The Acute Effects of Multiple Doses of Methamphetamine on Locomotor Activity and Anxiety-Like Behavior in Adolescent and Adult Mice

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The acute effects of multiple doses of methamphetamine on locomotor activity and anxiety-like behavior in adolescent and adult mice

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ABSTRACT

Methamphetamine (MA) is a highly addictive psychomotor stimulant drug. Research has shown that the acute effects of MA can be modulated by age, although previous findings from our lab do not find age differences in the effects of MA. Relatively little research has examined the effects of adolescent MA exposure; thus, it is important to understand how MA affects adolescent behavior and brain function compared to adults. In order to better understand the age differences in the effects of acute MA exposure, this research examined the effects of MA exposure on locomotor and anxiety-like behavior and plasma corticosterone levels in adolescent and adult C57BL/6 J mice. Mice were exposed to saline, 2 mg/kg MA, or 4 mg/kg MA and behavior was measured in the open field test. Immediately following behavioral testing, serum was collected, and plasma corticosterone levels were measured. MA-exposed mice showed increased locomotor activity and anxiety-like behavior compared to saline controls, regardless of age and dose of MA. However, adolescent mice showed the greatest locomotor response to the high dose of MA (4 mg/kg), whereas the adult mice showed the greatest locomotor response to the low dose of MA (2 mg/kg). There were no differences in stereotyped behavior between the adolescent and adult mice exposed to the low dose of MA (2 mg/kg) and the high dose of MA (4 mg/kg). There was no effect of MA exposure on plasma corticosterone levels. These data suggest age modulates the locomotor response to MA and further research is warranted to determine the developmental neurobiological mechanism underlying the dose-response age differences in the response to acute MA exposure.

1. Introduction

Methamphetamine (MA) is a highly addictive central nervous system psychomotor stimulant with an estimated 1.6 million users annually [1, 2]. Much research has focused on the effects of MA in adults, while relatively little research has examined the effects of MA in adolescents. MA use among adolescents increased in the early 2000s, but has since remained relatively stable [3,4]. However, the effects of MA differ between adolescents and adults, and adolescents show greater reward sensitivity to MA compared to adults [5]. Additionally, adolescent MA users show high rates of depression [6] and other psychiatric symptoms [7]. As the adolescent brain is still developing, it is important to better understand how MA affects adolescent behavior and brain function and how these effects may differ from those seen in adults.

It is well known that MA acutely increases locomotor activity and long-term use of MA can lead to movement disorders (for a review, see [8]). Research in rodents suggests there are age differences in the effects of MA on locomotor activity. For example, MA increases locomotor activity to a lesser degree in adolescent rodents compared to adult rodents following acute exposure [9] and chronic exposure [10], despite similar MA brain concentrations [11]. However, studies examining the acute response to amphetamine show conflicting findings, with some studies reporting adolescent mice have an increased locomotor response [12] or a similar locomotor response [13] compared to adult mice, or that adolescent rodents have a decreased locomotor response compared to adult rodents [14–16]. Furthermore, the age differences in the locomotor effects of MA are dose-dependent. A greater magnitude of locomotor activation is seen in adult mice compared to adolescent mice with a low dose of MA (2 mg/kg) within 20 min post-exposure, whereas this age difference is not seen until 45 min post-exposure with a higher dose of MA (4 mg/kg) [9]. Previous research from our lab shows that acute MA exposure (4 mg/kg) increases locomotor activity in both adolescent...
and adult mice and there are no age differences in locomotor activation 20 min following exposure [17]. However, this study only examined behavior for 20 min following exposure and only with a single dose of MA. The goal of the current study was to replicate our previous research and to better understand potential age differences in the effects of MA by examining the effects of acute MA exposure in adolescent and adult mice with a longer testing period and including multiple doses of MA.

Adolescent and adult MA users show increased rates of anxiety [18–20] and acute MA exposure increases anxiety in adults [21]. Studies examining the effects on anxiety-like behavior in preclinical rodent models have produced conflicting results. For example, acute MA exposure increases anxiety-like behavior in adolescent [17,22] and adult [17,23] rodents. In contrast, acute MA exposure has also been shown to decrease anxiety-like behavior in adult rats [24–26]. Previous research from our lab shows no age differences in MA-induced increases in anxiety-like behavior up to 20 min following a high dose of MA (4 mg/kg) [17]. Further research is warranted to explore potential age differences in the anxiety response to acute MA exposure that may be evident at later time points or with different doses of MA.

MA-induced changes in anxiety-like behavior may be due in part to MA’s effects on the hypothalamic pituitary adrenal (HPA) axis. In human adolescent MA users, social stressors cause an increase in cortisol levels beyond what is seen in non-using controls, potentially contributing to enhanced feelings of anxiety [19]. Acute MA exposure increases corticosterone levels in neonatal rodents [27–29] and adult rodents [31–33], although research also shows that acute high dose (4 mg/kg) MA exposure does not alter corticosterone levels in adolescent or adult mice 20 min following exposure [17,22]. Further research is needed to better understand potential age differences in the corticosterone response to MA at different time points and with different doses of MA.

The goal of the present study was to expand upon previous research in our lab examining the effects of acute MA exposure on locomotor activity and anxiety-like behavior in adolescent and adult mice [17]. Our previous research found no age differences in the behavioral or corticosterone effects of acute MA exposure 20 min post-exposure with a high dose (4 mg/kg) of MA. In order to better understand potential age differences in the response to MA, we expanded upon our previous research in the current study by examining the effects of multiple doses of MA and examining behavior for a longer time period to determine if age-specific effects appeared following the 20 min time point. Based on the literature in mice, we predicted that both a lower and higher dose of MA would increase locomotor activity and anxiety-like behavior, but have no effect on corticosterone levels, in both adolescent and adult mice. It was also predicted that the increases in locomotor activity following acute MA exposure would be lower in adolescent mice compared to adult mice.

2. Material and methods

2.1. Mice

Twenty-seven adolescent and 27 adult male C57BL/6 J mice from The Jackson Laboratory (Bar Harbor, ME, USA) were used. Mice arrived in our colony on postnatal day (PND) 24 for adolescent mice and PND 90 for adult mice. Mice were housed according to age with 2 mice per cage in standard mouse cages with bedding and nesting material under a 12-h light/dark cycle (light on at 09:00). Mice had access to food and water ad libitum. There were 9 mice per age and treatment group. All procedures and protocols were approved by the University of St. Thomas Institutional Animal Care and Use Committee (IACUC).

2.2. Open field testing

The open field test was performed on PND 42 ± 1 for the adolescent mice and PND 108 ± 1 for the adult mice. The specific ages of MA exposure and open field testing were chosen to replicate previous research examining the acute effects of MA exposure in adolescence [17,22]. The open field test was used to measure locomotor activity and anxiety-like behavior [34]. Testing was conducted under bright light (540 lx) and with background white noise (54 dB). Mice were tested in 2 consecutive trials. Trial 1 (baseline trial) was 20 min and assessed baseline behavior prior to treatment exposure. Trial 2 (treatment trial) was 45 min and assessed behavior following treatment exposure. Consecutive open field trials to establish baseline behavior and behavioral changes following MA exposure have been used in previous studies to examine the age-dependent effect of MA exposure [10] and were used to replicate the previous testing protocol from our lab [17,22]. The open field arenas were 40 × 40 cm with clear Plexiglas walls. The center of the arenas was defined as the inner 20 × 20 cm area [35,36]. For the first 20-minute trial (baseline trial), all mice received intraperitoneal (IP) injections of 0.9 % sterile saline at a volume of 0.1 mL and were immediately placed in the center of the open field arena. Following baseline open field testing, mice were removed from the arenas and received an IP injection of either 0.9 % sterile saline at a volume of 0.1 mL (n = 9 per age group), 2 mg/kg (d)-MA hydrochloride (Sigma Aldrich, St. Louis, MO) dissolved in 0.9 % sterile saline at a volume of 0.1 mL (n = 9 per age group), or 4 mg/kg (d)-MA hydrochloride dissolved in 0.9 % sterile saline at a volume of 0.1 mL (n = 9 per age group). The high dose (4 mg/kg) was used to replicate previous research from our lab [17,22] and based on previous research showing 4 mg/kg MA increases locomotor activity to a greater degree than 1 mg/kg or 2 mg/kg MA in adolescent C57BL6/J mice [11]. The low dose of MA (2 mg/kg) was included to obtain a dose-response for the locomotor and anxiety effects of MA exposure. Treatments were counterbalanced between housing cages, age groups, and the days of testing. Mice were immediately placed back in the center of the open field arena for the second 45-minute trial (treatment trial) following treatment exposure. Anymaze Video Tracking program (Stoelting Co., Wood Dale, IL) was used to measure total distance moved, the percent time spent in the center of the open field arena, and the percent distance moved in the center of the open field arena in each trial. Percent time in the center and percent distance moved in the center of the arena were used as measures of anxiety-like behavior [34]. Arenas were cleaned with 70 % isopropyl alcohol between trials.

2.3. Stereotyped behavior

Following open field testing, exploratory observation and analysis was conducted to examine stereotyped behavior in the treatment trial of the open field test in the MA-exposed (2 mg/kg and 4 mg/kg) adolescent and adult mice. The stereotyped behaviors of head bobbing, continuous sniffing, and grooming were quantified for the treatment trial of the open field test. Video recordings of the trials were scored by 2 trained observers blind to MA treatment group. Behavior was assessed in 30-sec intervals and the predominant behavior that was observed during each interval was recorded and a sum score of the behavior was calculated [37]. A total sum score of all stereotyped behaviors were calculated for each MA-exposed mouse.

2.4. Corticosterone ELISA

Immediately after open field testing, mice were euthanized via cervical dislocation and decapitation. Trunk blood was collected and stored at −80 °C until use. Plasma corticosterone levels were measured in 6 mice per treatment group using a competitive ELISA kit according to the manufacturer’s instructions (Abcam, Cambridge, MA) and the absorbance was measured at 450 nm using a spectrophotometer.

2.5. Data analysis

The effects of trial (baseline trial and treatment trial, repeated measure), treatment (saline, 2 mg/kg MA, and 4 mg/kg MA), and age
(adult and adolescent) on total distance moved, percent time spent in the center, and percent distance moved in the center of the open field were assessed using a repeated measure analysis of variance (ANOVA). Significant interactions were explored with univariate ANOVA tests. Distance moved in the open field test was also assessed in 5-minute blocks for each trial using a 4-way repeated measure ANOVA, with block and trial as the repeated measures and treatment and age as the between-subject factors. Significant interactions were further explored in each trial separately until the analysis reached its simplest terms. Greenhouse-Geisser correction for the violation of the assumption of sphericity was used for repeated measures ANOVAs. The effects of treatment and age on stereotyped behaviors in the open field test and plasma corticosterone levels were assessed using a 2-way ANOVA. Tukey HSD post-hoc tests were used to explore significant main effects of treatment. All statistical analyses were conducted using SPSS software (IBM, Armonk, NY) with a significance level of $p < 0.05$.

3. Results

3.1. Open field testing

There was a significant trial x treatment x age interaction ($F(2, 48) = 23.80$, $p < 0.01$). Two-way ANOVAs were used to explore the effect of age and treatment in each trial separately. There was no main effect of treatment in the baseline trial ($F(2, 48) = 0.63$, $p = 0.54$), but there was a significant main effect of age in the baseline trial ($F(1, 48) = 17.61$, $p < 0.001$). Adults ($5066.67 \pm 252.23$ cm) moved a greater distance than adolescents ($3748.47 \pm 180.10$ cm) in the baseline trial (Fig. 1). There was a significant treatment x age interaction in the treatment trial ($F(2, 48) = 20.91$, $p < 0.001$). One-way ANOVAs were used to examine the effect of treatment in each age group separately in the treatment trial. In adolescent mice, there was a significant main effect of treatment ($F(2, 24) = 32.24$, $p < 0.001$). Tukey HSD post-hoc tests showed that the high MA group (4 mg/kg) moved significantly more than the low MA group (2 mg/kg) and the saline group, and the low MA group (2 mg/kg) moved significantly more than the saline group. In adult mice, there was a significant main effect of treatment ($F(2, 24) = 49.65$, $p < 0.001$). Tukey HSD post-hoc tests showed that the low MA group (2 mg/kg) moved significantly more than the high MA group (4 mg/kg) and the saline group, and the high MA group (4 mg/kg) moved significantly more than the saline group (Fig. 1).

Repeated measures ANOVA was also used to assess distance moved in the open field in 5 min blocks across the baseline trial and the treatment trial. When the assumption of sphericity was violated, a Greenhouse-Geisser correction was used for all repeated measures ANOVAs. We assessed distance moved in 5 min blocks for each trial separately. For the baseline trial, there was a significant main effect of block ($F(2.49, 119.37) = 7.43$, $p < 0.001$). Post-hoc comparisons showed that all mice moved a greater distance during the first block (0–5 min) and second block (5–10 min) compared to the fourth block (15–20 min). There was also a significant main effect of age ($F(1, 48) = 17.61$, $p < 0.001$). Adult mice moved more than adolescent mice in the baseline trial across all blocks. There was no main effect of treatment or interaction between treatment and block in the baseline trial (Fig. 2A).

For the treatment trial, there was a significant block x treatment x age interaction ($F(4.86, 116.71) = 6.21$, $p < 0.001$). Thus, we explored the effect of block and treatment in each age group separately. In the adolescent mice, there was a significant block x treatment interaction ($F(5.30, 63.57) = 7.07$, $p < 0.001$). To explore this interaction, the effect of treatment was assessed in each block. The effect of treatment was significant for all blocks. Tukey HSD post hoc tests showed that the high (4 mg/kg) and low (2 mg/kg) dose MA group were higher than saline but not different from each other in block 1 (0–5 min). In blocks 2–4 (5–15 min) and blocks 8–9 (35–45 min), the high dose MA group (4 mg/kg) was higher than both the low dose MA group (2 mg/kg) and saline group, which were not statistically different from each other. Finally, in blocks 5–7 (20–35 min), all groups were significantly different from each other, with the low dose MA group (2 mg/kg) moving more than the saline group and the high dose MA group (4 mg/kg) moving more than both the low dose MA (2 mg/kg) and saline groups (Fig. 2B). In the adult mice, there was a significant block x treatment interaction ($F(4.36, 52.35) = 10.41$, $p < 0.001$). To explore this interaction, the effect of treatment was assessed in each block and was found to be significant for all blocks. Tukey HSD post hoc tests showed that the high (4 mg/kg) and low (2 mg/kg) dose MA group were higher than saline but not different from each other in blocks 1–3 (0–15 min). In blocks 4–9 (15–45 min), all groups were significantly different from each other with the high dose MA group (4 mg/kg) moving more than the saline group and the low dose MA group (2 mg/kg) moving more than both the high dose MA (4 mg/kg) and saline groups (Fig. 2C).

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Total distance moved in the open field test. All mice received injections of saline immediately prior to the baseline trial (trial 1), and mice received injections of either saline, 2 mg/kg methamphetamine, or 4 mg/kg methamphetamine immediately prior to the treatment trial (trial 2). In the baseline trial, main effect of age, adults moved more than adolescents (*$p < 0.01$). In the treatment trial, age x treatment interaction. In adolescent mice, low MA (2 mg/kg) moved more than saline (*$p < 0.01$), high MA (4 mg/kg) moved more than both low MA (2 mg/kg) and saline (*$p < 0.01$). In adult mice, low MA (2 mg/kg) moved more than both saline and high MA (4 mg/kg) (*$p < 0.01$), high MA (4 mg/kg) moved more than saline (*$p < 0.01$). $N = 9$ mice per group.
Repeatecl measures ANOVA was used to assess the effect of trial, treatment, and age on the percent time spent in the center of the open field arena. There was a significant trial x treatment interaction (F(2, 48) = 19.91, p < 0.001). One-way ANOVAs were used to explore the effect of treatment in each trial separately. There was no main effect of treatment in the baseline trial (F(2, 51) = 0.96, p = 0.39). There was a main effect of treatment in the treatment trial (F(2, 51) = 41.85, p < 0.001), with both the high (4 mg/kg) and low (2 mg/kg) dose MA-exposed mice showing lower percent time spent in the center of the arena compared with saline-exposed mice (Fig. 3A). There was no main effect of age (F(1, 48) = 0.46, p = 0.50) or any interactions with age on percent time in the center of the open field arena.

For percent distance moved in the center of the open field arena, there was a significant trial x age interaction (F(1, 48) = 4.51, p = 0.04) and a significant trial x treatment interaction (F(2, 48) = 35.87, p < 0.001). One-way ANOVAs were used to explore the effect of age and treatment in each trial separately. In the baseline trial, there was no main effect of age on percent distance moved in the center of the arena (F(1, 52) = 0.99, p = 0.32). In the treatment trial, there was also no main significant effect of age on percent distance moved in the center of the arena (F(1, 52) = 0.61, p = 0.44). When exploring the trial x treatment interaction, there was no main effect of treatment in the baseline trial (F(2, 51) = 0.83, p = 0.44). There was a main effect of treatment in the treatment trial (F(2, 51) = 43.12, p < 0.001), with both the low (2 mg/kg) and high (4 mg/kg) dose MA-exposed mice showing lower percent distance moved in the center of the arena compared with saline-exposed mice (Fig. 3B).

### 3.2. Stereotyped behavior

In order to explore the possibility that stereotyped behavior may differ in the MA-treated mice, an exploratory analysis was conducted to examine stereotyped behavior in the treatment trial of the open field test in the MA-exposed (2 mg/kg and 4 mg/kg) adolescent and adult mice. The stereotyped behaviors of head bobbing, continuous sniffing, and grooming were quantified from video recordings for the treatment trial of the open field test [37]. There was no main effect of treatment (F(1, 32) = 0.02, p = 0.90) or age (F(1, 32) = 3.43, p = 0.07), nor an interaction between treatment and age, on the sum score of stereotyped behaviors in the treatment trial of the open field test in MA-exposed mice (Table 1). There was also no main effect of treatment or age, nor an interaction between treatment and age, on the individual scores for head bobbing, persistent sniffing, or grooming behaviors (Table 1).

### 3.3. Plasma corticosterone levels

There was no main effect of treatment (F(2, 30) = 1.06, p = 0.36) or age (F(1, 30) = 0.002, p = 0.96), nor an interaction between treatment and age, on plasma corticosterone levels (Table 2).

### 4. Discussion

The aim of the current study was to examine the acute dose-response effects of MA exposure on locomotor and anxiety-like behavior in the open field test and plasma corticosterone levels in adolescent and adult mice. We found that acute MA exposure increased locomotion and anxiety-like behavior in the open field compared to saline controls but...
did not alter plasma corticosterone levels. While both the high dose (4 mg/kg) and the low dose (2 mg/kg) of MA increased movement in adolescent and adult mice, the dose-response curves differed between the age groups, with adolescent mice showing the greatest locomotor response to the high dose of MA (4 mg/kg) and adult mice showing the greatest locomotor response to the low dose of MA (2 mg/kg).

MA increased movement in adolescent and adult mice at both the low (2 mg/kg) and high (4 mg/kg) doses, an effect that replicates much previous research (for a review, see [38]) and replicates previous findings from our lab [17,22]. Previous work from our lab examined the acute effects of the high dose of MA (4 mg/kg) for 20 min following MA exposure and found no age differences in the locomotor response [17]. In the current study we hypothesized that adolescent and adult mice would show different responses to the locomotor effects of acute MA exposure at different doses and with the longer testing observation of 45 min. The findings from this study showed that while both adolescent and adult mice increased average locomotor activity over the 45 min trial with both the low (2 mg/kg) and high (4 mg/kg) doses of MA, adolescent mice showed a more blunted effect at the low dose of MA (2 mg/kg) compared to adult mice. For the adolescent mice at the low dose of MA (2 mg/kg), locomotor activity increased over saline controls for the first 5 min of the trial and from 20–35 min of the trial, but this group was not significantly different from saline controls at other time periods of the trial. In contrast, adult mice exposed to the low dose of MA (2 mg/kg) showed increased locomotor activity over saline controls for the entire 45 min trial. This finding replicates previous research showing adolescent mice are less sensitive to the locomotor effects of MA at 2 mg/kg compared to adult mice [9]. Furthermore, this suggests that the lack of age differences found in our previous research [17] was likely due to the shorter duration of (20 min) of quantifying locomotor activity. Taken together with the previous literature, our data suggest that adolescents are less sensitive to the locomotor-stimulating effects of low doses of MA compared to adults.

Adolescent and adult mice showed differential dose-responses to the locomotor effects of acute MA exposure. Adolescent mice showed the greatest locomotor response to the high dose of MA (4 mg/kg) compared to the low dose of MA (2 mg/kg), whereas adult mice showed the greatest locomotor response to the low dose of MA (2 mg/kg) compared to the high dose of MA (4 mg/kg). Previous research shows that adult rodents are more likely to engage in stereotyped behaviors in response to dopaminergic drugs such as amphetamine compared to adolescent rodents [14,15]. Based on these studies, it is possible that the age

| Table 2 |
| Plasma corticosterone concentrations. |

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>Plasma corticosterone concentrations (ug/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent</td>
<td>Saline</td>
<td>63.49 ± 9.17</td>
</tr>
<tr>
<td>Adolescent</td>
<td>Methamphetamine 2 mg/kg</td>
<td>77.64 ± 10.94</td>
</tr>
<tr>
<td>Adolescent</td>
<td>Methamphetamine 4 mg/kg</td>
<td>75.09 ± 11.27</td>
</tr>
<tr>
<td>Adult</td>
<td>Saline</td>
<td>71.45 ± 15.52</td>
</tr>
<tr>
<td>Adult</td>
<td>Methamphetamine 2 mg/kg</td>
<td>56.95 ± 3.23</td>
</tr>
<tr>
<td>Adult</td>
<td>Methamphetamine 4 mg/kg</td>
<td>86.59 ± 10.11</td>
</tr>
</tbody>
</table>

Note: All measures shown as group means ± SEM. N = 6 mice per group.

| Table 1 |
| Stereotyped behaviors. |

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>Head bobbing</th>
<th>Grooming</th>
<th>Sniffing</th>
<th>Sum stereotyped behavior score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent</td>
<td>Methamphetamine 2 mg/kg</td>
<td>0.00 ± 0.00</td>
<td>2.22 ± 1.25</td>
<td>3.33 ± 2.62</td>
<td>5.56 ± 2.66</td>
</tr>
<tr>
<td>Adolescent</td>
<td>Methamphetamine 4 mg/kg</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>7.00 ± 5.23</td>
<td>7.00 ± 5.23</td>
</tr>
<tr>
<td>Adult</td>
<td>Methamphetamine 2 mg/kg</td>
<td>11.22 ± 9.49</td>
<td>20.22 ± 13.00</td>
<td>7.44 ± 21.59</td>
<td>33.56 ± 17.83</td>
</tr>
<tr>
<td>Adult</td>
<td>Methamphetamine 4 mg/kg</td>
<td>10.11 ± 9.99</td>
<td>7.33 ± 6.97</td>
<td>16.11 ± 10.16</td>
<td>33.56 ± 17.83</td>
</tr>
</tbody>
</table>

Note: All measures shown as group means ± SEM. N = 9 mice per group.
difference in the dose-response to acute MA exposure in the current study was due to age differences in the induction of stereotyped behaviors during the open field test. However, results from the current experiment showed no differences in stereotyped behavior between the age group nor between the 2 mg/kg MA and 4 mg/kg MA groups. Thus, it appears that differences in stereotyped behavior cannot explain the dose-response differences in locomotor activity observed between the adolescent and adult mice in the current study. Our data also suggest that the lower doses of MA used in this study were not high enough to induce stereotyped behaviors in the adolescent or adult mice, and higher doses of MA are often used to examine stereotyped behaviors in mice [37]. Further research is warranted to further explore the neurobiological mechanisms underlying the age difference in the response to acute MA exposure.

In the current experiment, two doses of MA (2 mg/kg and 4 mg/kg) were used to examined age differences in the response to acute MA exposure. It is possible, however, that using only two doses of MA may not adequately address potential age differences in the dose-response curve to acute MA exposure. Previous studies in rats show that differences between adolescents and adults in the response to acute amphetamine exposure depends on the dose of amphetamine, with adolescent rats showing reduced locomotor activation compared to adults following 0.5 mg/kg or 1.5 mg/kg amphetamine, but higher locomotor activation compared to adults following 5 mg/kg amphetamine [14]. Adolescent mice acutely exposed to amphetamine show greater locomotor activity compared to adult mice following 1 mg/kg or 2 mg/kg amphetamine, but not following 4 mg/kg amphetamine [12]. Based on this literature on amphetamine, future studies should examine more doses of MA in order to examine more thoroughly the potential age differences in the response to acute MA exposure.

Age differences in the dopamine system may be one neurobiological mechanism underlying the dose-response age difference in locomotor activity found in the current study. For example, adolescent rodents have lower tyrosine hydroxylase levels in the nucleus accumbens and medial prefrontal cortex [39], increased MA-induced activity of dopamine transporters in the striatum [40], and increased expression of dopamine receptors in the striatum and nucleus accumbens [41] compared to adult rodents. Furthermore, adolescent rats are resistant to the long-term effects of MA on tyrosine hydroxylase levels in the striatum compared to adult rats [42]. In contrast, MA does not alter hippocampal tyrosine hydroxylase or phosphorylated tyrosine hydroxylase levels in either adolescent or adult male mice [17]. Although not assessed in the current study, age differences in the striatal dopamine system may account for the reduced sensitivity of adolescent mice to the low (2 mg/kg) dose of MA compared to adult mice, as previous research has suggested that a higher dopaminergic tone may account for behavioral sub-sensitivity in adolescent rodents exposed to dopaminergic drugs [14,39]. Studies in rats have also shown that striatal and plasma MA levels remain higher in adult rats compared to adolescent rats 1 h following MA exposure [42], suggesting age differences in MA pharmacokinetics may account for age differences in the behavioral response to MA. In contrast, brain levels of MA do not differ between adolescent and adult male mice following various doses of MA, suggesting locomotor response differences between the age groups cannot be attributed to pharmacokinetic differences [11]. Future research is warranted to further examine the striatal dopaminergic system and MA pharmacokinetics in adolescent and adult mice exposed to acute low and high doses of MA.

MA increased anxiety-like behavior in the open field, regardless of age and dose of MA. This data replicates previous work in our lab in which acute MA exposure increased anxiety-like behavior in adolescent [17,22] and adult mice [17]. These findings also replicate other studies that have shown that acute MA exposure increases anxiety-like behavior in adult rodents [23]. MA decreases social interaction behavior in rats, which is interpreted as increased anxiety-like behavior [43,44]. Human adolescent [19] and adult [7] MA users also show increased anxiety levels. However, the finding of MA-induced increases in anxiety-like behavior contradicts previous research showing MA decreases anxiety-like behavior in adult rats in the open field test [24,25,45] and elevated plus maze [25,26,46]. Differences in dosing paradigms and the tests used to assess anxiety may account for these differential findings. However, our findings from the current study replicate previous research from our lab suggesting acute MA exposure at high (4 mg/kg) and low (2 mg/kg) doses increase anxiety-like behavior in the open field test in adolescent and adult male mice [17,22].

There was no effect of MA on plasma corticosterone levels following the open field test, regardless of age. This finding replicates previous research showing no effect of MA on plasma corticosterone levels in adolescent [17,22,32] and adult mice [17]. However, other studies have found that acute MA exposure increases plasma corticosterone levels in adult mice, an effect that persists for up to 120 min after MA exposure [33]. It is possible that the stress from the open field testing occurring immediately before serum collection masked any treatment differences in plasma corticosterone concentrations. Future studies should examine the effects of acute MA exposure on plasma corticosterone levels without prior behavioral differences and assess a time course of the effects of MA on the corticosterone response. Taken together with our previous research [17,22], findings from our lab suggest low and high doses of acute MA exposure do not immediately alter plasma corticosterone levels following open field testing in adolescent or adult male mice. These data further suggest that MA-induced changes in the HPA axis, and specifically corticosterone levels, are not the mechanism underlying MA-induced increases in anxiety-like behavior. MA causes changes in many neurotransmitter systems in the brain, including those involved in mood and anxiety behaviors, such as the dopamine, serotonin, and norepinephrine systems (for a review, see [47]). It is possible that MA-induced changes in these systems underlie the increased anxiety-like behavior we observed in the current study.

Previous research has shown sex differences in the response to psychomotor stimulants. For example, female rodents show greater MA-induced increases in locomotor activity compared to male rodents [48,49]. Adolescent female rats show lower locomotor activity following acute amphetamine exposure compared to adult female rats, an age difference that is not observed in male rats [50], suggesting there is an interaction between age and sex in the locomotor response to amphetamines. Adolescent female rats also show greater amphetamine-induced sensitization of locomotor activity compared to adolescent male rats [51]. The focus of the current study was to isolate and examine age differences in the response to acute MA exposure and to replicate previous research from our lab [17,22]. Thus, we only used male mice in the current study. However, future research is warranted to examine sex differences in the locomotor response to MA and how sex differences interact with the age differences in the response to acute MA exposure.

PND 27–60 is typically considered the time period in rodent development that mirrors adolescence and puberty in humans [52]. Adolescence and the onset of puberty during adolescence are associated with a surge in gonadal hormones, and these developmental periods are associated with widespread neurobiological and behavioral changes [53]. Exposure to stress during adolescent development can affect behavior and sensitivity to drugs later in life [54]. The stress from shipping the mice is one such stressor that should be taken into consideration in the current study as the mice were shipped during early adolescence for the adolescent group. The stress from shipping may differ between the adolescent and adult mice, thus adult mice may exhibit different behavioral responses to MA [55]. While this procedure of shipping replicates previous work from our lab [17,22], future studies may consider the role of this stressor in the response to MA.

The specific point during adolescence when psychomotor stimulant exposure takes place can affect the neurobiological and behavioral response (for a review, see [56]). Studies show that PND 45 adolescent mice exhibit an increased locomotor response to acute amphetamine exposure compared to adult mice [12], whereas PND 33 adolescent mice
show equal amounts of locomotor activation compared to adult mice following acute amphetamine exposure [13]. PND 30 mice and PND 35 rats show decreased locomotor activation following acute MA or amphetamine exposure, respectively, compared to adult mice [9] and adult rats [14]. Previous research from our lab shows no locomotor response differences between PND 42 or PND 43 adolescent mice and adult mice following acute MA exposure [17]. Taken together, these data suggest the exact age of acute psychomotor stimulant exposure can influence the locomotor response in adolescent mice compared to adult mice. The current experiment exposed adolescent mice to MA on PND 41, 42, or 43, but further research is needed to understand if the pattern of results differs with earlier or later adolescent MA exposure.

There is evidence of age differences in the locomotor response to long-term psychomotor stimulant exposure, and these age differences may depend on the exact timing of exposure during adolescence. For example, high doses of amphetamine in PND 33–43 adolescent rats result in greater sensitization of the locomotor response compared to adult rats [57], but there are no age differences in locomotor sensitization following repeated exposure to amphetamine in PND 37–47 adolescent rats compared to adult rats [59]. Rats exposed to amphetamine on PND 31 and PND 45 show greater sensitization to the locomotor effects in adulthood compared to rats exposed to amphetamine in adulthood [59]. Although the aim of the current study was to investigate the effects of acute MA exposure, modeling chronic human psychomotor stimulant use in rodents is an important area of research as age differences with acute versus chronic exposure may differ. Future studies should examine potential age differences in the acute and chronic response to MA at different developmental points during adolescence to further explore these age effects.

In summary, the purpose of this study was to expand upon our previous research and examine potential age differences in the behavioral and corticosterone response to a low (2 mg/kg) and high (4 mg/kg) dose of MA. We found that acute MA exposure increases locomotor activity and anxiety-like behavior in adolescent and adult male mice but does not alter plasma corticosterone levels. While there were no age differences in the anxiety and corticosterone response to acute MA exposure, adolescent mice were less sensitive to the increases in locomotor activity following the low dose of MA (2 mg/kg) compared to adult mice. Adult mice were also more sensitive to the locomotor effects of the low dose of MA (2 mg/kg) compared to the high dose of MA (4 mg/kg), whereas adolescent mice showed higher sensitivity to the high dose of MA (4 mg/kg). Further research is warranted to examine the neurobiological mechanisms underlying this age difference in the dose-response to the acute locomotor effects of MA.

CRediT authorship contribution statement

Hayley A. Ortman: Formal analysis, Investigation, Writing - original draft, Writing - review & editing. Mikayla L. Newby: Formal analysis, Investigation, Writing - original draft, Writing - review & editing. Jonathan Acevedo: Formal analysis, Investigation, Writing - original draft, Writing - review & editing. Jessica A. Siegel: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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