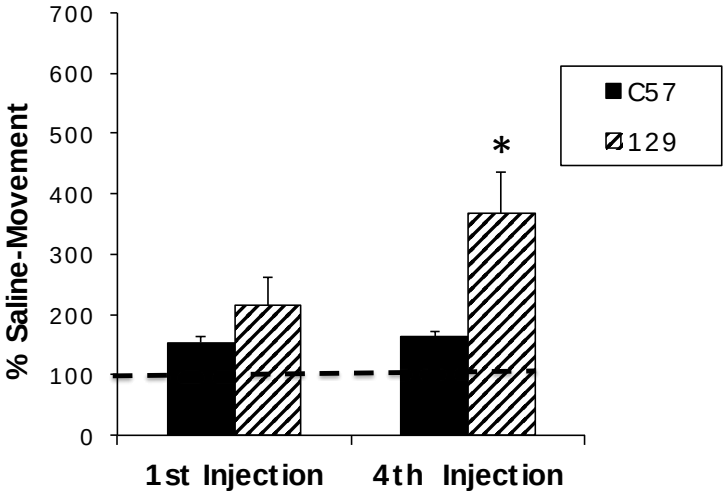

Figure 4. Locomotor activity of female C57Bl/6 (n =23) and 129/SvEv (n =15) mice during the first and fourth injection of methamphetamine. Data are expressed as percent change relative to the corresponding saline-treated control group, which was set to 100% (dashed line). *$p < 0.05$ versus C57Bl/6 during 4th injection of methamphetamine and versus 129/SvEv during 1st injection of methamphetamine.

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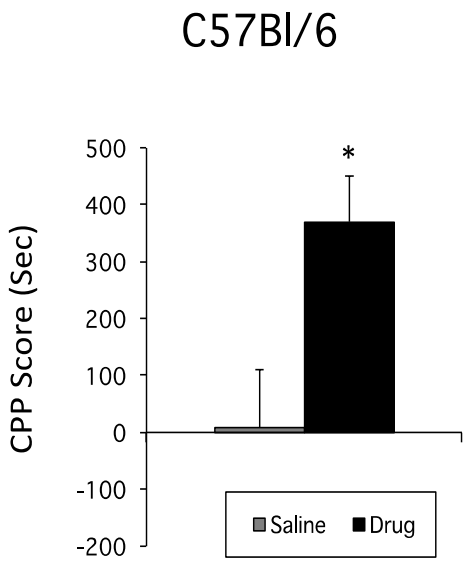

Figure 5. CPP score of adolescent female C57Bl/6 mice following treatment with methamphetamine (1mg/kg) (n=12) or saline (n=19), *$p < 0.05$.

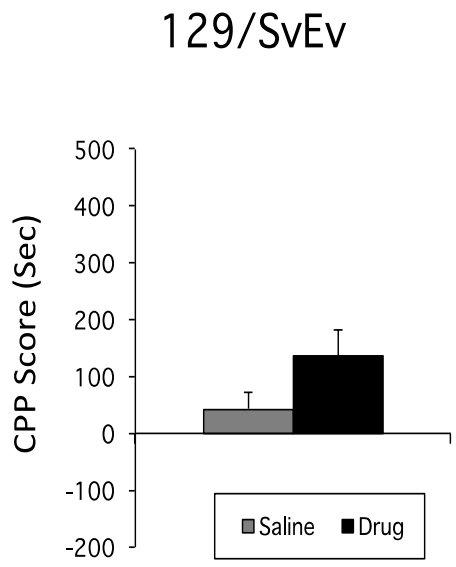

Figure 6. CPP score of adolescent female 129/SvEv mice following treatment with methamphetamine (1mg/kg) (n= 11) or saline (n= 20).

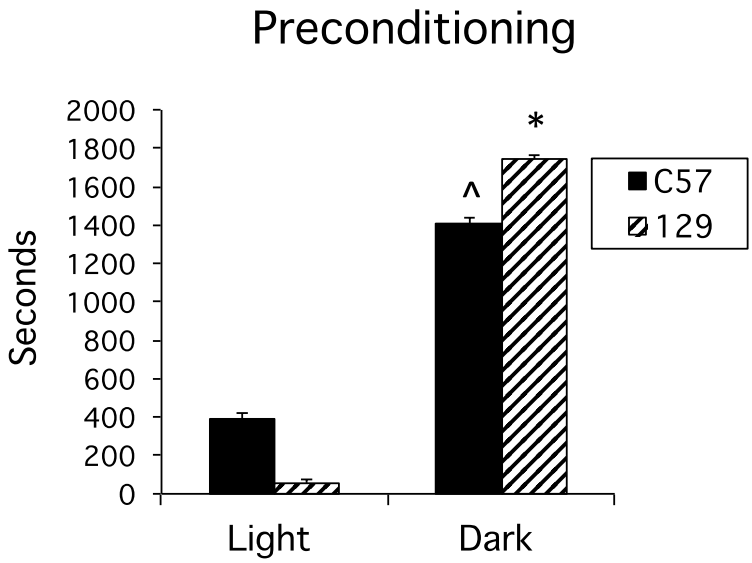

Figure 7. Mean time (seconds) that male C57Bl/6 (n =24) and 129/SvEv (n=23) mice spent in the light and dark compartments, prior to treatment. ^p <0.01 versus C57Bl/6 on light side; *p < 0.05 versus 129/SvEv on light side.

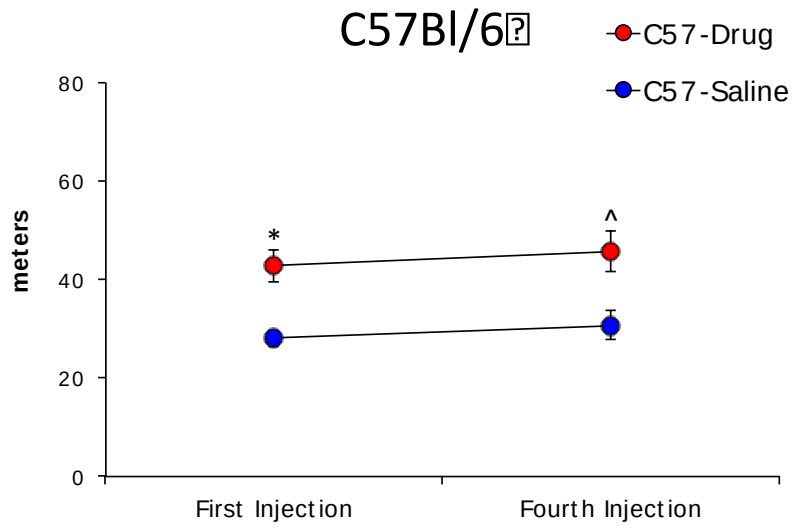

Figure 8. Locomotor activity of methamphetamine-treated (n= 12) and saline-treated (n=12) adolescent male C57Bl/6 mice on days that mice received their first (first injection) and last (fourth injection) exposure to methamphetamine. *$p < 0.05$ versus saline control after the first injection; ^$p < 0.01$ versus saline control after the fourth injection.

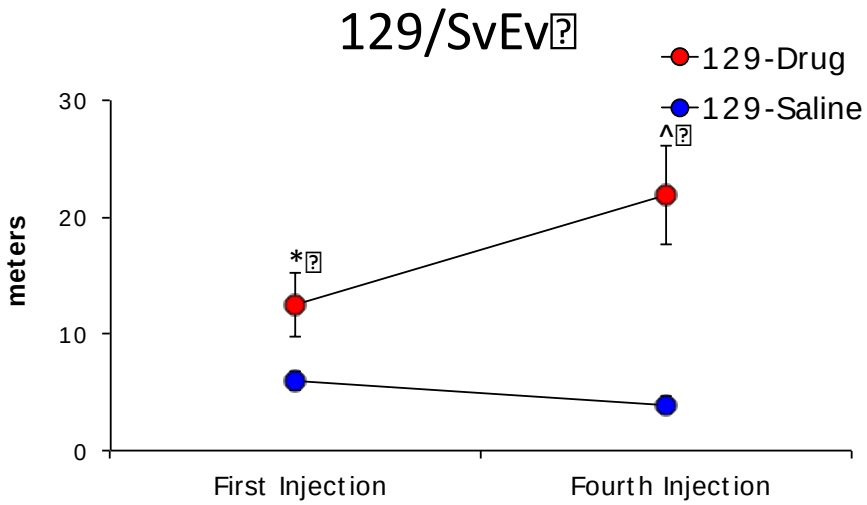

*Figure 9.*Locomotor activity of methamphetamine-treated (n=12) and saline-treated (n= 12) adolescent male 129/SvEv on days that mice received their first (first injection) and last (fourth injection) exposure to methamphetamine. *$p < 0.05$ versus saline control after the first injection;. ^$p < 0.01$ versus saline control after the fourth injection.

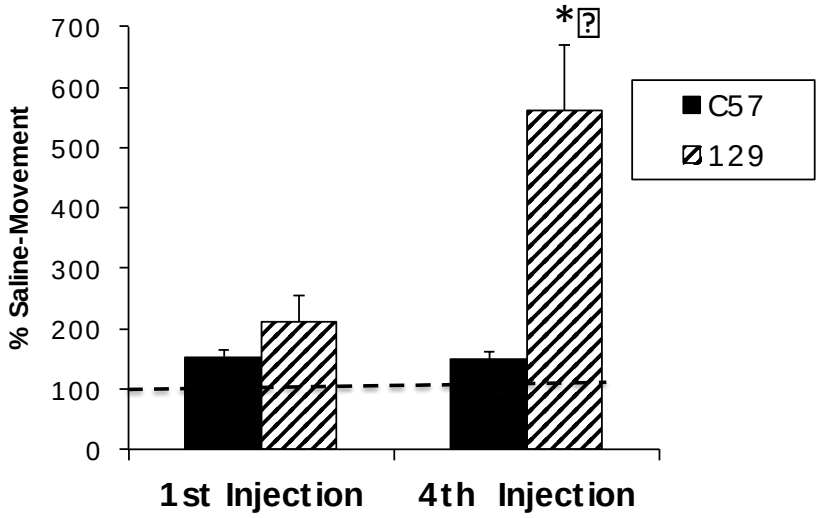

Figure 10. Locomotor activity of drug-treated C57Bl/6 (n =12) and 129/SvEv (n =12) male mice during the first and fourth injection of methamphetamine. Data are expressed as percent change relative to the corresponding saline-treated control group, which was set to 100% (dashed line). *$p < 0.01$ versus C57Bl/6 during the 4th injection of methamphetamine and versus 129/SvEv during the 1st injection of methamphetamine.

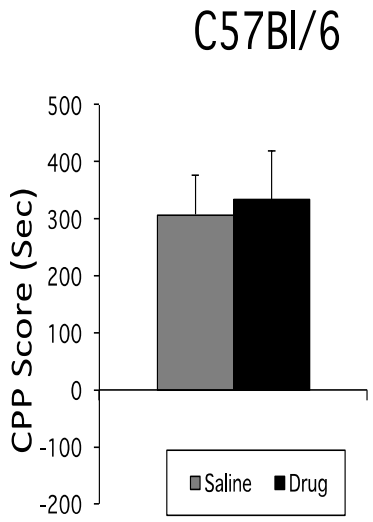

Figure 11. CPP score for adolescent male C57Bl/6 mice following treatment with methamphetamine (1mg/kg) (n= 12) or saline (n= 12).

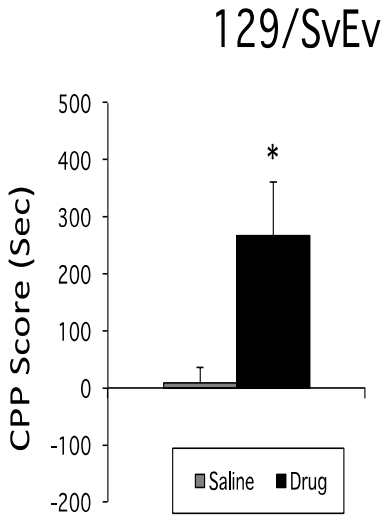

Figure 12. CPP score for adolescent male 129/SvEv mice following treatment with methamphetamine (1mg/kg) (n= 11) or saline (n= 12). *$p < 0.05$