Behavioural Brain Research 296 (2016) 125–128

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Extended access to methamphetamine self-administration up-regulates dopamine transporter levels 72 hours after withdrawal in rats

Christina D'Arcy[a,1], Joe E. Luevano[a,1], Manuel Miranda-Arango[a], Joseph A. Pipkin[b], Jonathan A. Jackson[b], Eddie Castañeda[b], Kristin L. Gosselink[a], Laura E. O'Dell[b,*]

[a] Departments of Biological Sciences, The University of Texas at El Paso, 500 West University Avenue, El Paso, TX 79968, USA
[b] Departments of Psychology, The University of Texas at El Paso, 500 West University Avenue, El Paso, TX 79968, USA

HIGHLIGHTS

- Extended access to METH IVSA produces larger intake versus limited access to this drug.
- Extended access to METH IVSA up-regulates dopamine transporters.
- Extended access to METH IVSA does not alter TH or D2 levels.

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 21 August 2015
Accepted 7 September 2015
Available online 12 September 2015

Keywords:
Withdrawal
Abstinence
Striatum
Methamphetamine
Dopamine transporter
IVSA

ABSTRACT

Previous studies have demonstrated that there are persistent changes in dopamine systems following withdrawal from methamphetamine (METH). This study examined changes in striatal dopamine transporter (DAT), tyrosine hydroxylase (TH) and dopamine receptor 2 (D2) 72 h after withdrawal from METH intravenous self- administration (IVSA). Rats were given limited (1 h) or extended (6 h) access to METH IVSA (0.05 mg/kg/0.1 ml infusion) for 22 days. Controls did not receive METH IVSA. The rats given extended access to IVSA displayed higher METH intake during the first hour of drug access compared to rats given limited access. Extended access to METH also produced a concomitant increase in striatal DAT levels relative to drug-naïve controls. There were no changes in TH or D2 levels across groups. Previous studies have reported a decrease in striatal DAT levels during protracted periods (>7 days) of withdrawal from METH IVSA. This study extends previous work by showing an increase in striatal DAT protein expression during an earlier time point of withdrawal from this drug. These results are an important step toward understanding the dynamic changes in dopamine systems that occur during different time points of withdrawal from METH IVSA.

1. Introduction

Clinical reports have shown that chronic exposure to methamphetamine (METH) produces a profound reduction in dopamine transporter (DAT) expression in the striatum [1,2]. Such alterations in DAT have been associated with METH addiction [3]. Consistent with clinical reports, METH intravenous self- administration (IVSA) in rodents produces long-term down-regulation of many dopaminergic markers within the striatum, including DAT, tyrosine hydroxylase (TH) and dopamine receptor 2 (D2). Although the latter effects have been largely observed following protracted periods of withdrawal (7–30 days) from METH IVSA [4–7], earlier time points of withdrawal (<7 days) have not been thoroughly investigated.

While there are species-specific differences in the rate and pathways of METH metabolism, the clearance of this drug and its psychoactive metabolites generally occurs 72 h after METH administration [8,9]. Also, the strongest symptoms of withdrawal are reported 24–72 h after abstinence from chronic METH use [10,11]. These studies suggest that 72 h after the initiation of METH withdrawal is a physiologically and psychologically relevant time point to examine changes in dopaminergic markers. A recent report also demonstrated that early in withdrawal from METH IVSA, rats given extended access (6 h) displayed a larger increase in depressive-like

* Corresponding author. Fax: +1 915 747 6553.
E-mail address: lodell@utep.edu (L.E. O'Dell).
[1] Both are primary contributing authors to this work.

http://dx.doi.org/10.1016/j.bbr.2015.09.010
0166-4328/

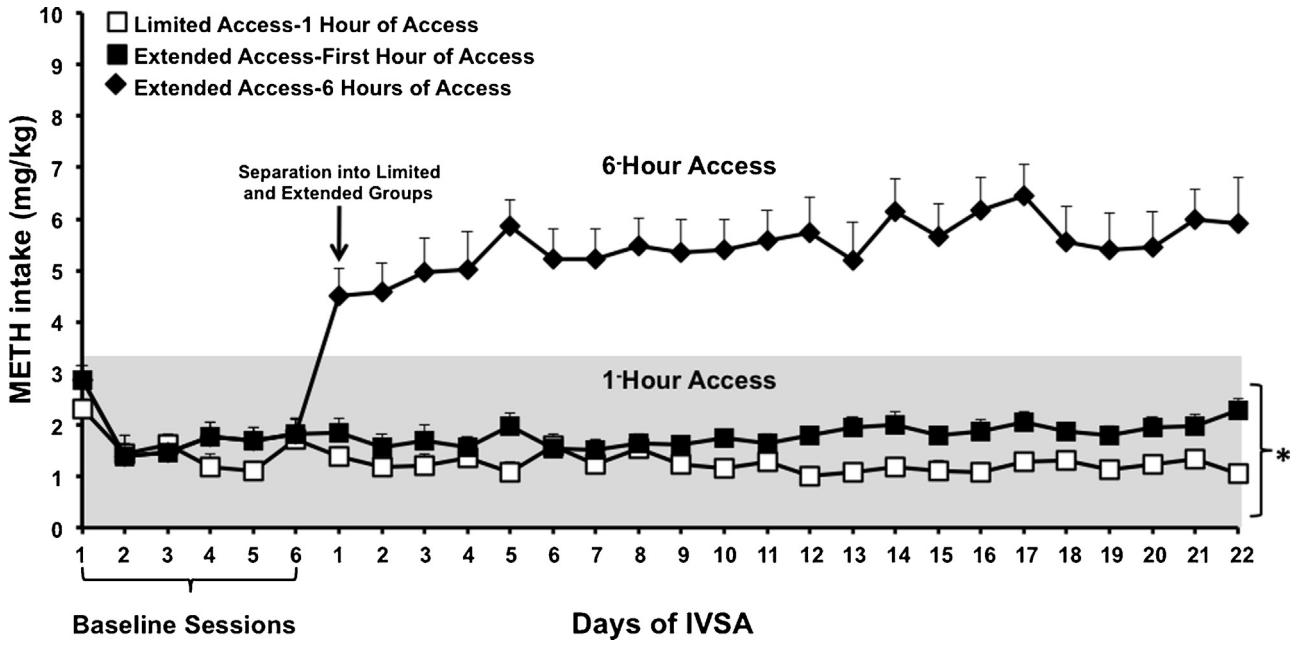

Fig. 1. These data reflect daily consumption (mg/kg ±SEM) during 6 baseline sessions and after the animals were separated into limited ($n = 7$) and extended ($n = 10$) access conditions. The data are presented during the 1st h of drug access (shaded region) and during 6 h of IVSA in the extended access group. Rats that were given extended access displayed higher levels of METH intake during the 1st h of METH access as compared to rats given limited access to this drug ($^*p \leq 0.05$).

states as compared to rats given limited (1 h) access [12]. Thus, the present study compared DAT, TH and D2 receptor levels in the striatum 72 h after withdrawal from limited and extended access to METH IVSA.

2. Methods

2.1. Subjects

Adult male Wistar rats (Harlan Inc.) weighing 300–350 g were pair-housed in a reversed light cycle vivarium. Food and water were provided ad libitum. However, the rats did not have access to food during the IVSA sessions and for approximately 1 h prior to the food training sessions. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the UTEP Institutional Animal Care and Use Committee. Ten rats were removed from the study due to catheter patency and/or mechanical issues.

2.2. Operant procedures

IVSA procedures have been described previously, with a few minor modifications in the current study [13]. Briefly, rats were trained to press for food pellets prior to IV catheterization. Following surgical recovery, the rats were given 6 sessions of METH IVSA (0.05 mg/kg/0.1 ml infusion) each lasting 1 h in order to establish baseline intake. Following each drug infusion, a 20-s cue light was illuminated over the drug lever that signaled a time-out period where responses on the lever had no scheduled consequences. The initial baseline values were similar across groups of rats that were given limited (1 h; mean baseline value = 1.56 mg/kg) or extended (6 h; mean baseline value = 1.83 mg/kg) access to METH IVSA. Following baseline testing, the rats were given limited or extended access to METH IVSA for 22 additional days. The dose and number of sessions were based on the work of Kitamura et al. [14]. Control rats were catheterized and post-operatively cared for in the same manner as the rats that received METH IVSA. The control rats did receive operant training for food pellets and they remained drug naïve throughout the duration of the study.

2.3. Protein analyses

Seventy-two hours after the final IVSA session, the rats were sacrificed and striatal tissue was bilaterally isolated. Homogenates were subsequently adjusted to 30 μg of protein in 20 μl volume and subjected to SDS-PAGE and Western blotting for DAT (SC-1433, Santa Cruz Biotechnologies), TH and D2 receptors (MAB318 and AB5084P respectively; Millipore Corporation) or actin (Sigma–Aldrich). Bands were visualized by chemiluminescence and evaluated via densitometry using the NIH ImageJ program. Total DAT (72 kDa), TH (56 kDa) and D2 receptor (52 kDa) protein levels were normalized to total actin (42 kDa).

2.4. Statistics

METH intake (mg/kg/day) was analyzed using repeated measures ANOVA with time (days of IVSA) as a repeated measure and access condition (limited versus extended) as a between subject variable. This approach was first applied to the 6 baseline days. Subsequent analyses were made using the first hour of METH access across 22 days of IVSA in limited versus extended access conditions. Separate analyses were conducted on each protein marker using one-way ANOVA, with subsequent post-hoc comparisons of group differences where appropriate. Significance was determined throughout our analyses at the $p \leq 0.05$ level.

3. Results

3.1. METH IVSA

Fig. 1 depicts METH consumption during baseline and subsequent METH IVSA days. Prior to separation into limited or extended access conditions, all rats were given 6 days of 1-h access to METH IVSA. Our analysis of METH consumption during baseline revealed that there were no pre-existing differences between rats