Behavioural Brain Research 349 (2018) 16–24

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Age- and sex-dependent effects of methamphetamine on cognitive flexibility and 5-HT_{2C} receptor localization in the orbitofrontal cortex of Sprague-Dawley rats

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ARTICLE INFO

Keywords:
Adolescence
Sex differences
Cognitive flexibility
Orbitofrontal cortex
Serotonin-2C receptors
Parvalbumin

ABSTRACT

Adolescents and females experience worse outcomes of drug use compared to adults and males. This could result from age- and sex-specific consequences of drug exposure on brain function and cognitive behavior. In the current study, we examined whether a history of intravenous methamphetamine (METH) self-administration impacted cognitive flexibility and 5-HT_{2C}R localization in the orbitofrontal cortex (OFC) in an age- and sex-dependent manner. Strategy shifting was assessed in male and female Sprague-Dawley rats that had self-administered METH (0.08 mg/kg/inf) or received non-contingent infusions of saline during periadolescence or young adulthood. After all rats reached adulthood, they were tested in an operant strategy shifting task and their brains were subsequently analyzed using immunofluorescence to quantify co-localization of 5-HT_{2C} receptors with parvalbumin interneurons in the OFC. We found that adolescent-onset females were the only group impaired during discrimination and reversal learning, but they did not exhibit changes in localization of 5-HT_{2C} receptors. In contrast, adult-onset males exhibited a significant increase in co-localization of 5-HT_{2C} receptors within parvalbumin interneurons in the left hemisphere of the OFC. These studies reveal that age and sex differences in drug-induced deficits in reversal learning and 5-HT_{2C}R co-localization with parvalbumin interneurons are dissociable and can manifest independently. In addition, these data highlight the potential for certain treatment approaches to be more suitable in some populations compared to others, such as alleviating drug-induced cognitive deficits as a focus for treatment in adolescent females.

1. Introduction

Females and adolescents disproportionately account for users that experience poor outcomes of drug use [1–4] and in these populations methamphetamine (METH) use is especially problematic [5]. Females initiate METH use at younger ages and transition to disordered use more rapidly than males [3,6], which is of particular concern given that METH use during adolescence is associated with increases in risky sexual behaviors, teen pregnancy, and the use of other drugs [7]. Moreover, the use of METH among adolescents has remained constant in recent years even though the rates of alcohol and tobacco use have declined [8]. To better understand and address the potentially unique complications of METH and other substance misuse in adolescents and females, a more complete understanding of drug-induced cognitive deficits and neuroadaptations in these populations is needed. In fact, one approach that has been used to understand differential vulnerability to substance use disorders (SUDs) has been investigation of the mechanisms underlying drug-induced cognitive dysfunction [9–12], particularly since deficits in working memory, attention, and inhibitory control are associated with poorer treatment outcomes [13–16].

In laboratory rodents, psychostimulant exposure during adolescence induces similar [17,18] or more severe [19–22] cognitive impairments than exposure during adulthood (for review see [12]). Disrupted inhibitory control of prefrontal cortex (PFC) neural circuits has been hypothesized to underlie drug-induced cognitive deficits [23,24] and recent work from our lab is consistent with this. Using in vitro whole-

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https://doi.org/10.1016/j.bbr.2018.04.047
Received 12 February 2018; Received in revised form 4 April 2018; Accepted 26 April 2018
Available online 30 April 2018
0166-4328/

cell recording we found that repeated amphetamine exposure during adolescence attenuated D_1 receptor-mediated inhibition in medial PFC pyramidal neurons in adulthood [25,26], potentially through reductions in D_1 receptor expression [27]. The important role of adaptations in medial PFC D_1 receptors in drug-induced cognitive dysfunctions was previously described by Fletcher et al. [28] who demonstrated that amphetamine-induced deficits in attention were alleviated by intra-PFC infusion of a D_1 receptor agonist.

In addition to dopamine, serotonergic signaling is critical for normal PFC function [29,30] and is thus another target through which vulnerability to develop SUDs may be influenced [31]. Modulation of inhibition in the PFC by serotonin is accomplished via multiple receptor subtypes that regulate excitability in a variety of neuronal populations [30,32,33]. This inhibitory modulation is mediated in part by 5-HT_{2C} receptors (5-HT_{2C}Rs; [34]), likely due to their preferential expression on parvalbumin-immunoreactive (PV-ir) interneurons [35]. These interneurons are the most abundant inhibitory neuron subtype in the PFC and they provide tight regulatory control of pyramidal cell output to subcortical structures [36,37]. As such, the preferential expression of 5-HT_{2C}Rs on PV neurons suggests that the behavioral effects associated with influencing their function may be the result of modulating inhibitory tone in the PFC. Reversal learning is one behavior that is sensitive to both 5-HT_{2C}R manipulations [38,39] and disrupted inhibitory transmission [40] in the orbitofrontal cortex (OFC), and is known to be impaired following repeated exposure to amphetamine or METH [17,41–43].

There is indirect behavioral evidence to suggest that repeated METH exposure changes 5-HT_{2C}Rs [44,45], but the impact of this exposure on 5-HT_{2C}R expression in the OFC is unknown. Based on the cellular distribution of 5-HT_{2C}Rs in the PFC [35] and evidence that antagonism of these receptors in the OFC improves reversal learning [39], we hypothesized that drug-induced increases in the co-localization of 5-HT_{2C}Rs on PV-ir interneurons could be a mechanism through which drug-induced deficits in cognitive flexibility manifest. To test this hypothesis, we examined strategy shifting and 5-HT_{2C}R co-localization with PV-ir interneurons in the OFC in rats with a history of intravenous METH self-administration that was initiated in adolescence or adulthood.

2. Methods

2.1. Subjects

Subjects were a total of 82 Sprague-Dawley rats (32 male, 50 female) that had previously been trained to self-administer *d*-methamphetamine HCl (0.08 mg/kg) or received non-contingent infusions of saline beginning during either adolescence or adulthood. The full methods outlining treatment of the METH rats used in this study and analysis of age- and sex-differences in METH self-administration was reported previously [46]. Of the 46 rats that initiated METH self-administration, 15 males and 25 females met acquisition criteria to be included in this study. The remaining male (n = 17) and female (n = 25) rats were littermates of the METH groups treated identically with the exception that they received non-contingent infusions of saline approximated to match the number of infusions earned by their METH counterparts. Briefly, subjects were offspring of rats bred in our colony, weaned on postnatal day (P) 22, and housed 2–3 per cage. Rats were housed on a 12 h light/dark cycle (lights off at 0900) and behavior was assessed during the dark cycle (0900–1700). Rats were implanted with indwelling jugular vein catheters on P32 or 82 (± 2 days) and had daily 2-h access to METH under increasing fixed ratio (FR) schedules of reinforcement (FR1 to FR5) or received non-contingent infusions of saline between P41-55 or P91-105, for adolescent- and adult-onset groups, respectively (Fig. 1). The last 7 days of self-administration (P56-62 and P106-112) consisted of four daily progressive ratio sessions (5-h) each separated by one 2-h maintenance session with METH available on an FR5 schedule. Rats were allowed ad libitum access to food and water except during the limited periods (≤ 4 h) noted below. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois, Urbana–Champaign, and were consistent with the Guide for the Care and Use of Laboratory Animals [47].

2.2. Apparatus

Strategy shifting took place in standard operant chambers (Coulbourn Instruments, Whitehall, PA) that were enclosed in sound-attenuating cubicles. The cubicles were equipped with fans that provided ventilation and masked extraneous noise. One wall of each operant chamber was equipped with a centrally located food trough outfitted on either side with retractable levers that were equidistant (87 mm) from the trough. White cue lights were located above each lever and a 2.9 kHz Sonalert speaker was located directly above the food trough. A white houselight was located near the chamber ceiling on the wall opposite the nosepoke ports. Graphic State software (v3.1; Coulbourn Instruments) was used for automated chamber control and data collection.

2.3. Strategy shifting

Five days after completion of self-administration sessions (on P67 or P117), male (n = 6–10/group) and female (n = 10–15/group) rats that received non-contingent infusions of saline or met acquisition criterion for METH self-administration (unit dose: 0.08 mg/kg/inf) began training for strategy shifting (Fig. 1). Food was removed 2 h prior to each daily session and returned 30 min to 2 h following its completion; rats were not otherwise food restricted and this procedure has no overall impact on body weight compared to rats fed ad libitum [48]. As we have described previously [17], rats were first trained across 4 sessions to lever press for a 45-mg food pellet (BioServ, F0021, Frenchtown, NJ). During the next 5 sessions, rats had to press the lever within 10-sec of its extension into the chamber to earn reinforcement. Subsequently, each rat's side bias was then established in a single session as described by Floresco et al. [49].

Daily strategy shifting sessions consisted of 120 trials that were separated by a 10-sec intertrial interval. Trials began when both levers were inserted into the chamber concurrent with illumination of one of the two cue lights located above the levers. Trials ended after either the rat pressed a lever or 10 s elapsed. Individual cue lights were presented pseudorandomly across trials, such that no cue light could be presented on more than two consecutive trials. Because all rats had prior exposure to cue lights during self-administration, the cue light illuminated 2-sec prior to trial onset during visual strategy sessions in order to increase its saliency [49].

Rats were first trained to use a visual strategy by reinforcing them (with delivery of a food pellet) for pressing the lever that had a cue light illuminated above it, regardless of the lever's spatial location (i.e., left or right side of pellet delivery trough). Trials continued until rats achieved eight consecutive correct choices and had completed at least 30 trials. On the day following acquisition of this performance criterion, rats were required to shift to an egocentric response strategy. This required that rats press a lever relative to its position, regardless of cue light location (e.g. always press the left lever). The location of the reinforced lever for the response strategy was determined individually for each rat such that it was the lever opposite of the rat's side bias that was observed during initial training (see above). Trials continued until rats achieved a criterion of eight consecutive correct choices.

Following acquisition of the response strategy, rats were overtrained on this response during a single session consisting of 150 trials. One day following the overtraining session, rats completed a within-session reversal of the learned response. During this session, rats were first required to demonstrate retention of the overtrained response by