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Low Dose Amphetamine Exposure in Adolescence Cross Sensitizes Adult Mice to Methamphetamine

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Low Dose Amphetamine Exposure in Adolescence Cross Sensitizes Adult Mice to

Methamphetamine

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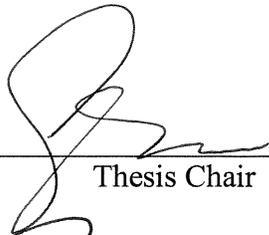Accepted by the Honors Faculty

of the University of North Georgia

in partial fulfillment of the requirements for the Degree of Bachelor of Science
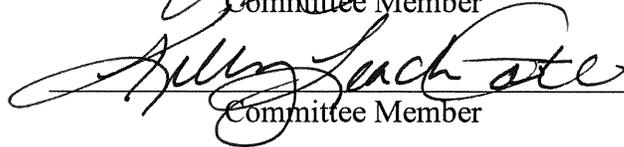
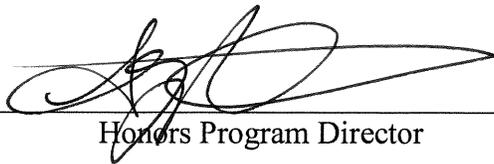Thesis Committee:

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Honors Program Director

Acknowledgments

Abstract

Amphetamine (AMPH) is a central nervous system stimulant and an effective treatment for ADHD among adolescents, in the form of Adderall®. The rise in the number of diagnoses of this disorder, however, comes with an increase in the possibility of wrongful AMPH prescription as well as an increased prevalence of AMPH abuse. Behavioral sensitization is a heightened behavioral response to a drug after repeated exposure, and it is a behavioral expression of neurological changes. These neurological changes are important in current models of addiction, so behavioral cross-sensitization with similarly acting drugs (e.g., methamphetamine (METH)) is useful as a measure of addiction. A previous study in this lab exposed adolescent mice to either a 1.0 mg/kg or a 10 mg/kg dose of AMPH during adolescence and tested for cross-sensitization to METH in adulthood. Considering that 1.0 mg/kg is at the higher end of a therapeutic AMPH dose, the current study examines more clinically relevant doses. In this study, male and female C57B1/6J mice were injected with either 0.01 mg/kg, 0.1 mg/kg, or 1 mg/kg of dextro-AMPH (Sigma-Aldrich, St. Louis, MO) or with saline for ten days during adolescence beginning on postnatal (P) day 22. Dextro-AMPH is one of the forms of AMPH found in Adderall®. On P90, they began testing in an open field chamber (OFC; Kinder Scientific, Poway, CA). Each experiment began with acclimation to the testing environment over 30min (i.e., habituation) followed by either a sub-acute (0.5mg/kg), i.p. challenge dose of METH (Sigma) or saline and a 70min OFC test session. Mice that were exposed to either the 0.1 mg/kg or the 1.0 mg/kg dose of AMPH during adolescence demonstrated increased sensitization to the sub-acute dose of METH in adulthood while the mice exposed to saline had no such response. Male mice also demonstrated increased sensitization when compared to females, but only at the 1.0 mg/kg AMPH dose.

Introduction

One in every five college students and one in every seven non-students of a similar age have reported abuse of prescription stimulants within the past year [1]. Additionally, diagnosis of Attention Deficit Hyperactivity Disorder (ADHD), a neurobehavioral disorder commonly treated using prescription stimulants, has risen in children from 9.5% in 2007 to 11.0% in 2011 and with this increase in diagnoses comes a potential increase in misdiagnoses [2]. Due to the high potential for both intentional and accidental abuse among adolescent populations, conducting research on the long-term effects of such abuse on the developing brain is important.

Adolescence is a period of neuronal maturation where many of the synapses that were formed during childhood are pruned away [3]. Which synapses are pruned is determined by the experience of the adolescent, as those synapses that are used the least are the ones that are lost. This maturation does not occur simultaneously across all areas of the brain, however, as the subcortical regions mature earlier than the prefrontal cortex. This early maturation of the subcortical regions, which contain the systems for emotion regulation and reward, compared to the prefrontal cortex, which is responsible for self-control, likely leads to the emotional imbalance and risk taking behavior common during adolescence [4]. There are also sexually dimorphic factors that play into the development of the brain. The onset of puberty marks a rise in hormones such as the gonadal steroids. These hormones play very different organizational roles in the brain depending on sex and the area of the brain in which the hormones are present. In certain areas of the brain in males, the presence of testicular steroids may inhibit cell death or initiate cell genesis, leading to these areas being larger for males than for females [5-6]. In females, the same thing can be seen in other areas of the brain, with the inhibition of cell death or the initiation of cell genesis caused by the ovarian steroids [5-6]. These differences in hormone

triggered development may be at least partly responsible for the sexually dimorphic behavioral differences typically observed in adolescents.

Some of the regions that undergo development during this time period are in the mesocorticolimbic pathways [7]. These pathways link such areas of the brain as the ventral tegmental area (VTA), the prefrontal cortex (PFC), and the nucleus accumbens (NAc). Hormonal differences, i.e. a greater presence of testosterone in males, have been indicated as a possible cause of the much higher degree of DA receptor expression in males in the PFC and the NAc during puberty [5]. This difference in DA receptor density and distribution between males and females could create sexually dimorphic differences in the reaction to drugs that work on the DA systems. This higher degree of expression in males could interact with any neuroplastic effects of prepubescent drug exposure and result in males becoming more susceptible to the long-term teratogenic effects of drugs that act on these pathways. Both the VTA and the PFC are important regions with regard to models of addiction, as the dopaminergic cell bodies residing in the VTA are thought to be responsible for motivation and behavior reinforcement, and the PFC is believed to play an important role in decision making and self-control [8]. The common mechanism of action of drugs of abuse and plasticity associated with drug exposure is in these areas of the brain and these cognitive factors are all important components of the development and maintenance of addiction. [9]. Therefore, the effects of prepubescent drug exposure may be exacerbated in males by this increase in receptor expression in males and may mean that males are more likely to experience addiction and drug-seeking behaviors than females. [10]. Considering that the NAc is also involved in reward and both the PFC and the NAc are regulators of motor activity, a direct link exists between the neurological basis of addiction and the motor behaviors presented by the addict [11]. Because of this link, any changes in the development of the mesocorticolimbic

pathways may also produce changes in locomotor output. Because there are no baseline differences in locomotor activity between males and females, these behaviors are useful to compare the differences in drug-induced neurological change between the sexes [12]

Amphetamine (AMPH) acts on the dopaminergic system by increasing the dopamine (DA) in the neuronal synapse. The drug causes DA to be dumped from the synaptic vesicles into the cytoplasm while also binding to the dopamine transporter (DAT) [13]. This blocks the DAT's ability to remove DA from the synapse and reverses the direction of DA transport so that the DAT moves DA into the synapse from within cell. AMPH also causes DA to be dumped from the synaptic vesicles into the cytoplasm. This dual mechanism of action causes AMPH to be a particularly potent stimulant. Prescription AMPH, such as Adderall®, is both a common treatment for ADHD and a common drug of abuse [14-15]. Because of the high degree of plasticity of the brain during adolescence and the powerful effects of AMPH on the DA systems, it is possible that abuse of AMPH during adolescent development could lead to lasting changes on the mesocorticolimbic pathways by interfering with synaptic pruning. Such changes could result in a cross-sensitization to other stimulant drugs during adulthood. Cross-sensitization occurs where the abuse of one drug causes an increase in the brain's response to a different drug, so AMPH abuse during adolescence may influence the brain's development to increase its sensitivity to other stimulants. One possible substance that adolescent AMPH abuse may cross-sensitize for is methamphetamine (METH). METH is a widely abused and highly addictive illicit drug with symptoms of dependence similar to symptoms of paranoid schizophrenia [16]. The effects of METH are quicker in onset than the effects of AMPH and typically produce a stronger response in high doses. However, METH is structurally similar to AMPH and acts on the same pathways [17]. These similarities could imply that any neuroplastic changes caused by

adolescent AMPH abuse may have a cross-sensitization effect for METH in adulthood that would increase the risk for adult METH abuse and addiction.

This study seeks to explore the possible relationship between adolescent AMPH abuse and adult cross-sensitization to METH by measuring the behavioral output of the mesocorticolimbic pathways, especially the NAc control of psychomotor responses. This behavioral output is called behavioral sensitization, or an increased motor response to a drug after repeated exposure [18-19]. An increase in behavioral sensitization is a hallmark of addiction, which operationalizes the neuroplastic changes caused by the drug [18-19]. For this study, three separate measures of behavioral sensitization were analyzed; X-Y ambulation, fine motor movement, and rearing. X-Y ambulation was the primary operationalization of behavioral sensitization, while fine motor movement and rearing were also used to operationalize behavioral sensitization, stereotypies, and anxiety. Stereotypies are purposeless, repetitive behaviors and, like behavioral sensitization, are hallmarks of addiction in that they are behavioral markers of drug-induced neurological change [20]. Anxiety is another side-effect of stimulant abuse, so measuring anxiety-related behaviors such as rearing is another effective way of measuring sensitization [18-19].

In a previous study by this lab, it was found that mice subjected to 1.0 mg/kg body weight and 10.0 mg/kg of AMPH during adolescence expressed a significantly increased behavioral response when given a sub-acute dose of METH in adulthood when compared to control animals [21]. Interestingly, male mice exhibited a significantly higher response to the 1.0 mg/kg dose than females, but this sex-effect disappeared at the higher dose. Considering that 1.0 mg/kg is at the higher end of a therapeutic AMPH dose, this study examines more clinically relevant doses. We hypothesized that the mice exposed to AMPH during adolescence would

show behavioral cross-sensitization to a sub-acute dose of METH during adulthood and that, at these smaller doses, males would demonstrate a higher response when compared to females. Such a response would indicate that AMPH abuse during this adolescent developmental window resulted in long lasting, sexually dimorphic neurological change.

Materials and Methods

Animals

Two batches of animals were used for this study. Five adult male and ten adult female C57Bl/6J mice were housed for harem breeding (Jackson Laboratories, Bar Harbor, ME). Due to low pup yield, ten more adult females were ordered and placed in three extra breeding cages. Male (n=19) and female (n=20) pups were born between August and December, 2014 and each was weaned on postnatal day 21 (P21). Animals were housed in Micro-Vent Hepa-filtered caging (Micro-Vent Caging System, Allentown Inc., Allentown, NJ) with access to food and water *ad libitum*, a 12 hour light-dark cycle with lights on at 0700, and constant temperature (20-22 °C) and humidity (55-60%).

Forty-four of the total number of mice were bred, treated, and tested one year prior to this study. These mice were kept under the same conditions as the more recent colony and were tested using the same procedures. Since these studies build upon one another, all data is included here.

Adolescent Pretreatment

To prevent litter effects, mice were randomly assigned to one of four dosing groups, with each group containing at least 6 males and 6 females (Figure 1). Prepubescent adolescent pretreatments were conducted in the animals' home cage. Pretreatments began on P22 with each mouse receiving once daily injections of either 1.0 mg/kg body weight (n=13; female=6;

male=7), 0.1 mg/kg (n=13; female=7; male=6), or 0.01 mg/kg (n=12; female=6; male=6) of dextro-amphetamine (Sigma-Aldrich, St. Louis, MO) for 10 days (P22-P31) between the hours of 0700 and 1000 (Figure 2). The fourth dosing group was injected on the same schedule with an equal volume of sterile saline to act as a control.

Adult Testing

Mice were aged until P90 without drug exposure (Figure 1). On P90, all mice were subjected to cross-sensitization testing in an Open Field Chamber (OFC, Kinder Scientific, Poway, CA). The OFC is a 41.5cm x 41.5cm x 38.0cm chamber that uses a 16 x 16 grid of photobeams to track movement. The system used was a smart frame open field system with rearing option and motor monitor host software. The OFC was housed and operated in a dark, quiet room separate from the animal housing facility. Each chamber was cleaned with 70% ethanol, soft soap, and water between trials.

Mice were tested for a total of 100 minutes. For the first 30 minutes of training, the mice were placed in the chamber prior to injections to allow for habituation to the new environment (Figure 2). After habituation, the mice were removed from the chamber and randomly assigned to one of two groups that received either an intraperitoneal sub-acute challenge dose (0.5 mg/kg) of methamphetamine (Sigma-Aldrich, St. Louis, MO) or and equal volume of sterile saline (0.9% NaCl) (Figure 2). A sub-acute dose is one that our lab has experimentally demonstrated to be small enough not to produce any effect in naïve animals, but to which sensitized animals exhibit a reaction. The mice were then placed back into the OFC for a 70 minute trial. The first 10 minutes of this trial was considered a rehabituation period while waiting for drug action (Figure 2). Activity measurements were collected in 10 minute intervals for the remainder of the test session. Measurements from 10 minutes to 40 minutes were binned and analyzed using IBM

SPSS (v21.0). Our lab has supported this time period to be the window of peak drug-related activity. Factorial ANOVAs were conducted to determine the effects of sex, treatment, and the cross-effects of sex and treatment. Post-hoc analysis using Fisher's LSD was conducted when appropriate.

F1
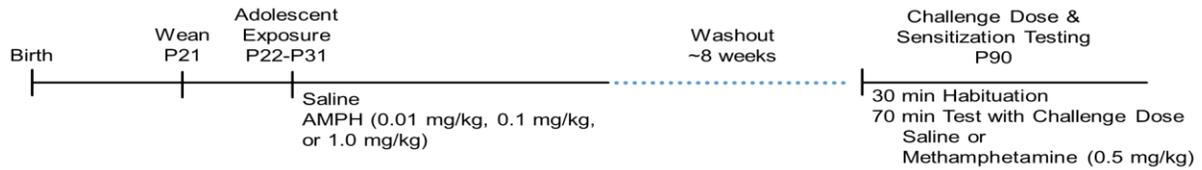
Fig 1 Experimental Timeline. Mice were weaned on the 21st day after birth (p21) and injection began on P22. Mice received either a low (0.01 mg/kg), medium (0.1 mg/kg), or high (1.0 mg/kg) dose of AMPH for 10 days, or an equivalent volume of saline for control. Mice were allowed to age for 8 weeks and were then tested in the OFC on P90. All mice were allowed 30 min of habituation before testing then were given either a sub-acute dose of METH or saline. Mice were placed back into the OFC for 70 min, with the first 10 min acting as rehabituation.

F2
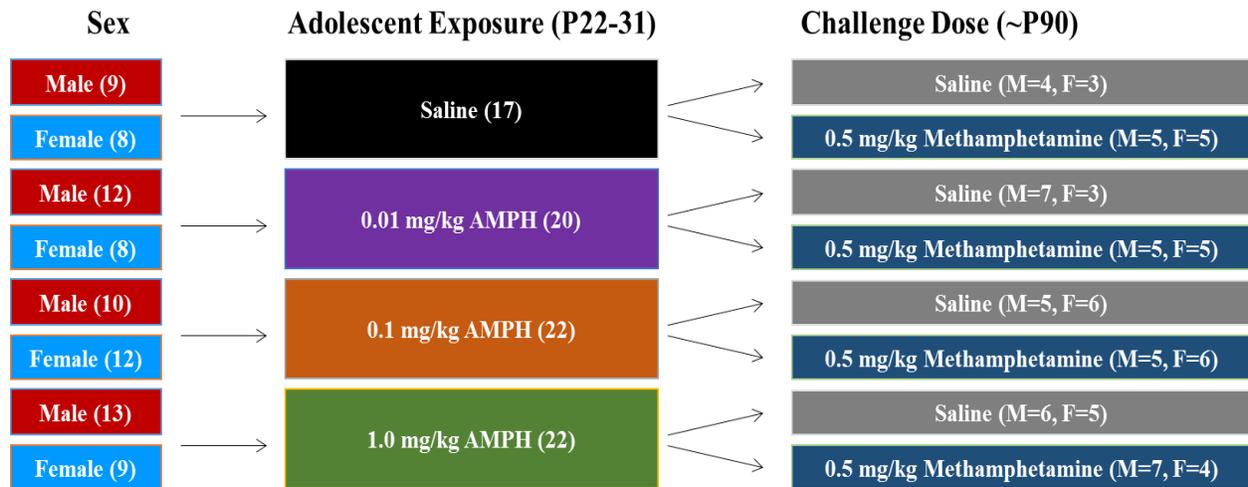
Fig 2 Experimental Dosing Paradigm. There were a total of 81 mice with 44 males and 37 females. Males and females were assigned to one of four adolescent pretreatment groups: saline, 0.01 mg/kg AMPH, 0.1 mg/kg AMPH, and 1.0 mg/kg AMPH. The males and females from each group were then assigned to one of two testing groups: saline or 0.5 mg/kg METH.

Results

The data were analyzed using an 8 by 2 ANOVA for fine motor movement, rearing, and x-y ambulation ((Saline treatment and saline challenge dose (SAL/SAL), saline treatment and METH challenge dose (SAL/METH), 0.01 mg/kg AMPH treatment and saline challenge dose (0.01 AMPH/SAL), 0.01 mg/kg AMPH treatment and METH challenge dose (0.01 AMPH/METH), 0.1 mg/kg AMPH treatment and saline challenge dose (0.1 AMPH/SAL), 0.1 mg/kg AMPH treatment and METH challenge dose (0.1 AMPH/METH), 1.0 mg/kg AMPH treatment and saline challenge dose (1.0 AMPH/SAL), 1.0 mg/kg AMPH treatment and METH challenge dose (1.0 AMPH/METH)) X (female, male)). Outliers were determined by examining habituation data for any mouse that exhibited a number of x-y ambulations that fell outside of two standard deviations of the mean (Figure 3). Only two outliers were removed from the analysis (female SAL/METH, female 0.1 AMPH/METH).

F3
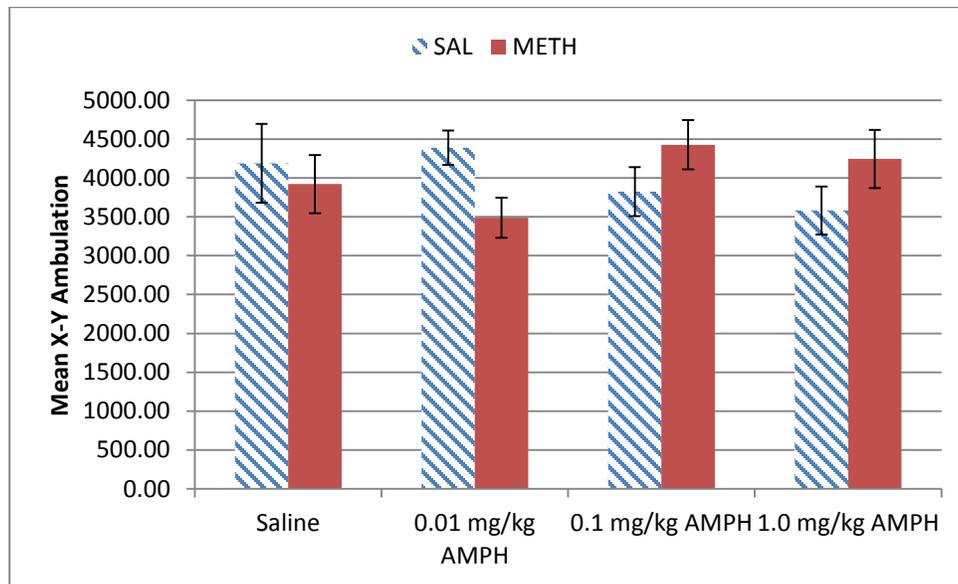
Fig 3 The mean X-Y ambulation from the habituation of all groups. After two outliers were removed, there was no observable difference between the mean number of x-y ambulations in any of the groups.

There was a main effect of treatment on fine motor movement, $F(7, 65)=3.652$, $p=.002$, $\eta_p^2 =0.282$. Post hoc analysis revealed that the 1.0 AMPH/METH (M=1256.92, SEM=99.123)

treatment group demonstrated significantly increased activity compared to all groups except for

0.1 AMPH/METH (p=.171) and SAL/METH (p=.918) (1.0 AMPH/SAL, 0.1 AMPH/SAL, 0.01

AMPH/METH, 0.01 AMPH/SAL and SAL/SAL; p=.002, p=.000, p=.010, p=.004, and p=.006,

respectively). The 0.1 AMPH/METH (M=1094.73, SEM=92.917) group also exhibited a

significant increase in activity compared to the 0.1 AMPH/SAL group (p=.025) and a marginal

increase in activity compared to the 1.0 AMPH/SAL group (p=.072). The SAL/METH

(M=1269.30, SEM=80.786) group did demonstrate a significant difference to all groups (1.0

AMPH/SAL, 0.1 AMPH/SAL, 0.01 AMPH/METH, 0.01 AMPH/SAL, SAL/SAL (p=.002,

p=.000, p=.009, p=.004, and p=.006, respectively) except for 1.0 AMPH/METH (p=.918) and

0.1 AMPH/METH (p=.150). There was no significant main effect of sex on fine motor

movement $F(1,65)$=0.226, p=.636, η_p^2=0.003, nor a treatment by sex effect $F(7,65)$=1.734,

p=.117, η_p^2=0.157 (Figure 4A-4D).

F4A
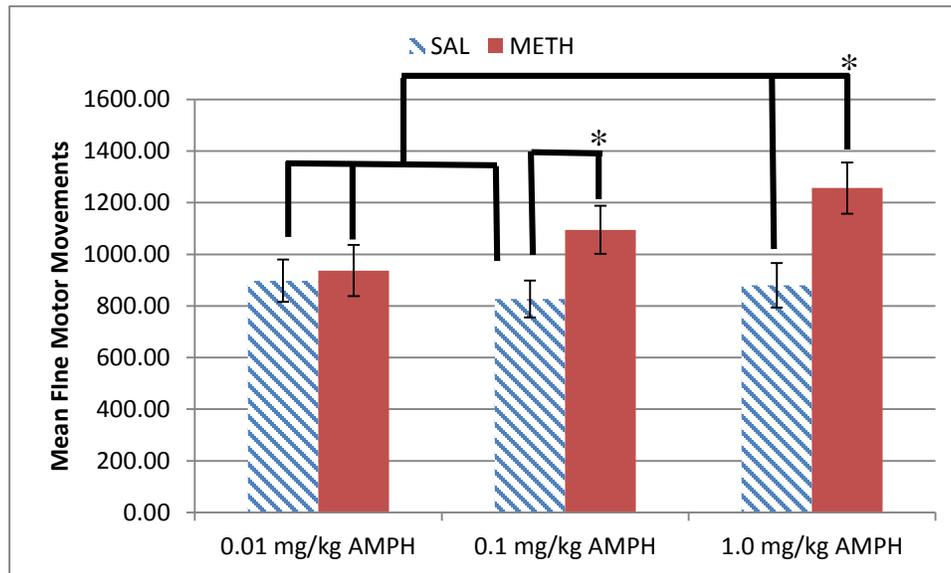
Fig 4A The mean number of fine motor movements each adolescent pretreatment group committed during OFC testing. Fine motor movements are used here as a marker for behavioral sensitization and stereotypic behavior. The 0.01 mg/kg AMPH saline and METH challenged groups, the 0.1 mg/kg AMPH saline challenged group and the 1.0 mg/kg AMPH saline challenged group all demonstrated a similar number of fine motor movements. A trend begins to develop at the 0.1 mg/kg AMPH METH challenged group, showing an increase in fine motor movement that continues into the 1.0 mg/kg AMPH METH challenged group.
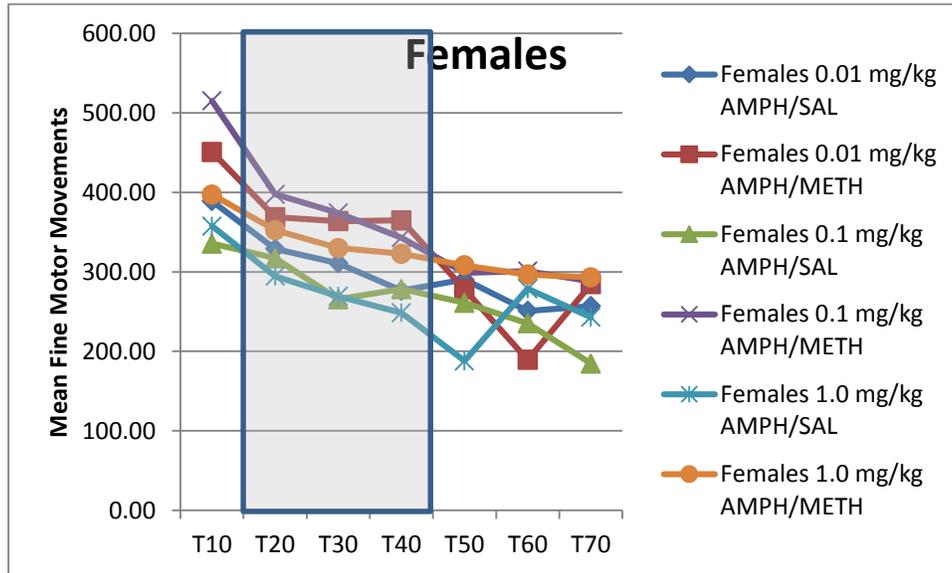
F4B
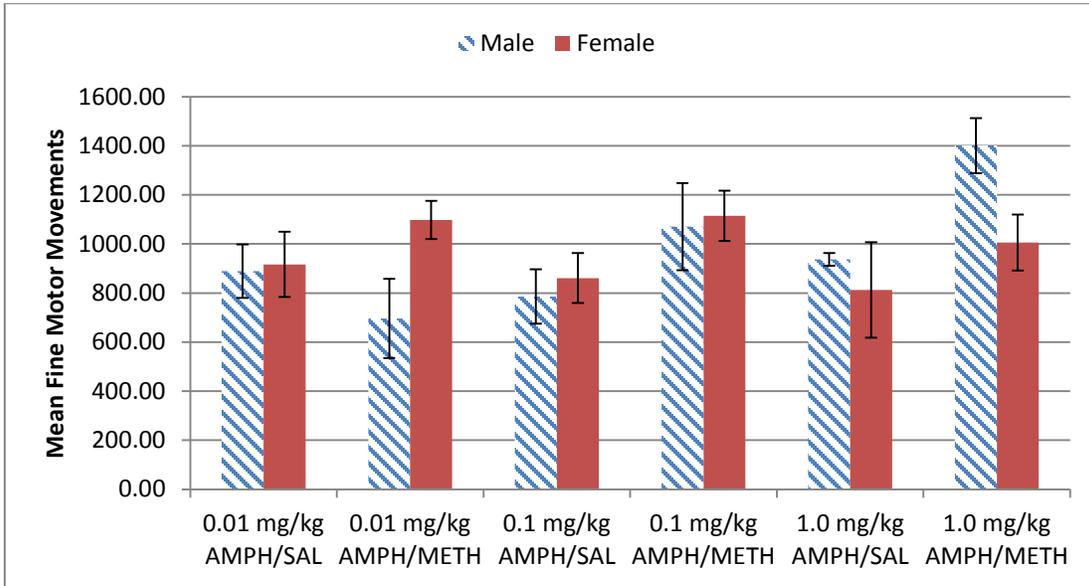
Fig 4B The difference in the means of fine motor movement between the male and female mice in each adolescent pretreatment group. There was no main effect of sex or any sex by treatment effect.
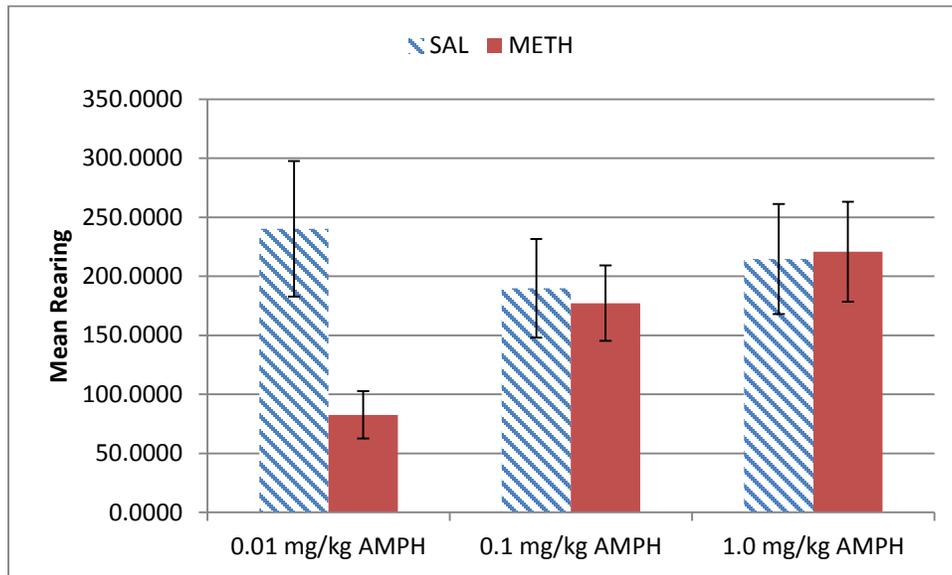
F4C
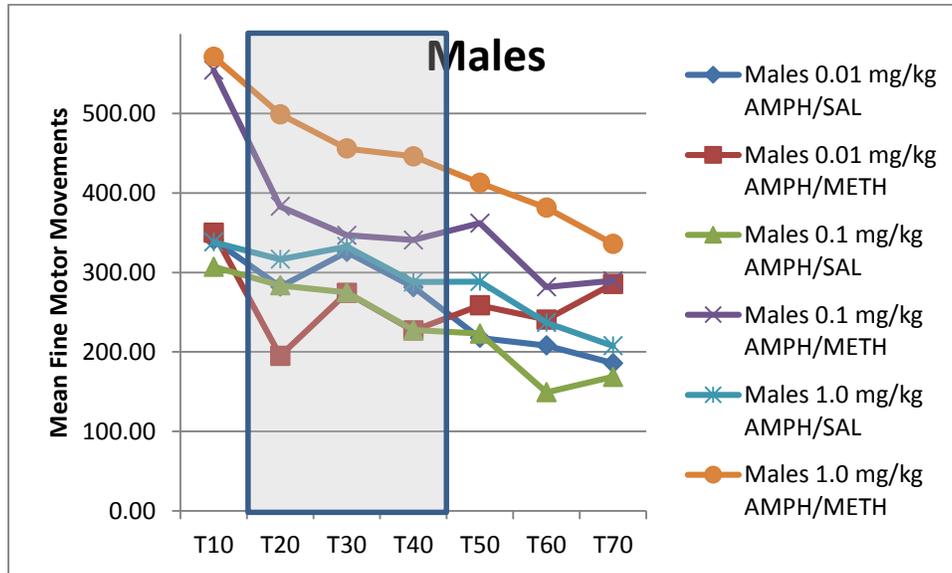
F4C

Fig 4C and 4D The change in fine motor movement during testing for each adolescent pretreatment group over time. Figure 4C shows this change in males and Figure 4D shows this change in females. The peak of drug related activity occurs between the 10 (T10) and 40 (T40) min mark and is highlighted.

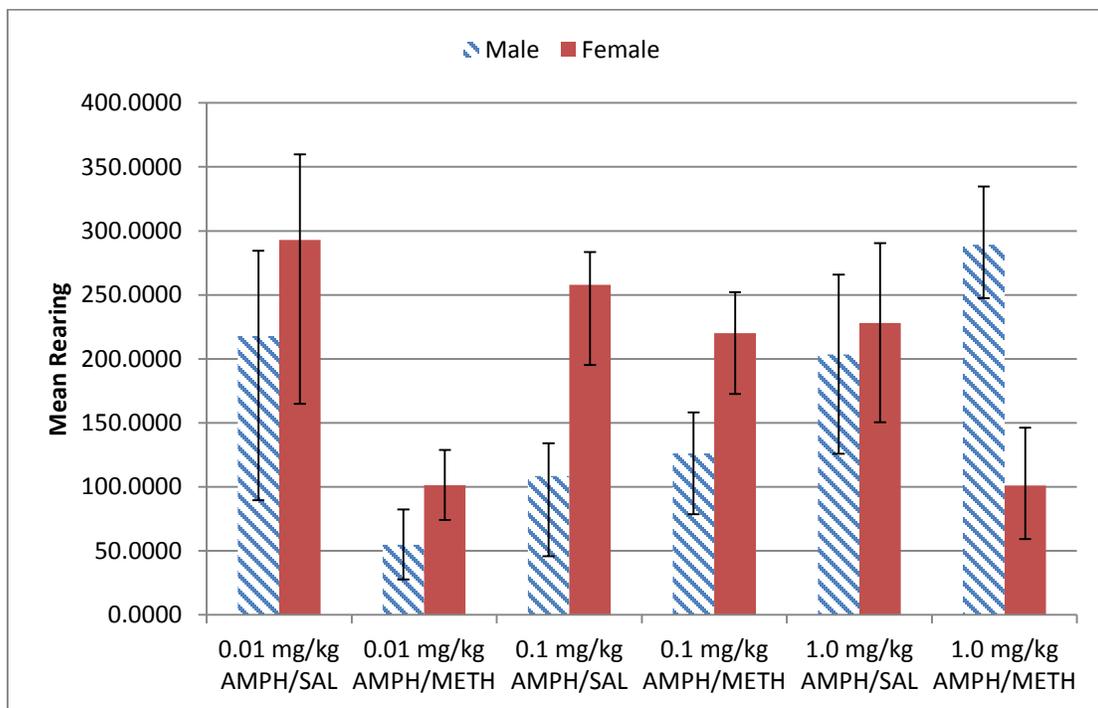There was no main effect of treatment on rearing $F(7,65)=1.481$, $p=.190$, $\eta_p^2=0.138$.

There was also no main effect of sex on rearing $F(1,65)=0.253$, $p=.617$, $\eta_p^2=0.004$ or any

treatment by sex interaction $F(7,65)=1.642$, $p=.140$, $\eta_p^2= 0.150$ (Figure 5A-5C).

F5A

Fig 5A The mean amount of rearing each adolescent pretreatment group committed during OFC testing. Rearing is used here as a marker for behavioral sensitization and anxiety related behavior. There was no main effect of treatment on rearing.

F5B

Fig 5B The difference in the mean amount of rearing between the male and female mice in each adolescent pretreatment group. There was no main effect of sex or any sex by treatment effect.

F5C
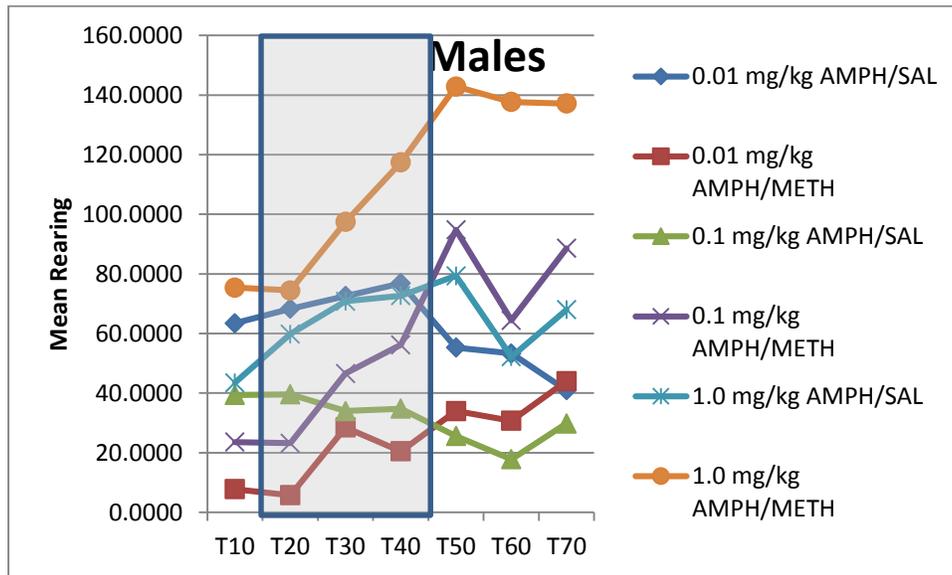
F5D
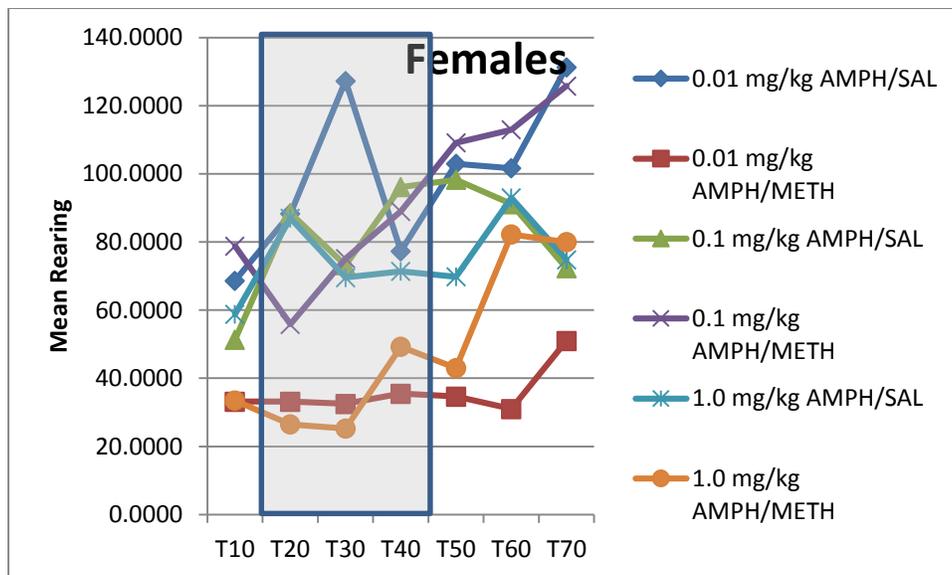
Fig 5C and 5D The change in rearing behavior during testing for each adolescent pretreatment group. Figure 5C shows this change in males and Figure 5D shows this change in females. The peak of drug related activity occurs between the 10 (T10) and 40 (T40) min mark and is highlighted.

A main effect of treatment on x-y ambulation was found $F(7,65)=2.225$, $p=.043$,

$\eta_p^2=0.193$. The 1.0 AMPH/METH (M=3050.09, SEM=503.101) mice demonstrated significantly

(p=.608) (1.0 AMPH/SAL, 0.1 AMPH/SAL, 0.01 AMPH/METH, 0.01 AMPH/SAL, and

SAL/SAL; (p=.012, p=.006, p=.004, p=.018, and p=.018, respectively). The 0.1 AMPH/METH

(M=2708.55, SEM=442.228) group expressed significantly more activity than the 0.1

AMPH/SAL (p=.039) and the 0.01 AMPH/METH (p=.028) groups while exhibiting a marginally

significant increase in activity compared to the 1.0 AMPH/SAL (p=.066), the 0.01 AMPH/SAL

(p=.090), and the SAL/SAL (p=.077) groups. The SAL/METH group (M=2801.60,

SEM=295.673) demonstrated a significant increase in activity as well when compared to the 1.0

AMPH/SAL (p=.047), the 0.1 AMPH/SAL (p=.028), and the 0.01 AMPH/METH (p=.020)

groups as well as a marginally significant increase in activity when compared to 0.01

AMPH/SAL (p=.066) and SAL/SAL (p=.057). No significant main effect of sex on x-y

ambulation was found $F(1,65)=0.269$, p=.606, $\eta_p^2=0.004$. The treatment by sex interaction effect

on x-y ambulation was only marginally significant $F(7,65)=1.968$, p=.073, $\eta_p^2=0.175$ (Figure

6A-6D).

F6A
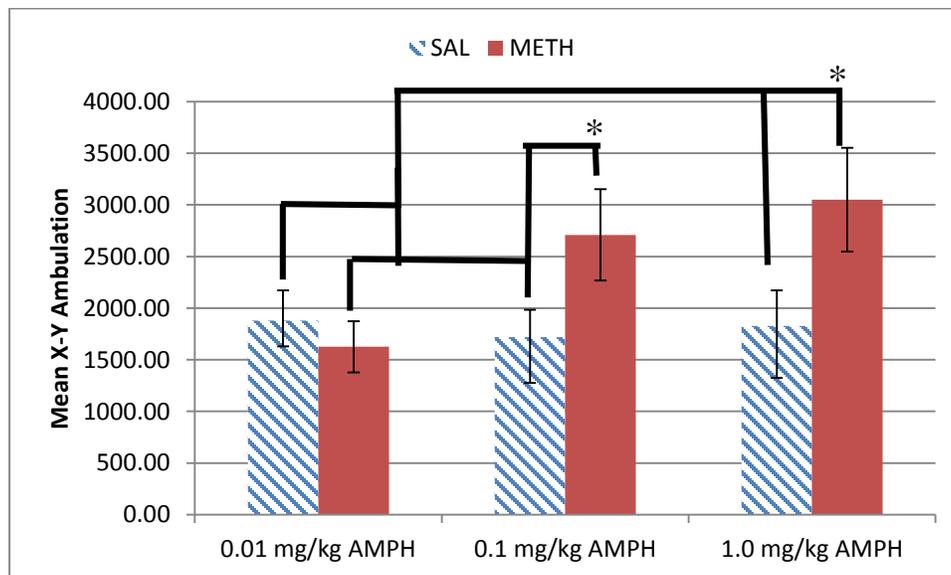
Fig 6A The mean number of X-Y ambulations each adolescent pretreatment group committed during OFC testing. X-Y ambulations are used here as a marker for behavioral sensitization. The 0.01 mg/kg AMPH saline and METH challenged groups, the 0.1 mg/kg AMPH saline

challenged group and the 1.0 mg/kg AMPH saline challenged group all demonstrated a similar number of X-Y ambulations. A trend develops at the 0.1 mg/kg AMPH METH challenged group, showing an increase in X-Y ambulations that continues into the 1.0 mg/kg AMPH METH challenged group.
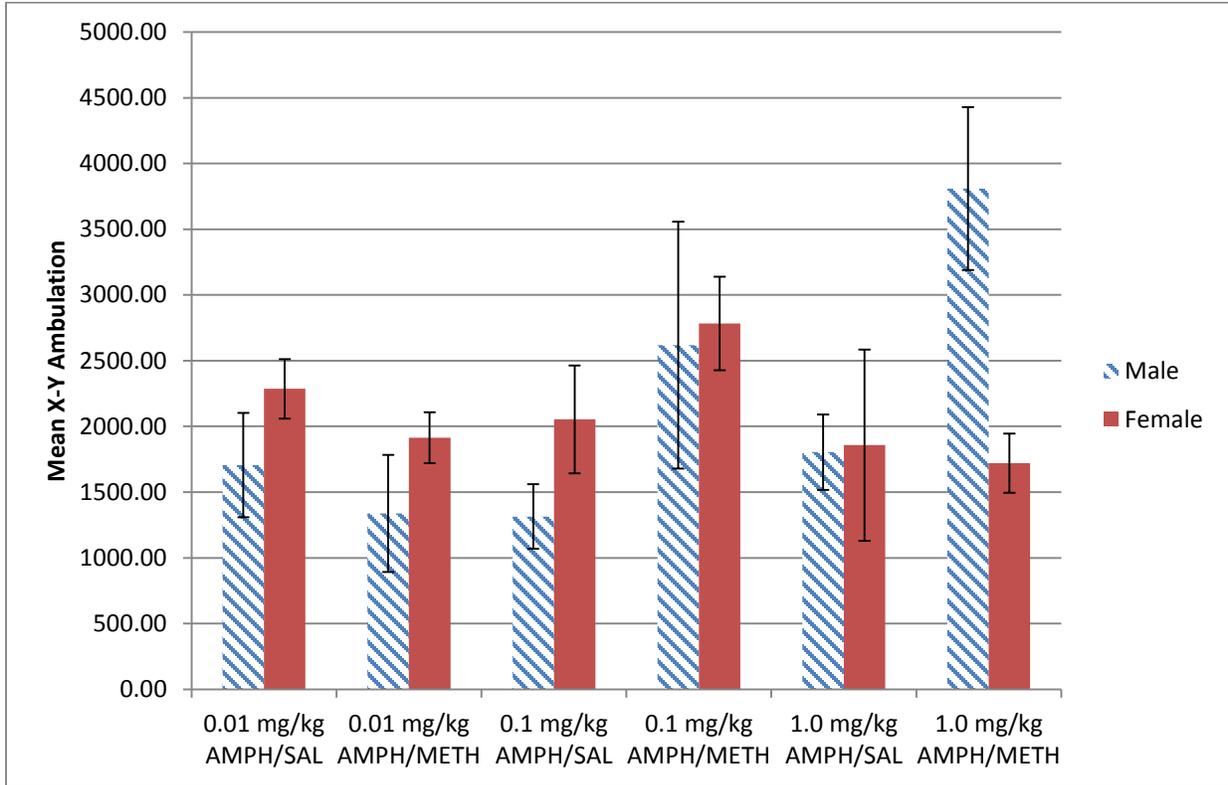
F6B

Fig 6B The difference in the means of X-Y ambulations between the male and female mice in each adolescent pretreatment group. The 0.01 mg/kg AMPH saline and METH challenged groups, the 0.1 mg/kg AMPH saline challenged group, and the 1.0 mg/kg AMPH saline challenged group all show a similar mean. The 0.1 mg/kg AMPH METH challenged group does show an increase in both male and female animals, however, the 1.0 AMPH METH challenge group only shows an increase in X-Y ambulations for males.

F6C
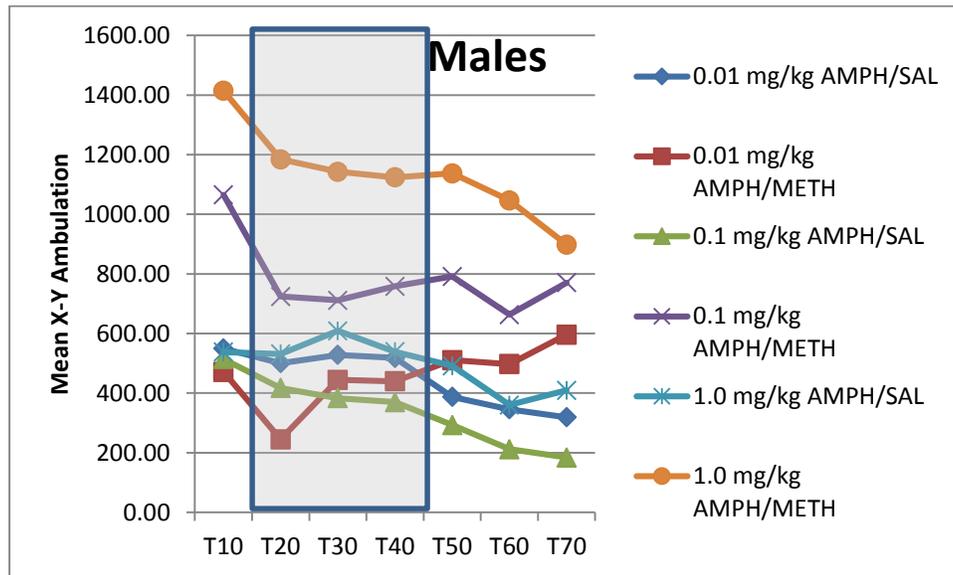
F6D
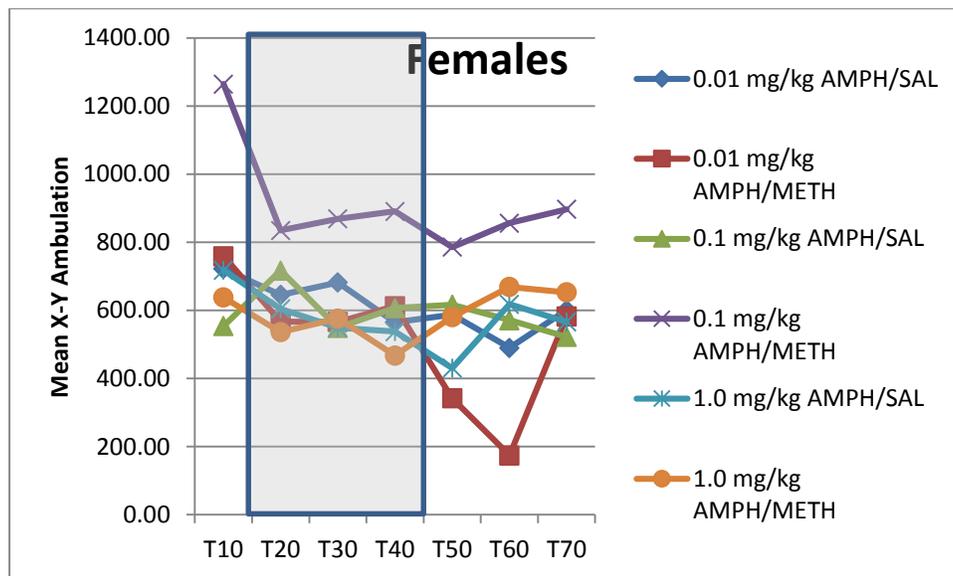
Fig6C and 6D The change in X-Y ambulations during testing for each adolescent pretreatment group. Figure 6 (left) shows this change in males and Figure 7 (right) shows this change in females. The peak of drug related activity occurs between the 10 (T10) and 40 (T40) min mark and is highlighted.

Discussion

While the SAL/SAL and SAL/METH control groups were included in data analysis, they

were not included in any of the graphed representations of our data. During analysis, the

SAL/METH group exhibited significantly increased sensitization compared to all other groups

except for 0.1 AMPH/METH and 1.0 AMPH/METH (Figure 7A). Previous experiments in this

lab using the 0.5 mg/kg METH challenge dose have consistently demonstrated that this dose acts

at a sub-acute level (Figure 7B) [17]. We believe the problem with these groups in the present

study may be procedural. It is possible that some of the mice in the SAL/METH group were

handled and injected incorrectly or with the wrong dose of METH. Our lab is currently looking

to rerun this control with a new group of mice in order to assess this problem; however, the

current data still reports interesting and significant trends that deserve discussion. The rest of this

section will discuss only the data that was represented in the graphs.

F7A
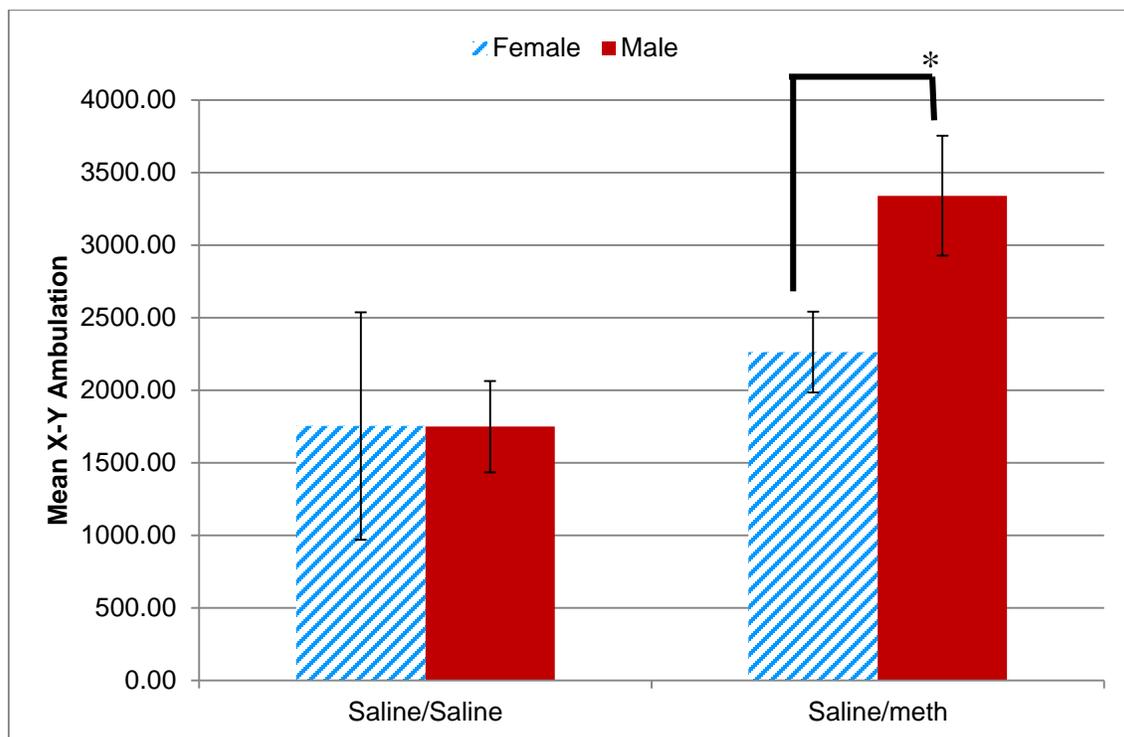
Fig 7A The mean of X-Y ambulations for the control groups in this study. There is no significant difference between the female and male saline challenged groups and the female METH challenged group. Contrary to the previous findings of this lab, a significant difference was found between male METH challenged group and all other groups.

F7B
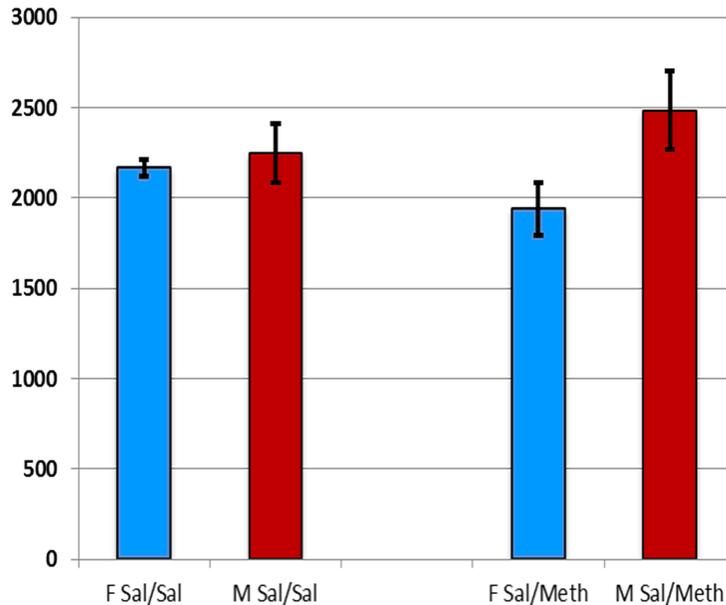
Fig 7B The results from the control groups in previous studies in this lab. There was no observable difference between the mean number of X-Y ambulations in the saline challenged mice compared to the METH challenged mice for the adolescent group treated with saline.

The results of the fine motor movement data demonstrate a general upwards trend in drug related activity as the adolescent pretreatment dose increases (Figure 4A). At the lowest dose (0.01 mg/kg AMPH), there is no difference between the METH challenged group and the control. This indicates that this low level of adolescent exposure may not be enough to cause long lasting changes in the brain. A reaction to the challenge dose begins to appear at 0.1 mg/kg AMPH, but the most significance was found at the 1.0 mg/kg AMPH dose. This reaction, however, only seemed to exist within the male population (Figure 4B). This is interesting as, though there was an increase in fine motor movements at the 0.1 mg/kg dose, the increase was expressed in both the males and the females. A sexually dimorphic reaction, therefore, was only exhibited at the 1.0 mg/kg dose.

The X-Y ambulation data demonstrated a similar trend as fine motor movement (Figure 6A). Both the 0.1 AMPH/METH and the 1.0 AMPH/METH groups expressed a significant increase in sensitization compared to all controls and the 0.01 AMPH/METH. There was no

difference between the 0.01 AMPH/SAL and the 0.01 AMPH/METH groups, indicating that this dose level may be too small to elicit a reaction to the sub-acute METH challenge. Once again, a sexually dimorphic reaction was not seen at 0.1 mg/kg AMPH level, but was present at the 1.0 mg/kg AMPH dose level (Figure 6B). At this level, the effect appears to lie in the males, as the females expressed no significant difference to any of the controls.

Adolescence is a time of high neural plasticity before the brain develops to become a mature brain of adulthood. Many of the characteristics typical of adolescents, such as high risk-taking and poor self-control, are due to the relatively early development of subcortical regions compared to the PFC [22]. These characteristics increase the likelihood of drug abuse, and many of these drugs act on the pathways in the brain that are undergoing development. Psychostimulants are one such drug, and they act on the mesocorticolimbic pathway, which connects many of the structures in subcortical regions as well as the PFC. These structures include the VTA, which is responsible for reward, and the NAc, which, along with the PFC, plays a role in movement [8]. Interfering with the functioning of these areas during their development could influence how they stabilize in the brain. One possible outcome of this interference is that adolescent stimulant exposure may cause a cross-sensitization to other stimulants. This cross-sensitization indicates that AMPH is causing a permanent change in the developing brain, and this may prime the adolescent to use, abuse, and become addicted to other drugs, such as METH, in adulthood. The NAc is involved in motor movement, so this cross-sensitization could result in an increased behavioral response called behavioral sensitization [18-19].

Our data demonstrates that AMPH abuse during adolescence may lead to behavioral sensitization to METH in adulthood, when AMPH exposure is above a certain dose. The 0.01

mg/kg dose did not produce any behavioral effects, but both the 0.1 mg/kg and the 1.0 mg/kg doses exhibited an increased locomotor response in both fine motor movement and X-Y ambulation. Previous studies in our lab demonstrated that at incredibly high doses of AMPH (10 mg/kg), X-Y ambulation decreases while fine motor movement dramatically increases. This trend may mean that the mice being tested are experiencing psychosis, in which their large movements are replaced by smaller stereotypies [20]. Stereotypies are purposeless, repetitive movements and are markers of addiction in that they are locomotor expressions of neurological change [20]. Rearing behavior can also be a stereotypy as well as a behavior produced by anxiety [23]. The fact that the fine motor movement and X-Y ambulation data mirrored one another and the fact there were no significant effects or discernable trends on rearing indicates that we were measuring behavioral sensitization, not stereotypies. Therefore, we may not have been measuring psychosis, but instead were measuring what we set out to measure, behavioral cross-sensitization.

By using clinically relevant doses of AMPH in adolescence, our data demonstrated that exposure to prescription levels of AMPH higher that 0.01 mg/kg lead to a cross-sensitization to METH in adulthood. Hormonal differences between males and females influence adolescent development during puberty, and we found that prepubescent exposure to certain doses of AMPH may cause neurological changes that effect these developments. Our lab has previously shown that males in the 1.0 AMPH/METH group show a greater increase in behavioral sensitization than females, but that this differences disappears at the higher dose of 10 mg/kg [21]. Our study's results mirror this finding for the 1.0 AMPH/METH group and also show that this effect disappears at lower doses (0.1 and 0.01 mg/kg AMPH).

More research needs to be done to determine what occurs with the 1.0 mg/kg dose for males that does not occur with females or at other doses. Future studies could be conducted to determine in what specific regions of the mesocorticolimbic pathway these long-term, sexually dimorphic changes occur. Studies should also look to see what molecular mechanisms are responsible for these changes. Of particular interest is the expression of D1 and D2 receptors. DA receptor expression undergoes considerable change during adolescence and the ratio of excitatory D1 receptors to inhibitory D2 receptors could play an important role in the development of sensitization [24]. The activation of DA receptors can trigger genetic changes that lead to differences in DA receptor expression, so the mechanisms of these changes could be an area of future research [4]. It would also be beneficial to look at other markers of addiction in order to see if this sexually dimorphic reaction only occurs with sensitization. A focus of behavioral research should be on determining the developmental window(s) in which AMPH exposure has the greatest effect. These windows are caused by hormonal changes throughout adolescence and are different between males and females, with females typically maturing earlier [4]. This means that exposure to AMPH during different time periods in adolescence could result in greater or weaker adult cross-sensitization for males when exposed to AMPH in certain developmental windows and females when exposed in others. Work should also be done to find the limits of the length of drug exposure that may produce a reaction and how this may interact with these developmental windows, the dose of the drug, and sex. Lastly, more work needs to be done with the current study in order to increase the n-value of all groups and rectify the issues with the SAL/METH control. This study demonstrated that adolescent AMPH exposure at certain doses leads to adult cross-sensitization to METH, especially in males. Adolescent abuse

of stimulants such as AMPH may therefore lead to long-lasting neurological changes that prime

adults for METH abuse and addiction.

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