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Research report

Age of exposure-dependent effects of amphetamine on behavioral flexibility

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HIGHLIGHTS

- Amphetamine impairs reversal learning early, but not late, in a set-shifting task.
- Adolescent amphetamine exposure facilitates strategy reversal learning.
- Amphetamine-induced sensitization is greater in rats exposed during adulthood.
- Results suggest that amphetamine induces lasting changes in cognitive flexibility.

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ABSTRACT

Drug use typically begins during adolescence, which is a period of ongoing neurobiological development that may confer heightened vulnerability to develop drug dependence. Previously, our lab has shown that amphetamine (AMPH)-induced deficits in a medial prefrontal cortex (mPFC)-sensitive working memory task are greater in rats exposed to the drug during adolescence compared to adulthood. Here, we examine potential age-dependent effects of AMPH exposure on behavioral flexibility tasks that are sensitive to disruptions in mPFC and orbitofrontal cortex (OFC) function. Male Sprague-Dawley rats were injected (i.p.) with saline or 3 mg/kg AMPH every other day between postnatal days (PNDs) 27–45 and PNDs 85–103. Starting around PND 125, rats were tested in an attentional set-shifting task and a subset of those was then tested in an operant strategy shifting task. Following completion of the operant task, rats were challenged with 3 mg/kg AMPH and monitored in open field chambers. Our results demonstrate that AMPH-exposed rats were faster to acquire simple and compound discriminations, but were impaired during the first stimulus-reward reversal when compared to controls. In the operant strategy shifting task, adolescent-exposed rats shifted more rapidly between strategies and completed reversals faster than adult-exposed and control rats, respectively. The final AMPH challenge revealed evidence for sensitization in drug pre-exposed rats, with adult-exposed animals exhibiting the most significant effects. Together, these results suggest that AMPH induces long-lasting changes in behavioral flexibility that are at least partially dependent on age of exposure and may be due to adaptations in OFC function.

1. Introduction

Adolescence is a transitional period between childhood and adulthood, which in humans is estimated to range from 12 to as late as 25 years of age [1,2]. There are normal, yet substantial, neurobiological and cognitive changes occurring during this period [2–4], which also coincides with the peak time period of initiation of drug use [5]. This is of significant concern because an early onset of drug use is associated with a number of negative outcomes, including continued use into late adulthood [6], a greater likelihood to transition to dependence [7] and the development of significant cognitive dysfunction [8]. Importantly, cognitive impairment resulting from drug exposure during adolescent development may play a significant role in the heightened vulnerability of adolescents to developing addiction. Indeed, cognitive deficits in tasks sensitive to the prefrontal cortex (PFC) are observed in drug abusers [9,10] and have been linked to poorer treatment outcomes [11].

Studies in humans and laboratory animals have demonstrated that the PFC is among the last brain regions to fully mature [12]. For example, longitudinal MRI studies have demonstrated that gray matter in the PFC follows an inverted U-shaped curve that

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peaks during early to mid-adolescence and declines to stable levels by the early twenties [13]. Furthermore, post-mortem findings have shown that synapse elimination continues to occur in the PFC during adolescence [14,15]. Similar effects have been observed in rodents during their adolescent period, which has been defined as broadly as postnatal days (PNDs) 21–60 [16], but more conservatively as PNDs 28–45 [2]. During this time, there is a loss of neurons in the medial PFC (mPFC) [17] and changes in the molecular composition of synapses in this region [18]. Widespread changes in neurochemical signaling and connectivity with other brain regions is also ongoing during this time. For example, there are significant changes in the density of monoamine transporters [19] and dopaminergic fibers in the PFC [20], as well as alterations in the number of receptor sites. In the case of dopamine D_1 and D_2 receptors in the PFC and nucleus accumbens (NAc), there is an initial period of overproduction followed by elimination as animals reach young adulthood [21,22]. Importantly, these receptors are known to differentially modulate NMDA receptors in the PFC and NAc between adolescence and adulthood [23–26].

In psychostimulant abusers, deficits have been observed in PFC-sensitive tasks assessing attention [27–29], behavioral inhibition [30], and decision-making [31]. In rodents exposed to amphetamine (AMPH) during adulthood, there is evidence for impairments in extradimensional shifts and reversal learning in an attentional set shifting task (ASST) [32], impaired visual attention in a five-choice serial reaction time task [33], and impaired impulse control in a differential reinforcement of low rates of responding task [34]. Recent studies, which have focused on the potential for age-dependent differences in the effects of repeated drug exposure on cognition, have suggested that adolescents may be more vulnerable to drug-induced deficits in performance [35]. For example, studies from our laboratory have shown that pre-exposure to AMPH leads to more significant deficits in working memory when that exposure occurs during adolescence compared to adulthood [36].

The goal of the current study was to further examine the potential for age of exposure-dependent effects of AMPH on cognition. To do this, we tested adult rats exposed to saline or AMPH during adolescence or young adulthood in an ASST that has previously been shown to be sensitive to manipulations that disrupt functioning in the PFC [37]. Specifically, performance on the extradimensional shift is known to be sensitive to lesions of the mPFC [38], whereas lesions to the orbitofrontal cortex (OFC) impair reversal learning [39]. Based on our previous findings of AMPH-induced disruptions in working memory [36] and the long lasting effects of adolescent AMPH exposure on response perseveration in adulthood [40], we hypothesized that rats treated with AMPH would be impaired on reversal learning and the extradimensional shift. A subset of rats that completed the ASST were tested in an operant strategy shifting task that has been argued [41] to assess behavioral flexibility and be sensitive to mPFC function in a manner similar to the ASST. Thus, we hypothesized that performance in the ASST would correlate to that on the operant-based task, with AMPH pre-exposure causing deficits in behavior. In order to determine if there were lasting effects of drug pre-exposure on AMPH-induced motor activity, rats that concluded operant testing were subsequently observed in an open-field arena following a challenge injection of AMPH.

2. Methods

2.1. Subjects

Subjects were sixty-six male Sprague-Dawley rats born from vendor-obtained breeders (Harlan; Indianapolis, IN, USA) that were maintained in our facility. They were weaned on PND 22 and housed 2–3 per cage with same-sex littermates. Starting between PND 119–120, rats were food restricted so that they could be maintained at ≥85% of their free-feeding body weights; water was available ad libitum throughout the study. Rats were kept on a 12-h light/dark cycle (lights on at 0800) with experiments performed between 0830 and 1830. 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* (National Research Council, 2011).

Table 1
Order of training for the seven discriminations sets used in the ID/ED set-shifting task. In the example illustrated here, texture (T) was the constant stimulus dimension across all discriminations and the stimulus depicted in bold was baited. For SD through IDR, odor (O) was the relevant dimension and digging medium (D) was irrelevant. For EDS and EDR, D was the relevant dimension and O was the irrelevant dimension. Numbers refer to specific stimuli (e.g., O1 = Lavender).

Discrimination	Odor	Digging medium	Texture
Simple discrimination (SD)	**O1**, O2	None	None
Compound discrimination (CD)	**O1**, O2	D1, D2	T1
Compound discrimination reversal (CDR)	O1, **O2**	D1, D2	T1
Intradimensional shift (IDS)	**O3**, O4	D3, D4	T2
Intradimensional reversal (IDR)	O3, **O4**	D3, D4	T2
Extradimensional shift (EDS)	O5, O6	**D5**, D6	T3
Extradimensional reversal (EDR)	O5, O6	D5, **D6**	T3

2.2. Pre-treatment

Rats were assigned to one of three treatment groups – control, adolescent-exposed, or adult-exposed – such that all groups were represented in each litter. Injections (i.p.) were given every other day during both adolescence (PND 27–45) and young adulthood (PND 85–103) as follows: those assigned to the control group were given 0.9% saline (1 ml/kg) at both time points, those in the adolescent-exposed groups were given 3 mg/kg *d*-AMPH sulfate during adolescence and saline during adulthood, and those in the adult-exposed groups were given saline during adolescence and 3 mg/kg *d*-AMPH sulfate during adulthood. This dose of AMPH, which was calculated based on the weight of the salt, was chosen based on our previous studies demonstrating its ability to induce long-lasting changes in behavior following adolescent or adult exposure [40,42]. For this treatment, animals were transported to a testing room, given their assigned injection, and placed individually in a clear plastic tub (46 cm × 25 cm × 22 cm) lined with hardwood bedding. After 60 min, rats were returned to their home cages and the colony room.

2.3. Attentional set-shifting task

Starting on ~PND 125, which was at least 5 days after they began food restriction, rats started training in an ID/ED version of an ASST (adapted from [38] and [43]). Training and testing took place in a clear plastic arena (74 cm × 30 cm × 50 cm), with access to either side of the arena controlled by insertion or removal of a divider. Terra-cotta pots with a height of 10 cm and an internal diameter of 11 cm were used and outfitted with stimuli for discriminations as described below. Rats were first trained to dig in a pot filled with corncob bedding for food reinforcement (45-mg Bioserv pellets). Food pellets were buried increasingly deeper in the pot until rats were reliably digging to retrieve pellets. Subsequently, they were trained to discriminate between pots based on one of three stimulus dimensions (texture, odor, or digging medium). A pair of pots that differed along one stimulus dimension (e.g. digging medium) was presented to rats by removing the divider to expose one end of the testing arena. On each trial, one of the pots was baited with a food pellet and the other remained un-baited. Residue from a crushed food pellet was placed in the un-baited pot to encourage the use of the presented stimulus dimension as the basis for discrimination rather than olfactory cues of the food pellet. At the start of each discrimination, rats were given four discovery trials during which they could dig in both pots without consequence. Choice of a particular pot was defined as displacement of the digging medium with the snout or paws. Following discovery trials, rats were allowed to dig in one pot only, as the non-chosen pot was immediately removed from the testing arena. Rats were trained in each discrimination until they met a criterion of six consecutive correct choices.

On the following day, rats were trained on a series of seven discriminations, with their movement to the next discrimination dependent on them meeting the training criterion of six consecutive correct choices (Table 1). First, rats were given a simple discrimination (SD) where the pots differed on a single stimulus dimension (e.g. odor). Next, they were progressed to a compound discrimination (CD) where all three stimulus dimensions were present on the pots. In this discrimination, the correct stimulus during the SD was maintained, an irrelevant dimension was added (e.g. digging medium) and the third dimension added was kept constant across all pots