Drug and Alcohol Dependence 178 (2017) 70–74

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

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

Short communication

The neurochemical consequences of methamphetamine self-administration in male and female rats

Andrew Johansen[a], Lisa M. McFadden[a,b,*]

[a] Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84103, United States
[b] Division of Basic Biomedical Sciences, University of South Dakota, Vermillion, SD 57069, United States

ARTICLE INFO

Keywords:
Methamphetamine
Female
Self-administration
Dopamine transporter
Brain-derived neurotrophic factor

ABSTRACT

Background: Methamphetamine (METH) is an addictive substance that is used in both males and females. Few preclinical studies have focused on understanding sex-differences in the neurochemical consequences of contingent METH. The purpose of the current study was to investigate potential sex-differences in the neurochemical consequences of METH self-administration.
Methods: Male and female adult rats were given extended access to METH or saline self-administration for 7 d. Following self-administration, hippocampal brain-derived neurotrophic factor (BDNF) and striatal dopamine transporter (DAT) were assessed via western blotting.
Results: Male and female rats had similar METH intake. METH self-administration reduced striatal DAT in both sexes, but only males that self-administered METH had elevated hippocampal BDNF levels.
Conclusions: Sex-differences exist in the neurochemical consequences of METH self-administration. These differences may lead to sex-specific vulnerability to the toxic effects of METH.

1. Introduction

The use of the highly addictive psychostimulant, methamphetamine (METH), occurs in both genders. Within the United States, females make up approximately 40% of lifetime METH users and approximately 50% of adolescent METH users (Chen et al., 2014). Recent studies suggest that gender differences exist in the use of METH, and its behavioral and psychological effects. Reports suggest that females start using METH at a younger age, transition from recreational use to addiction more quickly, and initiate the injection METH earlier than their male counterparts (Dluzen and Liu, 2008; Hadland et al., 2010; Liu et al., 2013; Rawson et al., 2005). Drug craving was also significantly correlated with depression and anxiety measures in male METH users but not females (Hartwell et al., 2016). Increased rates of depression, psychosis, and suicide have also been reported in females compared to males (Glasner-Edwards et al., 2008a, 2008b; Mahoney et al., 2010). Female METH users also reported a greater severity of drug use and psychological burden than their male counterpart (Simpson et al., 2016), further demonstrating the importance of investigating gender differences in METH users.

Recent clinical findings suggest that female METH/stimulant users show greater changes in the brain compared to their male counterparts. Female METH users currently in abstinence had reductions in hippocampal volumes compared to control females, whereas no difference was observed between METH-abusing and control male subjects (Du et al., 2015). Furthermore, female METH users had significantly lower phosphocreatine levels in the frontal lobe compared to male METH users (Sung et al., 2013). Researchers have also found that female METH/stimulant users displayed wide-spread reductions in grey matter volumes following prolonged abstinence (Regner et al., 2015). These changes in the female brain may in turn contribute to addictive behaviors and an increased risk of neurodegenerative diseases later in life.

Similar to findings in humans, preclinical studies utilizing METH self-administration have found sex-differences in drug use parameters. Female rats acquired METH self-administration more quickly than males (Kucerova et al., 2009; Reichel et al., 2012; Roth and Carroll, 2004). Further, female rats with a history of METH self-administration more vigorously reinstated drug-seeking compared to males (Cox et al., 2013; Holtz et al., 2012; Reichel et al., 2012). However, few studies have investigated the neurochemical consequences of METH self-administration in male and female rats. Previous research in male rats suggests METH self-administration increases brain-derived neurotrophic factor (BDNF) in various areas of the brain which may reduce the toxic effects of METH including attenuating the reduction of dopamine transporter (DAT) in the striatum (Krasnova et al., 2013;

* Corresponding author at: Division of Basic Biomedical Sciences, University of South Dakota, 414 E. Clark St, Vermillion, SD 57069, United States.
E-mail address: lisa.mcfadden@usd.edu (L.M. McFadden).

http://dx.doi.org/10.1016/j.drugalcdep.2017.04.011
Received 15 November 2016; Received in revised form 5 April 2017; Accepted 10 April 2017
Available online 06 June 2017
0376-8716/

McFadden et al., 2014). This increase in BDNF declines during abstinences from METH (Krasnova et al., 2013). Therefore, the current study investigated the acute neurochemical consequences of METH self-administration in male and female rats.

2. Methods

2.1. Animals

Male and female Sprague-Dawley rats (postnatal day 59–62; Charles River Laboratories) were housed two rats/cage. After surgery, rats were individually housed in transparent plastic cages. Water was available *ad libitum*. During food training, rats were food restricted such that no rat dropped below 90% of their starting body weight. Rats were maintained in a 14:10 h light/dark cycle. Animals were euthanized by rapid decapitation. All experiments were approved by the University of Utah's Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Racemic-METH hydrochloride (supplied by the National Institute on Drug Abuse, Research Triangle Institute, Research Triangle Park, NC) was dissolved in 0.9% sterile saline and is expressed as free base. Rats were anesthetized using ketamine (90 mg/kg; Hospira Inc., Lake Forest, IL, USA) and xylazine (5–7 mg/kg; Sigma-Aldrich, St. Louis, MO, USA). Heparinized saline (63.33 U/ml; Sigma, St. Louis, MO, USA) was used to dissolve the antibiotic cefazolin (10 mg/ml; Schein Pharmaceutical, Florham Park, NJ, USA). Flunixin meglumine (1.1 mg/kg; MWI Veterinary Supply, Meridian, ID, USA) was given for post-surgery analgesia. Methohexital sodium (10 mg/ml; JHP Pharmaceuticals, Rochester, MI) was used to assess catheter patency.

2.3. Food training and surgery

Food training and self-administration occurred in an operant chamber as previously described (McFadden et al., 2012a, 2012b). Prior to surgery, each rat was trained to press a lever for a 45-mg food pellet during four overnight 14-h sessions. Following food training, an indwelling catheter (see Frankel et al., 2008 for construction details) was implanted. Flunixin meglumine was given on the day of the surgery and the day following the surgery. Immediately following surgery and daily thereafter, each rat was infused with 0.1 ml of cefazolin, 0.05 ml of heparinized saline, and 0.05 ml of heparinized glycerol. Catheter patency was confirmed by infusing 0.1 ml (10 mg/ml) of methohexital sodium on the day prior to self-administration in all animals and throughout at signs of a potential loss in patency.

2.4. Self-Administration

Rats underwent 7 days of self-administration (8 h/session; FR1; 0.12 mg/infusion METH for males, 0.09 mg/infusion METH for females, or 10 µl saline) during the light cycle in a room maintained at 28 ± 1 °C to promote lever pressing (Cornish et al., 2008). An active lever press resulted in the retraction of the levers, a 10 µl infusion of saline or METH over 5 s, followed by an additional 20 s of retracted levers as described previously (McFadden et al., 2012a). Rectal temperatures were recorded approximately 30 min following each session (Physitemp Instruments, Clifton, NJ). Animals were sacrificed 1 h after the end of the last self-administration session, brains were removed and hemisected, and the dorsal striatum and hippocampus were dissected from the left hemisphere.

2.5. Western blotting

Synaptosomes were prepared as previously described (Hadlock et al., 2009; McFadden et al., 2015). Equal quantities of protein (10 µg) were loaded into each well of a 4–12% NuPAGE Novex Bis-Tris Midi gradient gel (Invitrogen, Carlsbad, CA) and electrophoresed using a XCell4 Surelock Midi-cell (Invitrogen). Membranes were blocked for 45 min with Starting Block Blocking Buffer (Pierce Chemical, Rockford, IL) and incubated for 1 h at room temperature with an anti-brain-derived neurotrophic factor (BDNF) polyclonal (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-dopamine transporter (DAT) polyclonal antibody (C-20; 1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were then washed and developed as previously described (McFadden et al., 2014). The resulting band densities were normalized to the percentage of average saline female immunoreactivity for ease of comparisons. The Bradford Protein Assay was used to quantify protein concentrations.

2.6. Statistical analysis

Statistical analysis was conducted in SAS Studios. Only METH rats that met the criteria for high pressers (1: average of more than 10 active lever presses per day; and 2: the ratio of active/inactive lever presses of 2:1) were included in analysis, resulting in the exclusion of one male. Drug intake was normalized to the individual animal's body weight (kg) on the day of the session. Statistical analyses among groups were conducted using an analysis of variance (ANOVA) or repeated-measures ANOVA followed by a Newman-Keuls posthoc analyses. The data represent means ± standard error of the mean (S.E.M.) of 10–11 rats/group.

3. Results

Saline self-administering rats decreased active lever pressing over the course of the 7 d, whereas METH rats increased lever pressing (Group x Day: $F(6,228) = 26.44$, $p < 0.05$; Fig. 1A). No sex differences were found (Sex: $F(1,28) = 1.74$, ns; Sex x Day: $F(6,228) = 0.49$, ns; Sex x Group x Day: $F(6,228) = 0.46$, ns). Due to sex-differences in body weight, daily METH intake was normalized to body weight. Male and female rats increased daily METH intake over the course of the 7 days ($F(6,108) = 6.35$, $p < 0.05$; Fig. 1B), but the increase was similar between the sexes (Sex: $F(1,108) = 0.14$, ns; Sex x Day: $F = (6, 108) = 0.70$, ns). However, female rats and METH rats had higher body temperatures on Days 2–7 and Days 3–7 of self-administration, respectively (Sex x Day: $F(6,228) = 3.04$, $p < 0.05$; Group x Day: $F(6,228) = 9.78$, $p < 0.05$; Fig. 1C). Given the unique temperature profiles between groups, body temperatures were analyzed separately. Further analysis found among METH self-administering rats, female rats had increasing daily body temperatures whereas male rats had stable body temperatures (METH- Sex: $F(1,18) = 18.84$, $p < 0.05$; Day: $F(6,108) = 2.08$, ns; Sex x Day: $F(6,108) = 4.24$, $p < 0.05$). However, among saline self-administering rats, female rats overall had higher body temperatures, but both males' and females' daily body temperatures decreased in a similar pattern (Saline- Sex: $F(1,20) = 9.71$, $p < 0.05$; Day: $F(6,120) = 10.60$, $p < 0.05$; Sex x Day: $F(6,120) = 1.46$, ns).

To assess the effects of METH self-administration, striatal DAT and hippocampal BDNF immunoreactivity were evaluated. METH self-administration decreased DAT immunoreactivity in the striatum (Drug: $F(1,37) = 7.43$, $p < 0.05$; Fig. 2A). However, there was no significant effect of sex ($F(1,37) = 0.02$, ns) or Sex x Day interaction ($F(1,37) = 0.65$, ns). It should be noted that the magnitude of the change was greater in females than males that self-administered METH (44% decrease in females versus 25% decrease in males compared to sex-matched controls). Lastly, hippocampal BDNF immunoreactivity was significantly increased in METH self-administering males but not METH