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Caudate neuronal recording in freely behaving animals following acute and chronic dose response methylphenidate exposure

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ABSTRACT

The misuse and abuse of the psychostimulant, methylphenidate (MPD) the drug of choice in the treatment of attention deficit hyperactivity disorder (ADHD) has seen a sharp uprising in recent years among both youth and adults for its cognitive enhancing effects and for recreational purposes. This uprise in illicit use has lead to many questions concerning the long-term consequences of MPD exposure. The objective of this study was to record animal behavior concomitantly with the caudate nucleus (CN) neuronal activity following acute and repetitive (chronic) dose response exposure to methylphenidate (MPD). A saline control and three MPD dose (0.6, 2.5, and 10.0 mg/kg) groups were used.

Behaviorally, the same MPD dose in some animals following chronic MPD exposure elicited behavioral sensitization and other animals elicited behavioral tolerance. Based on this finding, the CN neuronal population recorded from animals expressing behavioral sensitization was also evaluated separately from CN neurons recorded from animals expressing behavioral tolerance to chronic MPD exposure, respectively.

Significant differences in CN neuronal population responses between the behaviorally sensitized and the behaviorally tolerant animals were observed for the 2.5 and 10.0 mg/kg MPD exposed groups. For 2.5 mg/kg MPD, behaviorally sensitized animals responded by decreasing their firing rates while behaviorally tolerant animals showed mainly an increase in their firing rates. The CN neuronal responses recorded from the behaviorally sensitized animals following 10.0 mg/kg MPD responded by increasing their firing rates whereas the CN neuronal recordings from the behaviorally tolerant animals showed that approximately half decreased their firing rates in response to 10.0 mg/kg MPD exposure. The comparison of percentage change in neuronal firing rates showed that the behaviorally tolerant animals trended to exhibit increases in their neuronal firing rates at ED1 following initial MPD exposure and oppositely at ED10 MPD rechallenge. While the behaviorally sensitized animals in general increased in their percentage change of firing rats were observed following acute 10.0 mg/kg MPD and the behaviorally sensitized 10.0 mg/kg MPD animals and a robust increase in neuronal firing rates at ED1 and ED10 rechallenge.

These results suggest the need to first individually analyze animal behavioral activity, and then to evaluate the neuronal responses to the drug based on the animals behavioral response to chronic MPD exposure.

1. Introduction

Methylphenidate (MPD) is an effective and readily prescribed treatment for attention deficit hyperactivity disorder (ADHD). MPD has been used successfully since the 1960s to treat ADHD symptoms. Despite its decade's old use, the long-term effects of this medication have remained largely uninvestigated. Owing to MPD's pharmacological similarity to cocaine (Bogle and Smith, 2009; Gainetdinov and Caron, 2003; Zhang et al., 2004), it is not surprising that there has been a rise in the illicit use of MPD (Greely et al., 2008). The neurochemical mechanism whereby MPD exerts its effects is still uncertain. It has been previously established that MPD acts predominately on the dopamine (DA) and norepinephrine (NE) system by binding to their transporter and thus blocking the reuptake of DA and NE into the presynaptic terminal, thus increasing the extracellular DA and NE levels in the synaptic cleft (Kuczenski and Segal, 2001; Teo et al., 2003; Nestler, 2001; Volkow et al., 1995). This increase in extracellular DA and NE following repetitive MPD exposure can lead to the phenomenon termed behavioral sensitization or behavioral tolerance.

Behavioral sensitization was defined as a significant further acceleration of locomotor activity following repetitive chronic MPD exposure when compared to the initial acute MPD response. Behavioral tolerance has been defined as an attenuation in locomotor activity following chronic drug exposure when compared to the initial effect of the drug or when no change in locomotion is observed from experimental day 10 (ED10) compared to ED1 (Dafny and Yang, 2006; Kalivas and

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Stewart, 1991; Robinson, 1984). Generally, it is believed that behavioral sensitization is due to synaptic plasticity in the CNS motive circuit, including the ventral tegmental area (VTA), the nucleus accumbens (NAc), prefrontal cortex (PFC), and CN (Pierce and Kalivas, 2007; Rebec, 2006). Some studies report that MPD elicits behavioral sensitization, whereas other studies report that the same dose of MPD results in behavioral tolerance (Barron et al., 2009; Eckerman et al., 1991; Gaytan et al., 1996, 2000; Yang et al., 2003, 2006a,b,c,d, 2007). The above observations lead to our hypothesis; some individual animals will display either behavioral sensitization while other individual animals will express behavioral tolerance to the same repetitive dose of MPD (0.6, 2.5, and 10.0 mg/kg).

Neuronal activation is known to be the underlying mechanism regulating behavior, therefore we endeavored to evaluate the CN neuronal responses recorded in animals exposed to acute and/or chronic MPD (0.6, 2.5, and 10.0 mg/kg) based on their behavioral response to repetitive (chronic) MPD exposure. Our second hypothesis is that the CN neuronal population responses following acute and chronic MPD exposure recorded from animals expressing behavioral sensitization to chronic MPD exposure will be different from the CN neuronal population responses to MPD recorded from animals expressing behavioral tolerance.

2. Methods

2.1. Animals

Forty-seven male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) were housed individually in clear acrylic cages upon arrival. Animals were allowed access to food and water ad libitum. The home cage was used also as the test cage throughout the experiment to eliminate environment interaction. Animals were allowed 5–7 days acclimation with 12 hour light/dark schedules (lights on at 6:00 am) prior to bilateral electrode implantation into the CN and during all the experimental days. Experiments were approved by our Animal Welfare Committee and carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.2. Surgeries

Prior to surgery, electrodes were made using twisted nickel–chromium diamel coated (fully insulated except at tips) 60 μm diameter wire secured to a 1 cm copper connector pin (A-M systems, Inc.). On the day of surgery the rats were anesthetized with 50 mg/kg pentobarbital intra-peritoneal (i.p.). The animal's head was shaved and the animal was placed into the stereotaxic apparatus where an incision was made on the scalp to remove the skin, connective tissue and muscle to expose the skull. Holes were drilled in the skull bilaterally above the CN at 0.5 mm anterior to bregma and 3.0 mm lateral from midline for electrode implantation in the CN. Coordinates were obtained from Paxinos and Watson (1986) brain atlas. Six anchor screws were inserted into the skull at various vacant spots to later secure the skull cap from being ripped out. Electrodes were inserted into the brain at an initial depth of 3.5 mm from the skull and neuronal activity was monitored using a Grass emitter Hi Z Probe connected to a Grass P511 series pre-amplifier aiming to obtain 3:1 signal to noise ratio spike activity. If the neuronal activity exhibited less than a 3:1 signal to noise ratio of spike activity, the electrode was lowered in 10 μm increments until a 3:1 ratio activity was observed to maximum depth of 4.5 mm (if ratio not obtained, electrode was removed to repeat steps) at which point the electrode was permanently secured to skull using web glue cyanoacrylate surgical adhesive (Claussen and Dafny, 2012; Dafny, 1980, 1982; Dafny and Terkel, 1990; Frolov et al., 2015). Similar procedures were followed for the second twisted electrode which was implanted in the CN of the opposite hemisphere. The four copper pins from the two twisted electrodes (four electrodes total per animal) were inserted into the amphenol plug which were secured to the skull using dental acrylic cement creating the skull cap. Animals were allowed three to five days recovery after electrode implantation, during these days, animals were placed in the experimental apparatus for two to three hours with their home cage and connected to the wireless (telemetric) head stage transmitter (Triangle BioSystems, Inc. (TBIS); Durham, NC, USA) for acclimation. On the first (initial) recording day, rats weighed approximately 200 g to 220 g.

2.3. Drugs

Methylphenidate hydrochloride (MPD) was obtained from Mallinckrodt (Hazelwood, MO, U.S.A.). MPD was dissolved in a 0.9% isotonic saline solution and the 0.6, 2.5 and 10.0 mg/kg MPD doses were calculated as a free base. Control injections consisted of 0.8 ml isotonic saline solution (0.9% NaCl), all injections were given i.p. and were equalized to a volume of 0.8 ml with 0.9% saline to keep injection volumes the same for all animals and were administered between 8:00 and 9:00 am.

2.4. Experimental protocol

During the recovery period from electrode implantation, the animal was placed in the faraday box with their home cage for daily acclimation to the wireless head stage for 2 to 3 h. On experimental day 1 (ED1), rats were placed again with their home cage in a Faraday testing box to reduce "noise" during recording. The wireless Triangle Bio Systems Int'l (TBSI), Durham, NC, USA; (Fan et al., 2011) head stage was connected to the electrode pins of the skull cap, and animals were allowed to acclimate to the recording system for an additional 30 min prior to the beginning of the recording session. During this acclimation time, the neuronal parameters of the neuronal recording were set to capture the electrophysiological activity. After acclimation, the animal received a saline injection of 0.8 ml (standardized for all injections) and the neuronal and behavioral activity was recorded concomitantly for one hour to obtain the baseline activity, followed by either saline or 0.6, 2.5, or 10.0 mg/kg MPD injection (depending on the group) with recordings resumed for an additional hour. At ED2–ED6 animals received daily injections in their home cage of saline or 0.6 or 2.5, or 10.0 mg/kg MPD without recordings followed by three days of washout (ED7–ED9) where no injections were given. At ED10, the animal, in his home cage, was again placed inside the faraday testing box to record the CN neuronal and behavioral activity similar to procedure done at ED1 (Table 1).

The wireless TBSI head stage sent neuronal activity signals to a receiver connected to a Cambridge Electronic Design (CED) analog-to-digital converter (Micro1401-3; Cambridge, England) which digitized the data and transports the digitized data to a PC using Spike 2.7 CED software for offline evaluation. To eliminate any environmental contribution,

Table 1
Summary of the experimental protocol. There were four groups of animals (saline, 0.6, 2.5 and 10.0 mg/kg MPD). On experimental day 1 (ED1) the behavioral and the neuronal activity was recorded for 1 h following saline injection and for another hour preceding initial saline, 0.6, 2.5 or 10.0 mg/kg methylphenidate (MPD) administration. On ED2 through ED6 a single saline, 0.6, 2.5 or 10.0 mg/kg MPD injections were given without recordings. Animals went through a washout stage on ED7 through ED9 in which no injections were given, recording (behavioral and neuronal) was resumed at ED10 following a saline injection and 1 h later following a rechallenge of either saline, 0.6, 2.5 or 10.0 mg/kg MPD administration.

Experimental days	1	2–6	7–9	10
Treatment group				
Saline	Saline/saline	Saline	Washout	Saline/saline
0.6 mg/kg	Saline/0.6 mg/kg	0.6 mg/kg	Washout	Saline/0.6 mg/kg
2.5 mg.kg	Saline/2.5 mg/kg	2.5 mg/kg	Washout	Saline/2.5 mg/kg
10.0 mg/kg	Saline/10.0 mg/kg	10.0 mg/kg	Washout	Saline/10.0 g/kg