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Adolescent exposure to methylphenidate impairs serial pattern learning in the serial multiple choice (SMC) task in adult rats

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ABSTRACT

The long-term effects of adolescent exposure to methylphenidate (MPD) on adult cognitive capacity are largely unknown. We utilized a serial multiple choice (SMC) task, which is a sequential learning paradigm for studying complex learning, to observe the effects of methylphenidate exposure during adolescence on later serial pattern acquisition during adulthood. Following 20.0 mg/kg/day MPD or saline exposure for 5 days/week for 5 weeks during adolescence, male rats were trained to produce a highly structured serial response pattern in an octagonal operant chamber for water reinforcement as adults. During a transfer phase, a violation to the previously-learned pattern structure was introduced as the last element of the sequential pattern. Results indicated that while rats in both groups were able to learn the training and transfer patterns, adolescent exposure to MPD impaired learning for some aspects of pattern learning in the training phase which are learned using discrimination learning or serial position learning. In contrast adolescent exposure to MPD had no effect on other aspects of pattern learning which have been shown to tap into rule learning mechanisms. Additionally, adolescent MPD exposure impaired learning for the violation element in the transfer phase. This indicates a deficit in multi-item learning previously shown to be responsible for violation element learning. Thus, these results clearly show that adolescent MPD produced multiple cognitive impairments in male rats that persisted into adulthood long after MPD exposure ended.

1. Introduction

Methylphenidate (MPD) is a psychostimulant that is related to caffeine, amphetamine, and cocaine (Urban and Gao, 2013). At the height of its use in the 1990s, more than 2 million children were prescribed MPD (Challman and Lipsky, 2000) and it continues to be the preferred pharmacotherapy for the treatment of attention-deficit–hyperactivity disorder (ADHD) (Gray et al., 2007; Teter et al., 2003; Urban and Gao, 2013). MPD has also been identified as a potential drug of abuse and its illicit use has been on the rise within the past decade (Teter et al., 2003). While acute and chronic low doses of MPD (as prescribed for licit use) have been shown to improve cognitive function in rodents (Arnsten and Dudley, 2005; Berridge et al., 2006; Mohamed et al., 2011) chronic high doses of MPD have been shown to create more deleterious effects on the brain which often persist into adulthood (Bolanos et al., 2003; Brandon et al., 2001; Carlezon et al., 2003; Gray et al., 2007; LeBlanc-Duchin and Taukulis, 2007; Mcdougall et al., 1999; Scherer et al., 2010). For example, adolescent rats chronically exposed to high doses of MPD demonstrate impaired emotional response, poor object memory, and increased cross-sensitivity to other stimulants in adulthood (Bolanos et al., 2003; Brandon et al., 2001; Carlezon et al., 2003; LeBlanc-Duchin and Taukulis, 2007). High doses of MPD in prenatal, juvenile, and adult animals have also been shown to cause cognitive deficits such as impairments in spatial memory, delayed alternation performance, and working memory (Arnsten and Dudley, 2005; Levin et al., 2011; Scherer et al., 2010).

These studies illustrate that the effects of prolonged exposure to MPD treatment on brain structure and function might vary according to the dose and pattern of drug administration, as well as the complexity of the task involved (e.g., Bethancourt et al., 2009). Given the widespread usage of MPD among humans during the developmentally sensitive periods of childhood and adolescence, understanding potential long-term effects on neuronal systems and resultant behaviors is desirable (e.g., Grund et al., 2006). However, research on the long-lasting effects of MPD during adolescence is limited, and little work has examined

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the effects of adolescent exposure to MPD on complex learning, especially effects that might persist after exposure ends. Thus the goal of the current study was to assess how exposure to MPD during adolescence affects complex learning and memory in adulthood in a rat model.

To assess potential long-lasting developmental effects of MPD, the current experiment utilized a dosing and testing schedule previously successful in studies of adolescent drug exposure effects on adult rodents (Fountain et al., 2008; Kelley and Middaugh, 1999; Kelley and Rowan, 2004; Pickens et al., 2013). Rats were exposed to either the drug or saline for a five-week period, then they were given a five-week drug-free period before behavioral assessment began. This procedure allowed the drug to clear the subject's system, enabling assessment of developmental effects as opposed to the direct effects of the drug.

To assess effects of adolescent MPD exposure on adult cognitive systems, the current experiment examined the effects of adolescent MPD exposure on serial pattern learning in a SMC task in adult rats. This task was designed to be a close analog of a nonverbal method used in human studies to evaluate higher-level cognitive functions (Fountain, 2006; Fountain and Benson, 2006; Fountain and Rowan, 2000; Fountain et al., 2007; Stempowski et al., 1999). Serial pattern learning requires the subject to learn to expect and react to a prearranged patterned series of events; that is, subjects must learn to produce highly-organized patterns of behavior (Fountain, 2006; Fountain and Benson, 2006; Fountain et al., 2008). In the SMC task, rats are required to learn complex serial patterns which have been shown to recruit multiple cognitive systems concurrently, including stimulus–response (S–R) learning, multiple item memory, and abstract rule learning (for a review see, Fountain et al., 2012). Furthermore, prior research has shown that adolescent exposure to another stimulant, nicotine, causes learning impairments in adulthood in the SMC task (Fountain et al., 2008; Pickens et al., 2013). Other work with the SMC task has also demonstrated that adolescent nicotine causes both impairment and facilitation of different aspects of pattern acquisition in the same adult rats (Renaud et al., 2015). The ability to characterize drug-related effects on multiple cognitive systems concurrently in the same animals makes serial pattern learning in the SMC task ideal for assessing the effects of adolescent exposure to MPD on complex learning in adulthood.

2. Methods

2.1. Animal care and drug treatment

All procedures were approved by the institution's Animal Care and Use Committee. 14 Long Evans male rats were received on postnatal day 21 (P21) and were individually housed in stainless steel hanging cages throughout the experiment with free access to food and water. They were randomly assigned to one of two treatment groups (saline = 6; MPD = 8) on P25. For five consecutive days each week for five weeks, subjects received daily intraperitoneal injections of 20.0 mg/kg of MPD in saline solution or saline based on their body weight (1 ml/kg). As this was the first experiment performed to assess the effects of adolescent exposure to MPD in the serial pattern learning task, the current study utilized a high dose model of MPD to maximize the likelihood of detecting possible effects lasting into adulthood. Following five consecutive days of dosing each week, rats received two consecutive days free of injections, thus mimicking the common clinical practice of giving children “weekend holidays” from methylphenidate (Martins et al., 2004). Following five weeks of this dosing schedule, rats were given a 35-day drug-free period prior to the initiation of serial pattern learning training in the SMC task described below.

2.2. Apparatus

Four Plexiglas shaping chambers (30 × 30 × 30 cm) with stainless steel mesh floors and a single nosepoke receptacle 5 cm above the floor on one wall were employed. Nosepoke receptacles were constructed from 3.0-cm diameter PVC pipe end caps painted flat black with infrared emitter-detector pairs mounted on the sides and a cue light mounted in the rear of the receptacle. A solenoid (General Valve Corp., 20 psig, 24 V) was attached by tubing to a water opening at the bottom of each receptacle. A 20 ml syringe served as a water reservoir for each receptacle. Each shaping chamber was housed in a separate particleboard sound-attenuating shell.

Four clear Plexiglas training/test chambers were octagonal in shape (15 cm wide × 30 cm tall with 40 cm separating opposing walls) with a stainless steel mesh floor (Fig. 1). One nosepoke receptacle, as described above, was centered 5 cm above the floor on each of the eight chamber walls. Each test chamber was housed in a separate particleboard sound-attenuating shell.

2.3. Procedure

2.3.1. Shaping procedure

A timeline of experimental procedures is depicted in Fig. 2. Before experimental testing began, all rats underwent shaping for two days after 48 h of water deprivation. In 1-h nosepoke shaping sessions, the receptacle light was illuminated at the beginning of each trial. When the rat responded, the light was extinguished and a water droplet was delivered. A 1-s intertrial interval separated shaping trials. Rats were required to complete 240 nosepokes per day in the shaping chambers in order to continue to the training phase. All rats were successful in completing the shaping training.

2.3.2. Training phase: acquisition of a perfect pattern

After every second day of training, rats drank freely until satiated (about 5 min); subsequently, the water was removed to continue water deprivation. Starting on P95, rats performed 5 repetitions of the following “perfect” serial pattern: 123-234-345-456-567-678-781-812, every day for 49 days. As described above, the integers refer to the clockwise position of the 8 nosepoke receptacles while dashes indicate 3-s intertrial intervals (ITIs) that served as phrasing cues. An intertrial interval of 1 s was imposed between elements within each 3-element chunk. Additionally, a 3-s pause was also positioned between patterns to serve as an interpattern interval. The first digit of each

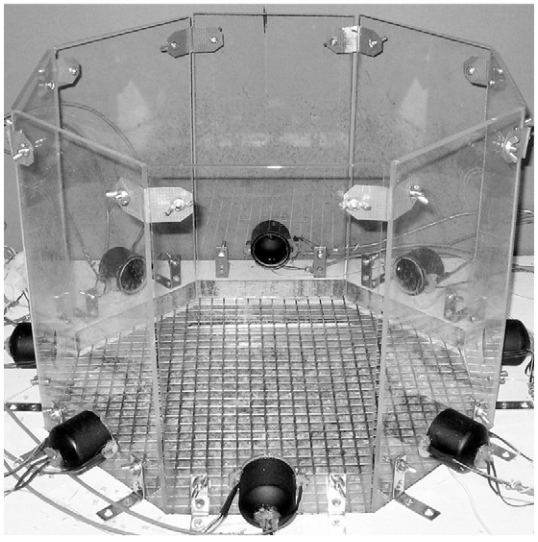

Fig. 1. Octagonal operant chamber used for serial pattern learning training. Chamber is made up of 8 walls each equipped with a nosepoke receptacle. Each receptacle contained an infra-red emitter and detector which were located on the left and right sides as well as a white LED cue light positioned on the back of the receptacle. An opening located at the bottom of each receptacle, connected to a solenoid and syringe by plastic tubing, served to deliver water to the chamber.