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Long-term effects of exposure to methamphetamine in adolescent rats

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ABSTRACT

Background: Flexible cognition is a set of processes mediated by the prefrontal cortex (PFC), an area of the brain that continues to develop during adolescence and into adulthood. Adult rodents exhibit impairments specific to reversal learning across various dosing regimens of methamphetamine (mAMPH). For adolescent rodents, ongoing PFC development can be assessed by discrimination reversal learning, a task dependent on frontostriatal integrity. The task may also index an increased vulnerability for mAMPH sampling in adulthood.
Methods: The purpose of the present study was to investigate the long-term effects of escalating, adolescent mAMPH exposure on reversal learning, a PFC-dependent task (Experiment 1) and the likelihood of later sampling of mAMPH in adulthood (Experiment 2).
Results: Unlike previous research in adult-treated rats, our results show more generalized learning impairments after adolescent mAMPH exposure to include both attenuated visual discrimination as well as reversal learning. Additionally, we found that rats pre-exposed to mAMPH during adolescence consumed significantly more drug in adulthood. Intake of mAMPH was positively correlated with this learning.

Taken together, these findings show that even modest exposure to mAMPH during adolescence may induce general learning impairments in adulthood, and an enduring sensitivity to the effects of mAMPH.

1. Introduction

A multitude of studies in human methamphetamine (mAMPH) users have documented cognitive impairments associated with protracted mAMPH abuse (Gonzalez et al., 2007; Woods et al., 2005; cf. Hart et al., 2012). Preclinical models of mAMPH exposure have been crucial in our understanding of the mechanisms by which mAMPH can lead to such impairments. Administration of either binge or escalating dose mAMPH in adult rats results in a broad array of learning and memory impairments (Belcher et al., 2005; Clark et al., 2007; Herring et al., 2010; Reichel et al., 2012; Kosheleff et al., 2012).

Cognitive flexibility and inhibitory control are constructs central to adaptive decision making, and the detrimental effects of mAMPH on these processes have received attention recently. These processes can be indexed, in part, by reversal learning across species (Izquierdo and Jentsch, 2012). Moderate to high binge doses of mAMPH result in impairments on response reversal (Cheng et al., 2007; cf. Daberkow et al., 2008), and visual discrimination reversal. Previously, we reported impairments specific to reversal learning after either binge dose, single dose, or escalating dose mAMPH, with discrimination learning and retention unaffected (Izquierdo et al., 2010; Kosheleff et al., 2012). Thus, as outlined here, there is abundant evidence for the long-term consequences of adult mAMPH exposure on cognitive flexibility as measured by reversal learning, yet this process in the adolescent period remains relatively unexplored. The study of this developmental period is of great value to understanding the progression to addiction since the initiation of drug use frequently occurs in adolescence in humans, particularly in the late teens (Patton et al., 2004; Schramm-Sapayta et al., 2009). Adolescents also represent a high prevalence of mAMPH users, with 1.4 million 12 years of age and older as documented users (SAMHSA, 2004).

The primary focus of the present study was to investigate the long-term effects of extended mAMPH exposure in adolescence on visual discrimination and reversal learning (Experiment 1). To our knowledge, there has not yet been a study that directly investigates the long-lasting effects of mAMPH in adolescence on this type of learning in adulthood. The secondary focus of the present study was to explore whether rats would exhibit differential voluntary

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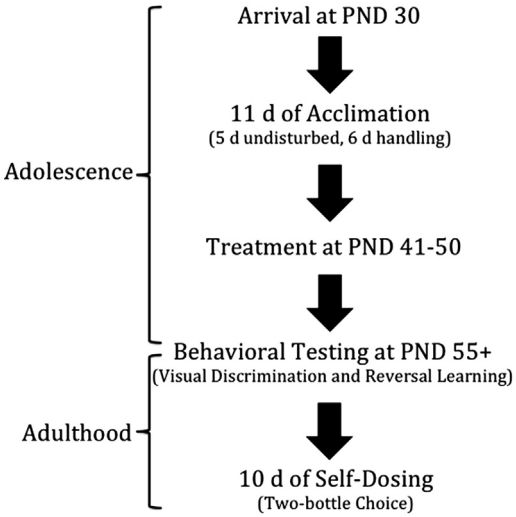

Fig. 1. Experiment timeline. Rats arrive at PND 28 followed by 12 d of habituation to the vivarium. Handling and weighing of rats began on the 7th d. Treatment began at PND 41–50, followed by a 5-d washout period. Behavioral pretraining began at PND 55. The self-dosing phase started after behavioral testing was completed (PND 119–173).

mAMPH sampling in adulthood after being exposed to mAMPH during adolescence (Experiment 2). In conjunction with Experiment 1, our findings may add a novel, longitudinal dimension to the literature on the cognitive effects of mAMPH.

2. Methods

2.1. Subjects

Eighteen male Long–Evans rats (Charles River Laboratories, Inc.) arrived at postnatal day (PND) 28 weighing between 76 and 100 g, and were socially housed 2 per cage, except during behavioral testing in Experiment 1 and during the 10-d duration of Experiment 2 (see Section 2.7). Rats were habituated to the vivarium from PND 28 to 33, and experimenter handling began at PND 34. Each rat was handled for a minimum of 10 min, once per day, and weights were recorded 3 times per week. The vivarium maintained a 12-h light/12-h dark cycle, with the temperature constant at 22 °C. Food and water were available ad libitum until behavioral testing. Treatment and behavioral testing took place between 0800 and 1600 h, as previously reported (Izquierdo et al., 2010; Kosheleff et al., 2011). All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, 2013) and approved by the CSULA Institutional Animal Care and Use Committee. See Fig. 1 for experimental timeline.

2.2. Experiment 1

Rats in this experiment were treated with mAMPH or SAL from PND 41 to 50 (late adolescence), and were assessed on post-treatment learning assays in adulthood. The testing paradigm has been used in previous work from our lab (Izquierdo et al., 2010).

2.3. Apparatus

Operant conditioning chambers measuring 35 cm in length, 28 cm wide, and 34 cm high (#80004, Lafayette Instrument Co., Lafayette, IN) were housed within sound- and light-attenuating cubicles (#83018DDP, Lafayette Instrument Co.). Each chamber was equipped with a houselight, tone generator, and a 12″ LCD touchscreen (EloTouch, Menlo Park, CA) in lieu of the wall opposing the pellet dispenser. The pellet dispenser delivered single 45 mg dustless sucrose pellets (BioServ, Frenchtown, NJ). Custom software (Ryklin Software Inc., NY) controlled touchscreen stimuli presentation, tone generation, houselight illumination, and pellet dispensation.

2.4. Drug treatment

Spear (2000) considered the age range of Post Natal Day (PND) 28–42 as the early adolescent period in the rat, paralleling human adolescence ages 12–18 years old. More recent studies affirm that PND 28–60 encapsulates the entire adolescent period in the rat (Laviola et al., 2003; Marco et al., 2011). Additionally, two other groups have treated rats with brief, high-dose mAMPH during late adolescence (PND 50–51) and have observed learning deficits (Vorhees et al., 2005; White et al., 2009). We chose our treatment period of PND 41–50, with these factors in mind. Rats were transferred from their housing colony room to a treatment room and given subcutaneous injections of D-mAMPH (Sigma, St. Louis, MO) or physiological saline (SAL) (10 ml/kg) once per d, for 10 consecutive d from PND 41 to 50. Before daily injections, rats were acclimated to the treatment room and left undisturbed for 30 min. Rats were randomly assigned to two treatment groups: (1) mAMPH group ($n = 10$) received mAMPH beginning at 0.3 mg/kg and escalating in 0.3 mg/kg increments per d, culminating to 3.0 mg/kg, (2) SAL group ($n = 8$) received a SAL treatment regimen identical to the mAMPH group. The order of injections was administered according to a latin-square design.

2.5. Behavioral testing

2.5.1. General. Rats were tested in three cohorts of 6 rats each. All behavioral training and testing took place five d per week, one session per d, with each session lasting a maximum of 45 min. After the last d of drug treatment (PND 50), rats received a 5-d washout period. During this period, rats were individually housed one per cage and left undisturbed with food and water ad libitum. During the last two d of this period, rats were fed 10 sucrose pellets in their homecage to familiarize them with the food rewards.

2.5.2. Food restriction. Beginning on the final d of the washout period, all rats were then single-housed and food-restricted to no less than 85% of their free-feeding body weight, while water was always available ad libitum. The weight of each rat was recorded three times per week to ensure a healthy body weight. New 85% minimum weights were calculated and observed throughout the study. Age-matched growth curves provided by the vendor were used for comparison to ensure mAMPH-treated rats fell within normal growth range.

2.5.3. Visual discrimination learning. After a series of pretraining phases outlined in detail previously (Izquierdo et al., 2012; Izquierdo et al., 2013), rats were shown two concurrently presented stimuli on each trial. One stimulus coincided with a reward and the other, a punishment. The designation of the reward stimulus was counterbalanced across each treatment group and the presentation of both stimuli alternated on the left and right side of the screen in a pseudorandom order predetermined by the custom software. The appearance of the stimuli remained on screen for 20 s, the absence of a nosepoke on either stimuli within the allotted time resulted in an 'omitted' trial. Each trial was separated with a 10-s inter-trial interval (ITI) before the initiation of the next trial. In order to advance to the next stage of testing, rats were required to reach a criterion of at least 85% correct nosepokes (minimum of 60 correct responses with all pellets consumed) within 45 min, for two consecutive d.

2.5.4. Reversal learning. Rats were required to respond to a reversal of the reward contingency: a nosepoke on the previously correct stimulus results in a punishment, and nosepoking the previously incorrect stimulus now results in a sucrose pellet reward. Methods and criterion were identical to those described above.

2.6. Experiment 2

Upon completion of behavioral testing, consumption of mAMPH vs H_2O (5 d) and then quinine vs H_2O (5 d) was measured, using methods similar to previous work performed in mice (Wheeler et al., 2009; Shabani et al., 2011).

2.6.1. Self-dosing. Rats that had received mAMPH or SAL during adolescence had voluntary access to consume pure H_2O or the drug or bitter tastant dissolved in H_2O for 18 h each d. H_2O was offered during the remaining 6 h of each d. The 18-h period was based on studies in mice showing that mAMPH intake is greater under an intermittent access schedule, compared to 24-h access (Phillips, unpublished).

2.6.2. Drug and tastant. Ten mg of mAMPH or 5.6025 mg of quinine hemisulfate salt monohydrate (Sigma, St. Louis, MO) was dissolved in 1 l of H_2O. Due to the bitter taste of mAMPH, the quinine tastant was used to ensure that taste could not account for a difference in consumption of the mAMPH between the adolescent exposure groups. These concentrations were reduced from those used in mice to account for somewhat greater drug sensitivity of rats, and to avoid rejection that can occur at higher concentrations (Shabani et al., 2011).

2.7. Two-bottle choice

2.7.1. General. Rats were first familiarized for 5 d drinking H_2O from two "bottles:" 50 ml plastic centrifuge tubes, sealed with a 3.8 cm diameter rubber stopper embedded with a 6.3 cm open tip stainless steel sipper tube. Rats were then offered the opportunity to voluntarily consume mAMPH and quinine in a two-bottle choice design in which each rat had concurrent access in the homecage to: (1) one bottle of pure H_2O and one bottle of H_2O mixed with mAMPH, and then (2) one bottle of pure H_2O and one bottle of H_2O mixed with quinine. Tubes filled with H_2O were placed in an empty cage to account for leakage.