Neuroscience Letters 633 (2016) 257–261

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

# Effects of early adolescent methamphetamine exposure on anxiety-like behavior and corticosterone levels in mice

Micaela A. Rud, Thao N. Do, Dr. Jessica A. Siegel *

*University of St. Thomas, 2115 Summit Ave, Saint Paul, MN 55105, USA*

## HIGHLIGHTS

- Adolescent methamphetamine exposure is understudied.
- We examined the effects of acute adolescent methamphetamine exposure in mice.
- Adolescent mice exposed to methamphetamine showed increased anxiety.
- Adolescent methamphetamine exposure had no effect on corticosterone levels.

## ARTICLE INFO

*Article history:*
Received 27 August 2016
Received in revised form
20 September 2016
Accepted 21 September 2016
Available online 22 September 2016

*Keywords:*
Methamphetamine
Corticosterone
Adolescence
Anxiety
Locomotor
Mice

## ABSTRACT

Methamphetamine (MA) is an addictive psychomotor stimulant that affects the central nervous system and alters behavior. The effects of MA are modulated by age, and while much research has examined the effects of MA use in adults, relatively little research has examined the effects in adolescents. As the brain is developing during adolescence, it is important that we understand the effects of MA exposure in adolescence. This research examined the effects of acute MA exposure on locomotor and anxiety-like behavior in the open field test and plasma corticosterone levels in adolescent male C57BL/6J mice. Baseline locomotor and anxiety-like behaviors were assessed in the open field test. Immediately following baseline measurements, mice were exposed to saline or 4 mg/kg MA and locomotor and anxiety-like behavior were measured. Serum was collected immediately after testing and plasma corticosterone levels measured. There were no group differences in baseline behavioral measurements. MA-exposed adolescent mice showed increased locomotor activity and anxiety-like behavior in the open field compared with saline controls. There was no effect of MA on plasma corticosterone levels. These data suggest that acute MA exposure during adolescence increases locomotor activity and anxiety-like behavior, but does not alter plasma corticosterone levels.



## 1. Introduction

Methamphetamine (MA) is a psychomotor stimulant that increases synaptic dopamine levels [1]. The rates of adolescents seeking treatment for MA abuse increased in the early 2000s [2,3], and those seeking treatment show higher rates of depression compared with adolescents in treatment for other drugs of abuse [4]. However, relatively little research has examined the effects of adolescent MA use on the brain and behavior. As the brain continues to develop during adolescence, it is possible that MA exposure may affect adolescents differently than adults. Therefore, it is important to investigate the effects of MA exposure during adolescence.

Previous research in humans and rodents shows that acute MA exposure increases locomotor activity, and long-term MA use effects motor behaviors and the underlying subcortical dopaminergic mechanisms (for a review, see [5]). These effects are modified by age in rodents. Adolescent mice show lower MA-induced increases in locomotor activity compared with adult mice following acute MA exposure, despite similar MA brain concentrations [6]. In addition, adolescent rats show lower MA-induced increases in locomotor activity compared with adult rats following 5 consecutive days of MA exposure [7].

Adolescent and adult MA users show increases in anxiety, with adolescent users also showing a higher incidence of paranoia [8–11]. Studies examining the effects of MA on anxiety in rodents show conflicting findings. For example, acute MA exposure in adult

* Corresponding author at: 2115 Summit Ave, JRC LL56, Saint Paul, MN 55105, USA.
*E-mail addresses:* micaela.rud@stthomas.edu (M.A. Rud), do326441@stthomas.edu (T.N. Do), jessica.siegel@stthomas.edu (J.A. Siegel).

http://dx.doi.org/10.1016/j.neulet.2016.09.036
0304-3940/

rats decreases anxiety-like behavior in the open field [12,13] and elevated plus maze [13–15]. In contrast, MA increases anxiety-like behavior in the light-dark test a week following MA exposure in adult mice [16], in the elevated plus maze immediately following a low dose in adult rats [17], and 3 and 5 days post-exposure in adult mice [18]. To the best of our knowledge, no studies have examined the effects of acute MA exposure on anxiety-like behaviors in adolescent rodents.

Activity of the hypothalamic-pituitary-adrenal (HPA) axis can alter anxiety and stress behaviors [19,20], and MA's effects on the HPA axis may underlie some of the drug's effects on anxiety behavior. While adolescent MA users have basal cortisol levels similar to non-users, adolescent MA users show increased cortisol levels following a social stressor [11]. However, abstinent adult MA users show decreases in plasma cortisol levels [21]. Acute MA exposure increases serum corticosterone levels in neonatal rats [22,23], neonatal mice [24,25], adult rats [15,26,27], and adult mice [28]. MA-treated adult mice also have increased plasma corticosterone levels several weeks after MA exposure [29]. However, it remains unclear how MA exposure alters plasma corticosterone levels in adolescent rodents. Therefore, in the current study, we examined the effects of acute MA exposure during adolescence on locomotor activity and anxiety-like behavior in the open field test and plasma corticosterone levels in male mice.

## 2. Material and methods

### 2.1. Mice

Sixteen male C57BL/6J mice from The Jackson Laboratory (Bar Harbor, ME, USA) were used. Mice arrived to our colony on postnatal day (PND) 24 and were housed 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 *ad libitum* access to food and water. In order to habituate the mice to handling and injections, all mice received inter-peritoneal (IP) injections of 0.9% sterile saline (0.1 mL) 3 days a week for 2 consecutive weeks from PND 30–41. 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 used to assess locomotor activity and anxiety-like behavior [30]. On PND 42 or PND 43, corresponding to late adolescence [31], mice were tested in 2 consecutive 20 min trials. Trial 1 was used to assess baseline behavior and trial 2 assessed behavior after treatment exposure. The open field arenas were 40 × 40 cm arenas with clear Plexiglas walls. The center of the arenas was defined as the inner 20 × 20 cm of the arena [32,33]. For the first 20 min trial (trial 1), all mice received IP injections of 0.9% sterile saline (0.1 mL) and were immediately placed in the center of the arena. Following baseline open field testing in trial 1, mice were removed from the arenas and received an IP injection of either 0.9% sterile saline (0.1 mL, $n = 8$) or 4 mg/kg (d)-MA hydrochloride (Sigma Aldrich, St. Louis, MO) dissolved in 0.9% sterile saline (0.1 mL, $n = 8$). This dose was based on previous research showing 4 mg/kg MA increased locomotor activity to a greater degree than 1 mg/kg or 2 mg/kg MA in adolescent C57BL6/J mice [6]. Treatments were counterbalanced between the mice in each cage and the days of testing. Mice were immediately placed back in the center of the open field arena for another 20 min trial (trial 2). Anymaze Video Tracking program (Stoelting Co., Wood Dale, IL) was used to record and 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 [30,32,34,35]. Arenas were cleaned with 70% isopropyl alcohol between trials.

### 2.3. Corticosterone ELISA

Immediately after open field testing, mice were euthanized via cervical dislocation and decapitation. Serum was collected and stored at −80 °C until use. Plasma corticosterone levels were measured in 5 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.

### 2.4. Data analysis

Two-way repeated measures analysis of variance (ANOVA) was used to assess the effect of trial (trial 1 and trial 2) and treatment (saline and MA) on the total distance moved, percent time spent in the center, and percent distance moved in the center of the open field arena. Significant interactions between trial and treatment were further explored with independent sample *t*-tests. Distance moved in the open field was also assessed in 5 min blocks for each trial using a 3-way repeated measures ANOVA, with block and trial as the repeated measures and treatment as the between subjects factor. Significant interactions were further explored in each trial separately until the analysis reached its simplest terms. An independent sample *t*-test was used to assess differences in plasma corticosterone levels between the treatment groups. All statistical analyses were conducted using SPSS software (IBM, Armonk, NY). A significance level of $p < 0.05$ was used.

## 3. Results

### 3.1. Open field testing

Repeated measures ANOVA was used to assess distance moved in the open field in 5 min blocks across trial 1 and trial 2. The analysis showed a significant 3-way block × trial × treatment interaction ($F(3, 42) = 41.16$, $p < 0.01$). To explore this interaction, we assessed distance moved in 5-min blocks for each trial separately. For trial 1, there was a significant main effect of block ($F(3, 42) = 7.92$, $p < 0.01$), but not main effect of treatment or interaction between treatment and block. Post-hoc comparisons showed that all mice moved a greater distance during the second block (5–10 min) compared with the first block (0–5 min) and the fourth block (15–20 min), and all mice moved a greater distance during the third block (10–15 min) compared with the fourth block (15–20 min). For trial 2, there was a significant interaction between block and treatment ($F(3, 42) = 41.33$, $p < 0.01$). Thus we explored the effect of block in each treatment group separately. There was no effect of block on distance moved for the saline mice ($F(3, 21) = 0.99$, $p = 0.42$). There was a main effect of block for the MA-treated mice ($F(3, 21) = 43.44$, $p < 0.01$), with MA-treated mice moving a greater distance during the third (10–15 min) and fourth (15–20 min) blocks compared with both the first (0–5 min) and second (5–10 min) blocks (Fig. 1a).

Repeated measures ANOVA was also used to assess the effect of trial and treatment on the total distance moved in the open field. There was a significant interaction between trial and treatment ($F(1, 14) = 78.83$, $p < 0.01$). Follow-up independent sample *t*-tests were used to assess the effects of treatment in trial 1 and trial 2 separately. There was no difference between treatment groups in trial 1 ($t(14) = -1.74$, $p = 0.10$). However, there was a difference between treatment groups in trial 2 ($t(14) = -8.40$, $p < 0.01$),