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Research report

Methylphenidate modulates dorsal raphe neuronal activity: Behavioral and neuronal recordings from adolescent rats

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ABSTRACT

Methylphenidate (MPD) is a widely prescribed psychostimulants used for the treatment of attention deficit hyperactive disorder (ADHD). Unlike the psychostimulants cocaine and amphetamine, MPD does not exhibit direct actions on the serotonin transporter, however there is evidence suggesting that the therapeutic effects of MPD may be mediated in part by alterations in serotonin transmission. This study aimed to investigate the role of the dorsal raphe (DR) nucleus, one of the major sources of serotonergic innervation in the mammalian brain, in the response to MPD exposure. Freely behaving adolescent rats previously implanted bilaterally with permanent electrodes were used. An open field assay and a wireless neuronal recording system were used to concomitantly record behavioral and DR electrophysiological activity following acute and chronic MPD exposure. Four groups were used: one control (saline) and three experimental groups treated with 0.6, 2.5, and 10.0 mg/kg MPD respectively. Animals received daily MPD or saline injections on experimental days 1–6, followed by 3 washout days and MPD rechallenge dose on experimental day (ED)10. The same chronic dose of MPD resulted in either behavioral sensitization or tolerance, and we found that neuronal activity recorded from the DR neuronal units of rats expressing behavioral sensitization to chronic MPD exposure responded significantly differently to MPD rechallenge on ED10 compared to the DR unit activity recorded from animals that expressed behavioral tolerance. This correlation between behavioral response and DR neuronal activity following chronic MPD exposure provides evidence that the DR is involved in the acute effects as well as the chronic effects of MPD in adolescent rats.

1. Introduction

Attention deficit hyperactive disorder (ADHD) is a neuropsychiatric disorder characterized by increased impulsivity, hyperactivity, and inattentiveness (Newcorn, 2000), although precise neurophysiologic deficits underlying ADHD remains undetermined (Krain and Castellanos, 2006; Dalley and Roiser, 2012). ADHD is commonly diagnosed during adolescence and management with psychostimulants often continues into adulthood (Arnsten, 2006; Homberg et al., 2016; Polanczyk and Rohde, 2007; Wilens, 2008). Methylphenidate (MPD) is the most widely prescribed stimulant in children and is the drug of choice for treatment of ADHD (Accardo and Blondis, 2001; Bolaños et al., 2003; Volkow et al., 1995). Adolescent and adult rats differ in both acute and long term responses to psychostimulants, and it was reported that chronic MPD exposure during development can modify long term behavioral responses to emotional stimuli, resulting in depression-like behavior in adult rats (Andersen et al., 2002; Bolaños et al., 2003, 2008). However, despite extensive use of MPD by children when the developing brain is still going through profound changes, the long term effects of chronic MPD administration on brain ontogenesis are poorly understood (Bolaños et al., 2003; Koda et al., 2010).

Research on MPD has focused primarily on its effects on dopamine and norepinephrine transmission (Arnsten and Dudley, 2005; Berridge et al., 2006). However, the role of 5HT in ADHD remains ambiguous (Oades, 2008). Behavioral and neurochemical studies (Kuczenski and Segal, 1997; Volkow et al., 2000) indicate that MPD affects the serotonergic system. Serotonin is a key mod-

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ulatory neurotransmitters in the CNS and has been implicated in the regulation of behavioral function (Cools et al., 2008). A major source of 5-HT in the brain is the serotonergic neurons of the dorsal raphe nucleus (Tao and Auerbach, 1995; Miyazaki et al., 2012). 5HT exhibits complex interactions with the other catecholamines, including selective regulation of DA release (Porras et al., 2002) and modulation of DR serotonergic neurons has been shown to result in increased action impulsivity and failure to wait for delayed rewards (Miyazaki et al., 2012) which warrant further investigation of its relationship with DA and NE and its role in ADHD (Gatley et al., 1999).

Repetitive exposure to psychostimulants has been shown to elicit dose dependent behavioral tolerance or sensitization (Lee and Dafny, 2014; Frolov et al., 2015) and it is this behavioral outcome that predicts drug abuse liability (Robinson and Berridge, 2001). Behavioral sensitization or tolerance is a phenomenon characterized by a further increased or decreased response to repeated drug exposure compared to the initial (acute) effect respectively (Claussen et al., 2014; Jones et al., 2014). At therapeutic doses, MPD is known to cause robust regulatory changes in dopaminergic output of the sensorimotor striatum; a region heavily involved in learning and habit formation (Yano and Steiner, 2005). Studies suggest that 5HT transmission is involved in the modification of gene regulation by psychostimulants and in the formation of addictive behaviors (Bhat and Baraban, 1993; Andersen et al., 2002; Van Waes et al., 2010). Paradoxically, chronic exposure to identical doses of MPD has been shown to elicit behavioral sensitization in some animals and tolerance in others (Frolov et al., 2015; Jones et al., 2014) however the reason for this dichotomy in adult rats is unclear.

The aim of this study is to further investigate the effects of acute and repetitive (chronic) MPD on both behavior and DR neuronal events in adolescent rats by concomitantly recording the behavioral and neuronal activity of DR neurons before and after acute and chronic exposure to MPD. The hypothesis of the study is 1) the dorsal raphe participates in MPD action, 2) the same MPD dose in some adolescent animals will elicit behavioral sensitization and in others behavioral tolerance and 3) the DR neuronal activity recorded from adolescent animals expressing behavioral sensitization will have a significantly different response to MPD exposure than those DR neurons recorded from animals expressing behavioral tolerance. Therefore, freely behaving animals previously implanted with permanent electrodes within the DR and MPD dose response protocol were used to concomitantly record the behavioral and the DR neuronal activity following acute and repetitive MPD injection.

2. Materials and methods

2.1. Animals

One hundred and sixty one (161) Male Sprague-Dawley rats at about post-natal day 30 (P-30) were purchased (Harlan, Indianapolis, IN, USA) and allowed 4–5 days of acclimation in our vivarium on a 12 h light/dark schedule (lights on 6:00am) prior to electrode implantation, while temperature ($21 \pm 2\,^{\circ}C$) and humidity (37–45%) were kept constant.

Food and water were given ad libitum. After electrode implantation, the animals were housed individually in clear acrylic standard cages that served as both home cage and test cage for the electrophysiological and behavioral recordings for an additional 5–6 days prior to the recording (experimental) days. Recording began on P-40 following saline (control) and MPD exposure. The experimental procedures were approved by the UTHSC Animal Welfare Committee and carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.2. Surgery

Animals were anesthetized with an intraperitoneal (i.p.) injection of 30 mg/kg pentobarbital prior to surgery. The animal's head was shaved and a lidocaine hydrochloride gel was applied for local anesthetic. The animal was then placed in a stereotaxic apparatus and an incision was made to expose the skull. Bilateral holes were drilled above the DR at 7.8 mm posterior to bregma and 0.2 mm lateral to the midline using the Sherwood and Timiras adolescent rat brain atlas (Sherwood and Timiras, 1970). An additional hole was drilled in the frontal sinus for the reference electrode, and six anchor screws were inserted at vacant spots in the skull. Two Nickel-Chromium Teflon coated (insulated except at tips, resistance approx. 80 mΩ) wires 60 μm in diameter were twisted and each was secured to a 1 cm copper connector pin and individually inserted into the DR on each side to be used as recording electrodes (four total recording electrodes). During the electrode placement, the DR unit activity was monitored using a Grass P511 amplifier with its cathode follower connected to an audiomonitor and oscilloscope. The electrodes were inserted at a depth of 6 mm and, if satisfactory neuronal activity was observed, they were secured to the anchor screws and to the skull by dental acrylic cement. Electrodes failing to detect satisfactory activity were lowered in steps of 5–10 μm increments until a 3:1 ratio spike activity was detected, to a maximum depth of 6.6 mm below the skull, and then secured with dental acrylic cement. Rats were allowed to recover from the surgical procedure for 5–6 days. During this recovery period, the animals were placed with their home cage in the experimental room for about two hours each day and connected to the wireless head stage (Triangle BioSystems Inc., TBSI, Durham, NC, USA) to adapt and acclimate to the neuronal and behavioral recording systems.

2.3. Drugs

Methylphenidate hydrochloride (MPD) was obtained from NIDA. Previous dose response MPD experiments (from 0.1 to 40.0 mg/kg i.p.) found that behavioral effects of MPD were observed from 0.6 mg/kg doses of MPD and higher (Gaytan et al., 1996; Algahim et al., 2009; Yang et al., 2011) therefore 0.6, 2.5, and 10.0 mg/kg doses were selected as low, moderate, and high experimental doses. MPD was dissolved in a 0.9% isotonic saline solution for injection. Control subjects received injections of 0.8 ml isotonic saline solution (0.9% NaCl). All MPD injections were titrated to a volume of 0.8 ml with 0.9% saline to equalize injection volumes for all animals. Injections were given i.p.

2.4. Experimental protocol and data acquisition

The rats were allowed to recover from electrode implantation and adapt to the behavioral and electrophysiological recording systems prior to recording for 5–6 days. On experimental day 1 (ED1), rats within their home cage were placed again within a Faraday cage to reduce background noise during recording sessions. The animals were allowed an additional 20 to 30 min for adaptation to the wireless head stage, during which the neuronal activity was monitored and the software parameters were selected for spike recording and sorting. The DR neuronal activity and the locomotive activity were recorded simultaneously using a wireless neuronal recording system (Triangle BioSystems Inc., TBSI, Durham, NC, USA) and an open field computerized animal activity system (Accuscan, Columbus, OH, USA). The TBSI wireless electrophysiological recording system consists of a head stage (weighing less than 5 g) and a remote receiver that was connected to an analog- to-digital converter (Micro1401-3; Cambridge Electronic Design (CED)) that