Brain Research Bulletin 87 (2012) 387–396

Contents lists available at SciVerse ScienceDirect

Brain Research Bulletin

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

Research report

Acute and chronic methylphenidate modulates the neuronal activity of the caudate nucleus recorded from freely behaving rats

Catherine Claussen, Nachum Dafny*

Dept. of Neurobiology and Anatomy, University of Texas at Houston Medical School, Houston, Texas suite 7.5616, TX 77030, United States

ARTICLE INFO

Article history:
Received 12 October 2011
Accepted 17 October 2011
Available online 21 October 2011

Keywords:
Ritalin
Caudate nucleus
Electrophysiology
Behavior sensitization/tolerance
Single unit activity

ABSTRACT

Methylphenidate (MPD) is currently one of the most prescribed drug therapies for attention deficit/hyperactivity disorder (ADHD) and moreover is abused for cognitive enhancement and used for recreation by the young and adults. Methylphenidate is used for prolonged periods of time and its mechanism of action on the brain is still unknown. The main action of MPD is known to act on the motive circuit of the brain, and one of these structures is the caudate nucleus (CN). The objective of this study was to investigate the neurophysiological properties of the CN neurons in response to acute and chronic administration of MPD in freely behaving animals, previously implanted with permanent semi microelectrodes. Twenty-six rats were permanently implanted with semi microelectrodes into the CN using general anesthesia. On experimental day one (ED1) the rat was placed into the testing chamber, and neuronal activity was recorded using a wireless (telemetric) headstage device following both a saline and a 2.5 mg/kg MPD injection. From ED2 to ED6 daily injections of 2.5 mg/kg MPD were administered without recordings to induce a chronic effect of the drug, preceded by three days of washout (ED7–ED9) where no injections were given. On ED10 rats were placed back into the testing chamber, the wireless headstage device was attached to skull cap and recordings were resumed for 1 h each following both a saline and re-challenge administration of 2.5 mg/kg MPD. Sixty-seven CN neuronal recorded units from twenty-six animals with identical shape and amplitude at ED1 and ED10 were evaluated. All the 67 CN units responded to MPD administration, 70% (47/67) CN units exhibited an increase in activity following initial 2.5 mg/kg MPD administration and 30% (20/67) exhibited a decrease in neuronal activity. On ED10 all the CN units showed a significant change in their firing rate baseline compared to ED1 baseline, 52% (35/67) exhibiting an increase in their ED10 baseline activity compared to ED1 baseline activity and 48% (32/67) of the CN units at ED10 exhibited decreasing activity. All the CN units responded significantly to MPD rechallenge at ED10, 57% (38/67) of the units exhibited increased neuronal activity while 43% (29/67) exhibited decreasing neuronal activity. The results indicate that the majority of the CN units exhibited neurophysiological sensitization.

1. Introduction

Methylphenidate (MPD) is a psychostimulant prescribed to treat attention deficit hyperactivity disorder (ADHD) and moreover is currently used by non-ADHD youth as a cognitive enhancement [20]. The increasing use of MPD by non-ADHD youth makes it imperative to investigate the long-term neuronal adaptions that occur following repetitive MPD exposure. MPD (Ritalin) is known to have a chemical structure similar to amphetamine and neuropharmacological effects similar to cocaine [27,44,45], both MPD and cocaine bind to the dopamine transport (DAT) system and block the reuptake of DA from the presynaptic terminal, thus elevating the extracellular DA levels within the synaptic cleft [45,60]. Chronic exposure to MPD has previously shown to result in dose dependent behavioral tolerance or sensitization. Behavioral sensitization is known as the progressive augmentation of behavioral activity in response to repetitive psychostimulant exposure [7,24,41,51,53]. Behavioral sensitization is an experimental marker used to characterize a drugs ability to elicit dependence [17,36,41,56]. MPD is known to exert its effects mainly on the central nervous system (CNS) by passageways particularly in the motive circuit structures of brain. The motive circuit consists of the prefrontal cortex (PFC), nucleus accumbens (NAc), ventral tegmentum area (VTA), caudate nucleus (CN) and other CNS sites. The CN contains elevated levels of DA and damage to the CN dopaminergic pathway is known to modulate motor performance [2]. It has been previously reported that the main CN output is to the thalamus via direct and indirect

* Corresponding author. Tel.: +1 713 500 5616.
E-mail address: Nachum.dafny@uth.tmc.edu (N. Dafny).

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doi:10.1016/j.brainresbull.2011.10.008

Table 1
The experimental protocol after surgery. On experimental day 1 (ED1) the neuronal recording for one hour was obtained following a saline injection and for another hour preceding initial 2.5 mg/kg methylphenidate (MPD) administration. On ED2 through ED7 2.5 mg/kg MPD injections were given without neuronal activity recordings. Animals went through a washout stage on ED7 through ED9 in which no injections were given, recording was resumed at ED10 following a saline injection and one hour later following a rechallenge of MPD administration of 2.5 mg/kg.

Experimental day	1	2–6	7–9	10
Treatment	Saline + 2.5 mg/kg MPD	2.5 mg/kg MPD	Washout (no injection)	Saline + 2.5 mg/kg MPD

pathways. These pathways act as a push–pull mechanism exhibiting excitatory and inhibitory effects on thalamic activity [61]. A recent behavioral study using bilateral non-selective and selective lesioning of the CN reported that MPD plays at least in part a modulatory role in the response to repetitive MPD exposure thus making the CN a structure in which neurophysiology activity following MPD administration is imperative for investigation [8].

Previous neurophysiological studies using psychostimulants targeted the mesocorticolimbic DA system [22,37,38,55]. One such study investigated the effects of amphetamine on auditory gating using in vivo recordings from anesthetized animals. The use of anesthetized animals is known to cause modulation of CNS activity [22]. Other studies employed in vitro analysis of MPD response on brain slices to analyze the post synaptic currents in the VTA [37,38]. Also, Yang et al. [55,56] previously studied the dose response property of MPD on sensory evoked potentials from several brain areas belonging to the motive circuit in freely behaving non-anesthetized rats. Yang et al. [56] demonstrated that MPD uniformly causes suppression of sensory evoked potentials. It is imperative to investigate the mechanism of action under this phenomena using a wireless telemetric device to study the MPD action on single unit activity in the CN of non-anesthetized animals. This has not been accomplished in the above studies; therefore the aim of this study was to investigate the effects of acute and chronic MPD administration on CN units by recording from non-anesthetized, freely behaving animals previously implanted with permanent semi-microelectrodes. Since non-anesthetized freely moving rats were used during this experiment, CN neuronal activity was recorded before and after MPD administration for an extended period of time, without modulation of the CNS due to anesthesia.

2. Methods

2.1. Animals

Twenty-six adult male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing between 150 and 175 g at the day of arrival were allowed 5–7 days acclimation in a room on a 12 h light/dark schedule (lights on 6:00 am). Food and water were given ad libitum. Animals were housed individually in clear acrylic cages that serve as both home cage and test cage for the recording. 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 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 an intra-peritoneal (i.p.) of 50 mg/kg pentobarbital. The head of the animal was shaved and lidocaine hydrochloride topical gel was applied to the shaved area as a numbing agent. The animal was then placed into the stereotaxic apparatus where an incision was made on scalp, and the muscle and connective tissue was removed to expose the skull. Using Paxinos and Watson [33] brain atlas coordinates, bilateral holes were created above the CN at .50 mm anterior to bregma and 3.0 mm lateral from midline. Six anchor screws were implanted in skull at vacant spots to secure the dental acrylic skull cap. Electrodes were then inserted into brain at an initial depth of 3.5 mm from the skull with neuronal activity monitiored by Grass emitter Hi Z Probe connected to a Grass P511 series pre-amplifier, when a 3:1 signal to noise ratio spike activity was exhibited, electrode was permanently secured to skull using web glue cyanoacrylate surgical adhesive. When the neuronal activity exhibited less than 3:1 signal to noise ratio 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 [10–12]. Similar procedures were followed for the second electrode which was implanted in the CN of the opposite hemisphere. The copper pins were inserted into amphenol plugs which were secured to the skull using dental acrylic cement creating the skull cap. Animals were allowed 4–7 days recovery after electrode implantation during which they were placed in experimental apparatus daily and connected to the wireless (telemetric) head stage transmitter (Triangle BioSystems, Inc. (TBIS); Durham, NC, USA) for acclimation. On initial recording day rats weighed approximately 200–220 g and approximately 300 g on the final recording day.

2.3. Drugs

Methylphenidate hydrochloride (MPD) was obtained from Mallinckrot (Hazelwood, MO, USA). MPD was dissolved in a 0.9% isotonic saline solution and the 2.5 mg/kg MPD was calculated as a free base. Control injections consisted of 0.8 ml isotonic saline solution (0.9% NaCl) administered i.p. All injections were equalized to a volume of 0.8 ml with 0.9% saline to keep injection volumes the same for all animals. Previous MPD dose response behavioral and neurophysiological sensory evoked potential procedure experiments from 0.1 mg/kg to 40 mg/kg MPD administration found that 2.5 mg/kg i.p. elicited behavioral and neurophysiological sensitization [1,17,18,28,36,50,52–56], therefore the MPD dose (2.5 mg/kg) was selected for this study.

2.4. Experimental protocol

On the experimental day one (ED1), rats were placed 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; [15]) head stage was connected to the electrode pins of the skull cap, and animals were allowed to acclimate for 30 min prior to the recording session. After acclimation the animal received a saline injection of 0.8 ml (standardized for all injections) and neuronal activity was recorded for 1 h followed by a 2.5 mg/kg MPD injection with recording resumed for additional hour post MPD injection. The wireless TBSI head stage sent neuronal activity signals to a receiver that was connected to a Cambridge Electronic Design (CED) analog-to-digital converter (Micro1401-3; Cambridge, England) which collected and stored the recorded data on a PC using Spike 2.7 CED software. On ED2 through ED6 animals received daily 2.5 mg/kg MPD injection in their homecage without neuronal recording. At ED7 through ED9 the animals underwent washout in which no injections were given proceeded by ED10 in which a saline injection was given with neuronal activity recorded 1 h post injection followed by a rechallenge administration of 2.5 mg/kg MPD and neuronal activity recorded for additional 1 h, the same as on ED1 (Table 1).

2.5. Data analysis

2.5.1. Data acquisition

Triangular BioSystem International (TBSI) telemetric headstage was attached to the electrode pins of the skull cap. The headstage sent neuronal activity signals to a receiver connected to the Cambridge Electronic Design (CED) analog-to-digital converter (Micro1401-3; Cambridge, England) which stored the data on a PC for offline analysis of spikes from ED1 and ED10 exhibiting similar waveform shape and amplitude pre and post MPD administration using Spike 2.7 CED software.

2.5.2. Spike sorting

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). There are two window levels, one for positive-going spikes and one for negative-going spikes. Spikes with a peak amplitude that were triggered by the window were used to create the templates. 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 spike event to find the best fitting template that yields the minimum residue variance. Secondly, a template matching procedure is then performed; when the distance between the template and waveform exceeds some threshold (80%) the waveforms are rejected. That means that the spike sorting accuracy in the reconstructed data is about 95%. All these parameters of spike sorting for each electrode were sorted and used for the activity recorded in