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Acute and chronic methylphenidate alters prefrontal cortex neuronal activity recorded from freely behaving rats

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ABSTRACT

Today's students around the world are striking deals to buy and sell the drug methylphenidate (MPD) for cognitive enhancement. Our knowledge on the effects of MPD on the brain is very limited. The present study was designed to investigate the acute and chronic effect of MPD on the prefrontal cortex (PFC) neurons. On experimental day 1 (ED1) recordings were obtained following saline injections and after 2.5 mg/kg MPD. On ED2 through ED6, daily single 2.5 mg/kg MPD was given followed by 3 washout days (ED7 to 9). On ED10, neuronal recordings were resumed from the same animal after saline and MPD injection similar to that obtained at ED1. Ninety PFC units were recorded, all responded to the initial MPD injection, 66 units (73%) increased their activity at ED10. Recordings were resumed for the 66 units that increased their firing rate at ED1, and following MPD injection 54 units (82%) exhibited significant increases in their baseline firing rates compared to ED1 baseline. When these 54 units were rechallenged (chronic effect) with MPD, 39/54 (72%) exhibited reduction in their firing rate which can be interpreted as tolerance. From the 24 (27%) units that responded to MPD at ED1 by decreasing their activity, 14 units (58%) exhibited a decrease in their baseline firing rates at ED10 compared to ED1 baseline. However, following MPD rechallenge of these 14 units, 11 units (79%) exhibited an increase in their firing rate which is interpreted as sensitization. In conclusion, all PFC units modified their neural baseline activity.

1. Introduction

The psychostimulant (methylphenidate MPD) has become the most prescribed drug for attention deficit hyperactivity disorder (ADHD) (Accardo and Blondis, 2001; Arnsten, 2006; Challman and Lipsky, 2000; Eichlseder, 1985; Solanto, 1998; Swanson et al., 1999). It is estimated that 5 to 15% of the USA population between 5 and 18 years old is being treated with MPD (Anderson et al., 1987; Barbaresi et al., 2002; Froehlich et al., 2007; Rowland et al., 2001). MPD is a psychostimulant that has a chemical structure closely related to the structures of amphetamine and methamphetamine (Kallman and Isaac, 1975; Patrics and Markowitz, 1997; Teo et al., 2003). Both MPD and cocaine elicit their effect by binding to dopamine transporter (DAT) and preventing the reuptake of dopamine to the presynaptic terminal thereby increasing the extracellular dopamine concentration in the synaptic cleft (Gatley et al., 1999; Volkow et al., 1995, 1999) as well as enhancing dopamine release (Kuczenski and Segal, 1997; Segal and Kuczenski, 1997). Dopamine modulates prefrontal cortex (PFC) function through the action of dopamine, D_1, and D_2 family receptors (Arnsten, 2006). This area is the target of this study since it plays a prominent role in a complex neuronal system that serves to regulate cognitive performance and motor function (Goldman-Rakic, 1987). The PFC has an important role in attention regulation, inhibits responses to distracting stimuli and suppresses irrelevant thoughts (Wood and Grafman, 2003), as well as guides behavior and attention using working memory, applying representational knowledge to inhibit inappropriate actions, thoughts, and feelings (Anderson et al., 1999; Arnsten, 2006), and is suggested to be one of the main central nervous system sites of MPD action (Yang et al., 2006a,b,c,d).

In previous behavioral and neurophysiological experiments using an MPD dose response protocol, it was observed that acute low dose of MPD (0.6 mg/kg i.p.) failed to elicit alteration in locomotion as well as sensory evoked responses while moderate MPD administration (2.5 mg/kg i.p.) elicits locomotor activation (Gaytan et al., 1997, 2000; Yang et al., 2000a,b, 2003, 2006a, 2007, 2010) and suppresses the sensory evoked responses recorded from the PFC (Yang et al., 2006b). Higher dose of MPD (10.0 mg/kg i.p.) elicits further excitation on locomotion and further attenuation of the average sensory evoked responses in the PFC. Moreover, chronic MPD application elicits behavioral sensitization but causes further attenuation of the sensory evoked responses component following all the three MPD doses (0.6, 2.5, and 10.0 mg/kg i.p.) (Yang et al., 2006a,b,c,d, 2007).

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In a similar experimental procedure MDMA (Ecstasy) instead of MPD, was administered and acceleration of the PFC average sensory evoked responses were observed (Atkins et al., 2009).

The objective of this study is to investigate the acute and the chronic effect of MPD on PFC single unit activity recorded from freely behaving animals previously implanted bilaterally with permanent electrodes. This study is critical since today, on university campuses around the world, students are striking deals to buy and sell prescription drugs such as MPD (Ritalin) (Greely et al., 2008).

2. Material and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 170–180 g on the purchase day were each housed individually in the animal facility room with an ambient temperature of 21 ± 2 °C and relative humidity of 58–64%: food pellets and water were provided ad libitum. The room was illuminated on a 12:12 light/dark cycle (light on at 06:00). The initial 5–7 days were used for acclimation.

2.2. Electrode implantation

On the last day of the acclimation period, the rat was weighed and anesthetized with 50 mg/kg i.p. phenobarbital and placed into a stereotoxic instrument. The head was shaved and the head skin and muscle were retracted from the cranium and bilateral holes of 0.5 mm in diameter were drilled over the prefrontal cortex, 3.2 mm anterior to the Bregma, 0.6 mm lateral from the midline using the coordinate derived from Paxinos and Watson (1986) atlas. Nichrom wire electrodes 60 μm in diameter, insulated over their whole length except at the tip by Teflon were inserted into the holes 2.8 mm below the skull aiming to be in the PFC area 3 i.e. cingulate cortex. Unit activity was monitored during electrode penetration using Grass P511 and its cathode follower. If at this location there was no spike activity with signal to noise ratio of at least 3:1, the electrodes were moved down in steps of about 5 μm until they showed spike activity with good signal to noise ratio. Once good signal was obtained, the electrode was permanently fixed to the skull with dental acrylic cement and the second electrode in the other hemisphere was implanted in a similar way (Dafny, 1980; Dafny and Terkel, 1990; Dafny et al., 1973, 1979, 1981, 1983; Yang et al., 2006b,c). The electrode leads were attached to an amphenol plug and the latter was cemented to the skull.

Rats were allowed to recover from the surgical procedure for 5 to 7 days. During this recovery time, every day for 2 to 3 h, the rats were acclimated to the testing cage and were connected to Alpha Omega wireless transmitter located on the back of the animals which allowed the animals to move freely around the testing cage. The Alpha Omega transmitter digitized the neuronal activity and sent the signals to a receiver that connects to a PC that stores the data. Off line Alpha Omega software was used to sort the spike activity to evaluate the sorted spikes (single unit). Housing condition and experimental procedure were approved by our animal welfare committee. All efforts were made to minimize the number of animals used and their suffering. This study was conducted in accordance to the declaration of Helinski and approved by our institutional animal welfare committee.

2.3. Drugs

Vehicle injections consisting of 0.8 cc isotonic saline solution (0.9% NaCl) were administered. Methylphenidate hydrochloride (MPD) was obtained from Mallinckrot (Hazelwood, MO). MPD was dissolved in a 0.9% isotonic saline solution and the 2.5 mg/kg MPD was calculated as a free base. All injections were given intra-peritoneally (i.p) and equalized to a volume of 0.8 cc with 0.9% saline so that the volume of each injection was the same for all animals. In our previous MPD dose response experiments using behavioral and neurophysiological sensory evoked potential procedure, it was found that 2.5 mg/kg i.p. was the dose that elicited behavioral and neurophysiological sensitization (Algahim et al., 2009; Gaytan et al., 1996, 2000; Lee et al., 2009; Podet et al., 2010; Yang et al., 2000a,b, 2003, 2006a,b,c,d, 2007, 2010). Therefore, this dose was selected for this single unit experiment.

2.4. Experimental protocol

At experimental day 1 (ED1), animals were connected to the recording system and allowed 20–30 min acclimation before the recording session. Saline was injected (0.9% in 0.8 cc) and baseline neuronal recording for 30 min started immediately post saline injection followed by 2.5 mg/kg i.p. MPD injection and recording was resumed for an additional 120 min. From ED2 to ED6 rats were injected once a day in the morning with 2.5 mg/kg MPD in the test cage. ED7 to ED9 were the washout days, i.e. no injections were given. At ED10, an identical experiment as that in ED1 was performed with the neuronal recording following saline and following 2.5 mg/kg MPD (see Table 1). Similar experimental protocol was used previously (Yang et al., 2006a,b,c, 2007, 2010).

2.5. Histological verification of electrode placement

At the conclusion of the recording at ED10, the rats were overdosed with sodium pentobarbital. A small lesion was produced at the tip of each of the electrodes by passing a 50 μA DC current for 30 s. The rat's brain was transcardialy perfused with 10% formalin solution containing 3% potassium ferrocyanide. Brain slices were cut serially at a thickness of 40–60 μm and histologically stained with Cresyl violet. The position of the electrode tip was identified by the location of the lesion and the Prussian blue spot using the Paxinos and Watson (1986) Rat Brain Atlas.

2.6. Data analysis

2.6.1. Spike sorting

Off line spike sorting by Alpha Omega software was used. The Spike 2 version 7 software (Cambridge Electronics Design — CED) was used for spike sorting. The data was captured by the program and processed using low and high pass filters (0.3–3 kHz). 1000 waveform data points were used to define a spike to create. The spikes were extracted when the input signal enters the amplitude window (previously determined). Spikes with peak amplitude outside these limits were rejected. The algorithm that we used to capture a spike allows the extraction of templates that provide high-dimensional reference points that can be used to perform accurate spike sorting, despite the influence of noise, spurious threshold crossing and waveform overlap. All temporally displaced templates are compared with the selected spike event to find the best fitting template that yields the minimum residue variance. Secondly, a template

Table 1
Experimental protocol.

Experimental day	1	2–6	7–9	10
Treatment	Saline 2.5 mg/kg	2.5 mg/kg	Washout	Saline 2.5 mg/kg

On experimental day 1, neuronal recordings were performed after saline injection (baseline) for 30 min and continued for 120 min following 2.5 mg/kg injection of MPD. Experimental days 2–6 rats received a 2.5 mg/kg MPD administration in their home cage without recording. Days 7–9 were washout days (drug abstinence). Neuronal recordings resumed on experimental day 10 after saline (baseline) and 2.5 mg/kg MPD. Experimental day 1 and day 10 were treated with identical protocol.