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Sex differences in the acute locomotor response to methamphetamine in BALB/c mice

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Abstract

Women use methamphetamine more frequently than men and are more vulnerable to its negative psychological effects. Rodent models have been an essential tool for evaluating the sex-dependent effects of psychostimulants; however, evidence of sex differences in the behavioral responses to methamphetamine in mice is lacking. In the present study, we investigated acute methamphetamine-induced (1 mg/kg and 4 mg/kg) locomotor activation in female and male BALB/c mice. We also evaluated whether basal locomotor activity was associated with the methamphetamine-induced locomotor response. The results indicated that female BALB/c mice displayed enhanced methamphetamine-induced locomotor activity compared to males, while basal locomotor activity was positively correlated with methamphetamine-induced activity in males, but not females. This study is the first to show sex-dependent locomotor effects of methamphetamine in BALB/c mice. Our observations emphasize the importance of considering sex when assessing behavioral responses to methamphetamine.

Keywords

methamphetamine; BALB/c mice; sex differences; locomotor activity

Methamphetamine (METH) use remains a significant public health concern in the United States and worldwide. METH is one of the most frequently used amphetamine-type stimulants (ATS) in the United States: out of the 1.6 million Americans who engage in illicit use of ATS, 35% reported using METH in 2014 [1]. Sex differences among METH users also exist. Women are more likely to begin using METH at an earlier age [2], report more METH-associated psychological symptoms [3], and use METH more frequently [4].

Rodent models have been essential in evaluating the effects of both acute and chronic METH exposure. As in humans, rats exhibit sex-dependent responses in that females are more sensitive to its acute locomotor activating effects[5], self-administer more METH than

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males [6] and are more likely to reinstate METH-seeking behavior following periods of abstinence [7]. Additionally, neonatal METH exposure results in sex-dependent differences on memory tasks [8] and striatal protein kinase A activity [9] in adult rats. Studies in mice have also revealed sex differences. For example, there are several reports that neurotoxic doses of METH induce greater striatal depletions of dopamine and serotonin in males compared to females [10]. Prenatal METH exposure results in sex-dependent deficits in hippocampal-dependent memory tasks [11]. Sex differences have also been observed in METH-induced immune responses [12] and hypothalamic-pituitary-adrenal (HPA) axis activation [13]. Yet, the influence of sex on acute METH-induced hyperactivity in mice has not been thoroughly examined. One group observed greater locomotor activity in females; however, the mice in that study were selectively bred for either low or high sensitivity to METH [14]. Another study indicated no sex differences in METH-induced locomotor activity [15]. Given the evidence of sex-dependent effects of other psychostimulants (i.e. cocaine, amphetamine) on locomotor activity in mice [16, 17], the first aim of the present study was to extend these findings by assessing whether acute METH-induced locomotor activation differs between male and female BALB/c mice.

In humans, the effects of METH use can also vary from one individual to another. Studies in rodents have shown that individual differences in the acute locomotor response to a novel environment can predict psychostimulant-induced behaviors [18]. There is also evidence that basal locomotor activity is positively correlated with subsequent responding to cocaine [19] and nicotine [20], however, this correlation has not been evaluated with METH in mice. Thus, the second aim of the present study was to determine whether basal locomotor activity correlates with the acute locomotor response to METH in BALB/c mice.

Female and male BALB/c (AnNHsd) mice (16–22 weeks old) were obtained from Envigo (formerly Harlan Laboratories; Houston, TX, USA). All mice were group-housed in plastic cages with isolator tops in a humidity and temperature-controlled room with a 12 h light/dark cycle (lights on at 6:00 a.m.). All experimental procedures were conducted during the light cycle and were approved by the Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals [21].

To evaluate the effects of METH on locomotor activity, mice were placed into an acrylic test chamber (27.5 in. × 27.5 in. × 8.0 in.; Opto-M3; Columbus Instruments; Columbus, OH) equipped with infrared sensors. All tests were conducted under dim lighting. Mice were first habituated to the test chambers for three consecutive days (60 min. /day). The day following habituation, mice were given a subcutaneous injection of sterile phosphate buffered saline (PBS; vehicle control) and placed in the chamber for 30 minutes to assess their baseline locomotor activity. Total locomotor activity was recorded in beam-breaks per 10 minutes by using software supplied by Columbus Instruments. After the baseline period, mice were given a subcutaneous injection of either a low dose (1 mg/kg; Female: n=5, Male n=9) or high dose (4mg/kg; Female: n=5, Male n=5) of METH [(+)-methamphetamine hydrochloride, Sigma-Aldrich; St. Louis, MO, USA] dissolved in sterile PBS. These doses were chosen based on our previous work assessing METH-induced locomotor activity in

female BALB/c mice [22]. After the injection, mice were placed back into the testing chamber and locomotor activity was recorded for an additional 90 min.

A Student's t-test was used to compare 30 min. baseline locomotor activity between males and females for each dose. Data were analyzed using a two-way (*sex* $\times$ *time*) repeated measures analysis of variance (ANOVA), followed by Bonferroni post-hoc tests. A linear regression analysis was also conducted to detect potential correlations between basal activity (last 10 min. of session) and METH-induced locomotor response (90 min. session; low and high doses were pooled). Significance level was defined as $p < 0.05$. Statistica Academic software (Statistica 12; Dell Software, Tulsa, OK) was used for all analyses. All data are shown as mean $\pm$ S.E.M.

As indicated in Table 1, 30 min. baseline locomotor activity did not differ between males and females for both low and high doses. At the low dose (Figure 1A), METH did not significantly increase locomotor activity above baseline in either sex, as no main effect of time ($F_{(9, 108)} = 1.843$; $p = 0.069$) or sex was observed ($F_{(1, 108)} = 2.423$; $p = 0.146$). At the high dose (Figure 1B), there were significant main effects of time ($F_{(9, 72)} = 12.015$; $p < 0.0001$) and sex ($F_{(1, 72)} = 14.201$; $p < 0.001$), as well as a time $\times$ sex interaction ($F_{(9, 72)} = 9.772$; $p < 0.0001$). Post hoc tests indicated that both sexes displayed increased METH-induced activity above baseline levels: males and females displayed peak activity at 20 min. ($p < 0.05$) and females continued to display increased activity from 30 to 90 min. ($p < 0.05$). Females also displayed significantly higher METH-induced activity than males ($p < 0.001$). Linear regression analysis revealed that basal locomotor activity was positively correlated with METH-induced locomotor activity in males ($R^2 = 0.5099$, $p = 0.004$), but not in females ($R^2 = 0.0839$, $p = 0.4169$; See Figure 2).

Our observations support and extend previous reports evaluating sex differences and the locomotor effects of psychostimulants in rodents. Following acute METH administration, female BALB/c mice displayed enhanced METH-induced locomotor activation compared to males, an observation that parallels previous mouse studies with cocaine [16, 17] and amphetamine [17]. BALB/c mice are generally regarded as having a low sensitivity to the locomotor-activating effects of amphetamine [23] and METH [24]. We observed that female BALB/c mice exhibited enhanced and prolonged locomotor responses to acute METH while males did not, which underscores the importance of considering the influence of sex on drug-induced locomotor activity in BALB/c mice. Contrary to our findings, Dluzen and Mickley (2005) observed no influence of sex on locomotor behaviors following METH administration in mice. This divergent finding may be explained, in part, by their use of CD-1 mice, which were administered a high dose of METH (40 mg/kg) [15]. High doses of METH reliably induce hypolocomotion [22] and stereotypy [25], which would likely mask any potential sex differences in locomotor activity.

Differences in pharmacokinetics between male and female mice may have contributed to the sex differences in METH-induced locomotor activity found in the present study. Since sex-dependent effects of METH on locomotor activity in mice parallel what has been observed in rats [5, 26], it is possible that similar pharmacokinetic sex differences in rats and mice also exist. Indeed, female C57BL/6J mice (at postnatal day 11) display significantly higher

plasma and brain METH levels than males at 120 min. post-injection [27]. Also, adult female rats metabolize and eliminate METH at slower rates than males, which results in significantly higher serum concentrations of METH in females over time [28]. Thus, the elevated and extended duration of METH-induced locomotor activity observed in females in the present study could reflect sex-dependent pharmacokinetics of METH. Still, further studies are needed to confirm these differences in BALB/c mice, as there is evidence that enhanced cocaine- and amphetamine-induced locomotor activity in adult female mice is not necessarily due to a sex-dependent metabolism of either drug [17].

Sex differences in dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) functions could have contributed to the enhanced locomotor response we observed in females. METH primarily exerts its actions at monoaminergic synapses where it elevates synaptic dopamine and norepinephrine through several mechanisms including reversing the functions of DAT and VMAT2. A series of experiments by Dluzen and colleagues revealed that striatal DATs [29] and VMAT2 [30], may be more functionally active in female mice, and the synergistic effects of these transporters could result in a more sensitive behavioral response to METH in females [31].

An important aspect of developing potential pharmacotherapies for METH use disorder is determining whether individual differences in behaviors associated with METH use could influence the effectiveness of potential treatments. Few, if any, preclinical studies have examined basal behavioral differences and its relationship to the locomotor response to METH in male and female mice. Thus, in the present study we demonstrated that basal locomotor activity was positively correlated with subsequent locomotor responses to METH in males, but not in females. Our observations corroborate previous work in male mice with cocaine [19] and nicotine [20]; however, our results indicate that this association may only be reliably detected in males and not in a cohort of freely cycling females, as was used in the present study. Therefore, it is possible that the lack of a correlation in females could reflect differences in the behavioral responses to METH across estrous cycle stages. For example, our previous work demonstrated that female rats display reduced locomotor responses to cocaine during estrus versus proestrus and diestrus [32]. Thus, in the present study it is possible that the variability in estrous cycle stages between freely cycling females could partially explain why basal activity was not correlated with METH-induced locomotor activity. Also, the variability in locomotor activity in females after the low dose of METH was greater than at the high dose, which could also explain the sex differences we observed. Future studies assessing the potential influence of the estrous cycle on the relationship between basal activity and METH-induced locomotor activity are needed.

Overall, our results indicate sex differences in the response to acute METH administration in BALB/c mice. Females displayed enhanced METH-induced locomotor activation compared to males, an observation that counters previous reports that BALB/c mice do not display robust hyperactivity following administration of psychostimulants [16, 23, 24]. In addition, basal locomotor activity correlated with METH-induced locomotor activity in males and not females. These results emphasize the importance of considering sex when assessing the behavioral effects of METH in BALB/c mice, particularly since behavioral assays are routinely used to test potential therapeutic treatments for METH use disorder.

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References

- Center for Behavioral Health Statistics and Quality. Behavioral health trends in the United States: Results from the 2014 National Survey on Drug Use and Health. 2015. (HHS Publication No. SMA 15-4927, NSDUH Series H-50)
- Hser YI, Evans E, Huang YC. Treatment outcomes among women and men methamphetamine abusers in California. *J Subst Abuse Treat*. 2005; 28(1):77–85. [PubMed: 15723735]
- Simpson JL, Grant KM, Daly PM, Kelley SG, Carlo G, Bevins RA. Psychological Burden and Gender Differences in Methamphetamine-Dependent Individuals in Treatment. *Journal of psychoactive drugs*. 2016; 48(4):261–9. [PubMed: 27541988]
- Dluzen DE, Liu B. Gender differences in methamphetamine use and responses: a review. *Gender medicine*. 2008; 5(1):24–35. [PubMed: 18420163]
- Milesi-Halle A, McMillan DE, Laurenzana EM, Byrnes-Blake KA, Owens SM. Sex differences in (+)-amphetamine- and (+)-methamphetamine-induced behavioral response in male and female Sprague-Dawley rats. *Pharmacol Biochem Behav*. 2007; 86(1):140–9. [PubMed: 17275894]
- Reichel CM, Chan CH, Ghee SM, See RE. Sex differences in escalation of methamphetamine self-administration: cognitive and motivational consequences in rats. *Psychopharmacology (Berl)*. 2012; 223(4):371–80. [PubMed: 22592902]
- Ruda-Kucerova J, Amchova P, Babinska Z, Dusek L, Micale V, Sulcova A. Sex Differences in the Reinstatement of Methamphetamine Seeking after Forced Abstinence in Sprague-Dawley Rats. *Frontiers in psychiatry*. 2015; 6:91. [PubMed: 26217239]
- Williams MT, Moran MS, Vorhees CV. Refining the critical period for methamphetamine-induced spatial deficits in the Morris water maze. *Psychopharmacology (Berl)*. 2003; 168(3):329–38. [PubMed: 12684734]
- Crawford CA, Williams MT, Newman ER, McDougall SA, Vorhees CV. Methamphetamine exposure during the preweanling period causes prolonged changes in dorsal striatal protein kinase A activity, dopamine D2-like binding sites, and dopamine content. *Synapse*. 2003; 48(3):131–7. [PubMed: 12645038]
- Yu L, Liao PC. Sexual differences and estrous cycle in methamphetamine-induced dopamine and serotonin depletions in the striatum of mice. *Journal of neural transmission*. 2000; 107(4):419–27. [PubMed: 11215753]
- Acevedo SF, de Esch IJ, Raber J. Sex- and histamine-dependent long-term cognitive effects of methamphetamine exposure. *Neuropsychopharmacology*. 2007; 32(3):665–72. [PubMed: 16710318]
- Saito M, Terada M, Kawata T, Ito H, Shigematsu N, Kromkhun P, Yokosuka M, Saito TR. Effects of single or repeated administrations of methamphetamine on immune response in mice. *Experimental animals /Japanese Association for Laboratory Animal Science*. 2008; 57(1):35–43.
- Zuloaga DG, Johnson LA, Agam M, Raber J. Sex differences in activation of the hypothalamic-pituitary-adrenal axis by methamphetamine. *J Neurochem*. 2014; 129(3):495–508. [PubMed: 24400874]
- Kamens HM, Burkhart-Kasch S, McKinnon CS, Li N, Reed C, Phillips TJ. Sensitivity to psychostimulants in mice bred for high and low stimulation to methamphetamine. *Genes, brain, and behavior*. 2005; 4(2):110–25.
- Dluzen DE, Mickley KR. Gender differences in modulatory effects of tamoxifen upon the nigrostriatal dopaminergic system. *Pharmacol Biochem Behav*. 2005; 80(1):27–33. [PubMed: 15652377]
- Thomsen M, Caine SB. Psychomotor stimulant effects of cocaine in rats and 15 mouse strains. *Exp Clin Psychopharmacol*. 2011; 19(5):321–41. [PubMed: 21843010]

17. Van Swearingen AE, Walker QD, Kuhn CM. Sex differences in novelty- and psychostimulant-induced behaviors of C57BL/6 mice. *Psychopharmacology (Berl)*. 2013; 225(3):707–18. [PubMed: 22975726]
18. Bevins RA, Peterson JL. Individual differences in rats' reactivity to novelty and the unconditioned and conditioned locomotor effects of methamphetamine. *Pharmacol Biochem Behav*. 2004; 79(1): 65–74. [PubMed: 15388285]
19. Eisener-Dorman AF, Grabowski-Boase L, Tarantino LM. Cocaine locomotor activation, sensitization and place preference in six inbred strains of mice. *Behavioral and brain functions : BBF*. 2011; 7:29. [PubMed: 21806802]
20. Bernardi RE, Spanagel R. Basal activity level in mice predicts the initial and sensitized locomotor response to nicotine only in high responders. *Behav Brain Res*. 2014; 264:143–50. [PubMed: 24508239]
21. National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals., Institute for Laboratory Animal Research (U.S.), National Academies Press (U.S.). *Guide for the care and use of laboratory animals*. National Academies Press; Washington, D.C: 2011. p. xxvp. 220
22. Singh RA, Kosten TA, Kinsey BM, Shen X, Lopez AY, Kosten TR, Orson FM. Dose-dependent changes in the locomotor responses to methamphetamine in BALB/c mice: low doses induce hypolocomotion. *Pharmacol Biochem Behav*. 2012; 103(2):230–6. [PubMed: 23010423]
23. Oliverio A, Eleftheriou BE, Bailey DW. Exploratory activity: genetic analysis of its modification by scopolamine and amphetamine. *Physiol Behav*. 1973; 10(5):893–9. [PubMed: 4715816]
24. Kitahama K, Valatx JL. Strain differences in amphetamine sensitivity in mice. I. A diallel analysis of open field activity. *Psychopharmacology (Berl)*. 1979; 66(2):189–94. [PubMed: 119262]
25. Kitanaka J, Kitanaka N, Tatsuta T, Miyoshi A, Koumoto A, Tanaka K, Nishiyama N, Morita Y, Takemura M. Pretreatment with l-histidine produces a shift from methamphetamine-induced stereotypical biting to persistent locomotion in mice. *Pharmacol Biochem Behav*. 2010; 94(3):464–70. [PubMed: 19895842]
26. Mattei R, Carlini EA. A comparative study of the anorectic and behavioral effects of fenproporex on male and female rats. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas /Sociedade Brasileira de Biofisica .. [et al.]*. 1996; 29(8):1025–30.
27. Acevedo SF, Pfankuch T, van Meer P, Raber J. Role of histamine in short- and long-term effects of methamphetamine on the developing mouse brain. *J Neurochem*. 2008; 107(4):976–86. [PubMed: 18786166]
28. Milesi-Halle A, Hendrickson HP, Laurenzana EM, Gentry WB, Owens SM. Sex- and dose-dependency in the pharmacokinetics and pharmacodynamics of (+)-methamphetamine and its metabolite (+)-amphetamine in rats. *Toxicology and applied pharmacology*. 2005; 209(3):203–13. [PubMed: 15916788]
29. Bhatt SD, Dluzen DE. Dopamine transporter function differences between male and female CD-1 mice. *Brain Res*. 2005; 1035(2):188–95. [PubMed: 15722058]
30. Dluzen DE, Bhatt S, McDermott JL. Differences in reserpine-induced striatal dopamine output and content between female and male mice: implications for sex differences in vesicular monoamine transporter 2 function. *Neuroscience*. 2008; 154(4):1488–96. [PubMed: 18515015]
31. Dluzen DE, McDermott JL. Sex differences in dopamine- and vesicular monoamine-transporter functions. *Ann N Y Acad Sci*. 2008; 1139:140–50. [PubMed: 18991858]
32. Kosten TA, Sanchez H, Jatlow PI, Kehoe P. Neonatal isolation alters the estrous cycle interactions on the acute behavioral effects of cocaine. *Psychoneuroendocrinology*. 2005; 30(8):753–61. [PubMed: 15919581]

Highlights

- Female and male BALB/c mice were administered methamphetamine and open-field locomotor activity was assessed.
- Female BALB/c mice displayed an enhanced response to the locomotor-activating effects of methamphetamine compared to males.
- Basal locomotor activity was positively correlated with locomotor responses to methamphetamine in male, but not female BALB/c mice.

A

B

Figure 1.

Basal locomotor activity after vehicle (BASE) and locomotor activity after (A) 1 mg/kg or (B) 4 mg/kg of METH during the 90 min. session. Female mice displayed enhanced locomotor responses to METH versus male mice. Values are mean $\pm$ SEM. (*) denotes a significant difference between females and males.

Figure 2.

Simple linear regression between basal locomotor activity and METH-induced locomotor activity in (A) females and (B) males. 1 mg/kg and 4 mg/kg METH doses were pooled for each sex. A significant correlation was observed in the males ($p < 0.001$), but not the females ($p > 0.05$).

Table 1Baseline Locomotor Activity¹ after Vehicle (PBS) Injection

METH Dose Group	Female (mean ± SEM)	Male (mean ± SEM)	P-value ²
1 mg/kg	7045.60 ± 417.27	4918.78 ± 950.52	0.14
4mg/kg	5121.80 ± 420.58	5862.40 ± 370.21	0.22

¹ Baseline activity during 30 min. session² Student's t-test