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Dorsal raphe neuronal activities are modulated by methylphenidate

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Abstract

This study investigated the electrophysiological properties of the dorsal raphe nucleus (DR) neurons in response to the acute and repetitive administration of methylphenidate (MPH). Activities of DR neurons were recorded from non-anesthetized, freely behaving rats previously implanted bilaterally with permanent semi microelectrodes. The main findings were: (1) after initial (acute) administration of MPH (2.5 mg/kg i.p.) on experimental day one (ED1), 56 % of DR units significantly changed their firing rates. The majority of the responsive units (88 %) exhibited increased firing rate; (2) daily MPH injections were given on ED2 through ED6 followed by 3 washout days. On ED10, 83 % of the DR units significantly changed their baseline activity compared to the baseline activity on ED1; (3) after rechallenge MPH administration on ED10, 63 % of DR units exhibited significant change in their firing rate; the majority of the responsive units (76 %) exhibited a significant increase in their firing rate; (4) The effect of rechallenge MPH administration on ED10 was compared to the effect of initial MPH on ED1, 47 % DR units exhibited a further significant increase in their firing rate while 53 % DR units exhibited decrease or non-change in their firing rate which can be interpreted as electrophysiological sensitization or tolerance. In conclusion, this study demonstrated that acute MPH administration modulated the DR neuronal activities. Repetitive MPH administration modulated the baseline activities of DR units and elicited neurophysiological sensitization or tolerance. The results indicated that MPH affects DR neuronal activity.

Keywords

Serotonin; Electrophysiology; Single unit activity; Sensitization; Tolerance

Introduction

Attention deficit/hyperactivity disorder (ADHD) is a complex and heterogeneous behavioral disorder characterized by symptoms of inattention, impulsivity, and/or hyperactivity. ADHD affects about 3–5 % of school aged children, but its etiology is still not completely understood. Dysregulated dopaminergic and noradrenergic neurotransmission has been

widely implicated in the pathophysiology of ADHD (Arnsten 2006; Biederman 2005; Del et al. 2011). However, the serotonergic system has been reported to be related to impulsive behavior in children (Halperin et al. 1997; Lucki 1998; Oades et al. 2008) and the literature further suggests that serotonin (5-HT) has a strong relationship with ADHD (Oades 2007, 2008; Sinzig and Lehmkuhl 2007; Zepf et al. 2008). Methylphenidate (MPH) is a psychostimulant medication most commonly prescribed for the treatment of ADHD (Gunther et al. 2010; Polanczyk et al. 2007). The majority of studies report that MPH treats the symptoms of ADHD by increasing the level of dopamine and norepinephrine in the brain (Berridge et al. 2006; Izenwasser et al. 1999; Jones et al. 1998; Massello and Carpenter 1999). Some evidence from neurochemical, histochemical and behavioral studies suggest that MPH affects the serotonergic system as well (Geyer et al. 1975; Kuczenski et al. 1987; Volkow et al. 2000). The dorsal raphe nucleus (DR) is known to be a major source of 5-HT in the mammalian brain, and 5-HT containing cell bodies in the DR are found to project to dopaminergic cells in the ventral tegmental area (VTA) which is the origin of the dopaminergic cell bodies of the mesocorticolimbic DA system (Van Bockstaele et al. 1993; van der Kooy and Hattori 1980). The DR also receives dopaminergic innervation from the VTA (Kalen et al. 1988; Peyron et al. 1995). In the light of this, it is apparent that the VTA and DR may functionally communicate with each other. It has been reported that the psychostimulant, cocaine, can elicit a dose-dependent inhibition of DR 5-HT neurons in anesthetized rats (Cunningham and Lakoski 1988, 1990), whereas long-term exposure of amphetamine has been shown to increase spontaneous firing rates in DR neurons in urethane anesthetized rats (Heidenreich et al. 1987). However, there is no electrophysiological study concerning the effect of acute and repetitive MPH on the DR neurons thus far. We hypothesize that the DR neurons participate in the response to acute and repetitive MPH exposure. Thus the aim of the study was to investigate the effect of acute and repetitive MPH exposure on the DR neuronal activity recorded from non-anesthetized, freely behaving rats previously implanted with permanent semi microelectrodes using new wireless (telemetric) recoding technology system. This study will provide electrophysiological evidence that the DR participates in the action of MPH.

Materials and methods

Animals

Male, 7- to 8-week-old Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing 150–175 g upon arrival were housed with free access to food and water for 3–5 days for acclimation prior to electrode implantation. The room temperature was maintained at 21 ± 2 °C with a relative humidity of 55–62 % under 12 h:12 h alternating light–dark cycle (light on at 06:00). After electrode implantation, animals were individually housed in clear acrylic cages which were used as both home cage and test cage for the duration of the electrophysiological recordings. On the first recording day, the animals' weighted approximately 200–220 g. All experimental procedures were approved by University of Texas Health Science Center Animal Welfare Committee and in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

Surgery

Animal was anesthetized with 50 mg/kg pentobarbital intraperitoneal injection (i.p.), the head was shaved and lidocaine hydrochloride topical gel was applied to the shaved area for local anesthetic. The animal was then placed into a stereotaxic instrument. A one inch incision was made on the scalp, the muscle was removed and the skull was exposed. Holes were drilled above the DR in both hemispheres (7.8 mm posterior to bregma, 0.2 mm lateral to midline according to the rat atlas, Paxinos and Watson 1986) and an additional hole in the frontal sinus for the reference electrode. Two twisted Nickel–Chromium 60 μ m in diameter electrodes (fully insulated except at tips, secured to 1 cm copper connector pins) were placed into the DR in each hemisphere (i.e. each animal had four recording electrodes). In the vacant spots six anchor screws were inserted to secure the skull cap with dental acrylic cement. During the placement of electrodes, the unit activity was monitored. Electrodes were fixed to the skull only when spike activity exhibited at least a 3:1 signal to noise ratio; Otherwise, the electrode was lowered in the steps of 10 μ m increments until a 3:1 ratio spike activity was observed, to maximum depth of 6.6 mm below the skull (Chong et al. 2012; Claussen and Dafny 2012; Dafny 1980, 1982, 1988; Dafny and Terkel 1990; Salek et al. 2012). The copper pins were inserted into an Amphenol plug which was fixed onto the skull with dental acrylic cement. Rats were allowed to recover from the surgical procedure for 4–7 days. During this recovery period, rats were placed daily with their home cage in the experimental room and connected to the wireless head stage (Triangle BioSystems Inc, TBSI, Durham, NC, USA) to adapt and acclimate to the recording system.

Drug

Methylphenidate hydrochloride was obtained from Mallinckrodt Inc. (St. Louis, MO, USA). MPH was dissolved in 0.9 % isotonic saline (NaCl) solution. To prepare 2.5 mg/kg i.p. injection, MPH was calculated as a free base, control injections consisted of 0.8 ml isotonic saline solution. It has been reported that administration of approximately 2–3 mg/kg MPH (i.p.) in rodents achieved plasma levels similar to clinical use (Brandon and Steiner 2003; Gerasimov et al. 2000). MPH in rodents often requires higher doses than in humans because of more rapid metabolism (Gatley et al. 1999). Previous dose response experiments using MPH from 0.1 to 40 mg/kg found that 2.5 mg/kg MPH (i.p.) elicited behavioral and neurophysiological sensitization (Gaytan et al. 1996, 2000b; Lee et al. 2009; Podet et al. 2010; Yang et al. 2003a, 2006a, b, 2007a). Based on the above studies, the 2.5 mg/kg MPH dosage was selected for this experiment. All injections (saline and MPH) were given i.p. and equalized to 0.8 ml with saline so that all injections volumes were the same for all animals.

Experimental protocol and data acquisition

Animals were randomly divided into saline control and MPH groups, on experimental day one (ED1), rats were placed with their home cage in a Faraday testing box to reduce noise during the recording session. The wireless head stage (TBSI) 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 and neuronal activity was recorded for 1 h followed by a saline injection (control) or MPH injection with recording resumed for an additional hour post-injection. The head stage sent neuronal

activity signals through a transmitter to a receiver (TBSI) that interfaces with an analog-to-digital converter [Micro1401-3; Cambridge Electronic Design (CED)] that collected and stored the recorded data from each electrode into a separate file on a PC using the CED Spike 2.7 software. On ED2 through ED6 animals received daily saline or MPH injection in their home cage. On ED7 to ED9 the animals underwent washout period, in those days no injections were given. There were no neuronal activities recording on ED2 to ED9. On ED10, after 30 min adaption, baseline neuronal activities were recorded for 1 h post-injection of saline and followed by either saline (control) or MPH rechallenge administration with neuronal activity recorded for additional 1 h, the same as that on ED1 to observe the repetitive effect of the drug (Table 1), similar protocol was used previously (Gaytan et al. 2000a; 2000b; 2002; Yang et al. 2000; 2003b; 2007b). In this study, DR neuronal activities were recorded from non-anesthetized, freely behaving rats previously implanted with permanent semi microelectrodes using new wireless (telemetric) recording technology system (Fan et al. 2011). This allows us to investigate the electrical activities of DR neurons before and after acute and repetitive MPH exposure without the interference of anesthesia.

Spike sorting and data analysis

The recorded neuronal activity was replayed offline for spike sorting and analyzing with the CED Spike 2.7 software fixed template matching system (FTMS). The data are captured by the program and processed using low and high pass filters (0.3–3.0 kHz). There are two window levels, one for positive-going spikes and one for negative-going spikes. Spikes with peak amplitude that were triggered by the window were used to create the templates. One thousand waveform data points were used to define a spike. The spikes were extracted when the input signal enters the previously determined amplitude window. The algorithm used to capture a spike allows the extraction of templates that provide high-dimensional reference points that can be used to perform consistent and accurate spike sorting. 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 matching procedure is then performed; when the distance between the template and waveform exceeds some threshold (80 %) the waveforms are rejected. Therefore, spike sorting accuracy in the reconstructed data is about 95 %. The templates that were used to analyze ED1 file were then loaded onto the ED10 file of the same electrode and the same animal to evaluate the neuronal activity on ED10. This ensured that the spike amplitude and pattern sorted from ED1 was the same on ED10. Once spike sorting was completed the data were exported into a spread sheet which calculated the average neuronal firing rate and produced a sequential firing rate graph (Chong et al. 2012; Claussen and Dafny 2012; Salek et al. 2012). The comparisons were made as follows: (1) DR unit firing rate following initial 2.5 mg/kg MPH administration on ED1 was compared to activity after saline injection; (2) Saline baseline activities of ED10 were compared to baseline activities of ED1; (3) DR units firing rate after rechallenge of 2.5 mg/kg MPH administration on ED10 was compared to saline baseline on ED10; and (4) DR neuronal activity after MPH administration on ED10 was compared to the activity following the initial 2.5 mg/kg MPH on ED1. Significance of change and direction of the change (decrease or increase) were determined by the critical ratio test ($C.R. = (E - C) / (\sqrt{E} + C) = \pm 1.96 = p < 0.05$) where C is the control and E is the drug activity (Claussen and Dafny 2012; Dafny 1980; Yang et al. 2006b). Statistical significance

between saline and MPH treatment groups was determined with Chi-square test. $p < 0.05$ was considered significant.

Histological verification of electrode placement

An overdose of sodium pentobarbital was administered to the rat at the end of the ED10 recording session. The rat was perfused intracardially with 10 % formalin solution containing 3 % potassium ferrocyanide, and a 50 μ A DC current was passed through the electrode connector pin for 30 s to produce a small lesion in the recording sites. The brain was excised and stored in 10 % formalin for subsequent histological processing. The position of the electrodes was confirmed by the location of the lesion and the Prussian blue spot using the Rat Brain Atlas (Paxinos and Watson 1986).

Results

Forty-eight electrodes implanted in nineteen animals were confirmed to be in the target (Fig. 1). One hundred and forty-three DR units exhibiting similar amplitude and pattern on ED1 and ED10 were evaluated.

Effect of saline

Effect of saline injection was recorded in 24 DR units to obtain the control for animal handling and the injection volume (Table 2). On ED1, DR units exhibited similar firing rate after the first and second saline injection (Fig. 2a). Daily saline injections were given on ED2 though ED6 followed by 3 washout days (ED7–9). On ED10, DR units exhibited similar firing rate after the first and second saline injection (Fig. 2b). The firing rate of DR units on ED10 and ED1 after saline injections was similar (Fig. 2), i.e. saline administration did not change the firing rate of DR units. Thus, any significant change following MPH compared to activity after saline injection was considered as the effects of the drug.

Acute effect of MPH on DR units

One hundred and nineteen DR units recorded from fifteen rats were used to observe the effect of MPH. On ED1, following the initial (acute) MPH (2.5 mg/kg) administration, 56 % (67/119) of DR units exhibited a significant ($p < 0.05$) response to the drug by changing (increase or decrease) their firing rates compared to their saline baseline activities. 88 % (59/67) of the DR units that responded to MPH exhibited a significant ($p < 0.05$) increase in their firing rate and only 12 % (8/67) of the DR units that responded to MPH exhibited a significant ($p < 0.05$) decrease in their activities after initial MPH administration (Table 2, acute effect). Figure 3 shows two representative DR units that responded to 2.5 mg/kg MPH administration by a significant increase of its firing rate in a, and by a significant decrease of the firing rate in b. In conclusion, three groups of DR units were identified based on their response to acute MPH administration (increased, decreased and unresponsive units).

Comparing ED10 baseline activities to ED1 baseline activities

After initial MPH administration and recording on ED1, the rats were injected in their home cage daily with a single 2.5 mg/kg MPH dosage for an additional 5 days (ED2–6), followed by 3 washout days (ED7–9) and recordings were resumed on ED10. Most of the DR units

(83 %, 99/119) exhibited a significant ($p < 0.05$) change of their baseline activity on ED10 compared to ED1 baseline activity. 64 % (63/99) of the responsive units showed a significant increase in their ED10 baseline activities while 36 % (36/99) of them exhibited a significant ($p < 0.05$) decrease in their ED10 baseline neuronal activity compared to their ED1 baseline firing rate (Table 2 baseline response).

In order to find out whether the response direction (increase or decrease) of DR units to acute MPH on ED1 affected their baseline activity on ED10, the baseline activity on ED1 of those three groups of DR units (increased, decreased and unresponsive units to MPH as shown in Table 2) was compared with their baseline on ED10 (Table 3A) as the results of the six daily MPH and three washout days. For the 59 DR units which exhibited a significant ($p < 0.05$) increase in their neuronal firing rate on ED1 following the initial MPH exposure, 54 % (32/59) of them exhibited a significant ($p < 0.05$) increase in their baseline firing rate on ED10, while 39 % (23/59) of them exhibited a significant ($p < 0.05$) decrease in their baseline firing rate on ED10 compared to ED1 baseline.

Only eight DR units exhibited a decrease in their neuronal activity to MPH on ED1, all of them exhibited a significant ($p < 0.05$) change in their ED10 baseline activity compared to their ED1 baseline activity. The majority (63 %, 5/8) of these units exhibited increases ($p < 0.05$) in their baseline activities on ED10, and 37 % (3/8) of them exhibited decreased ($p < 0.05$) baseline activity on ED10 compared to ED1 baseline (Table 3A).

Of the 52 unresponsive DR units to MPH exposure on ED1, 50 % (26/52) of them showed a significant ($p < 0.05$) increase in their baseline activity on ED10, and 19 % (10/52) of them showed a significant ($p < 0.05$) decrease in their baseline activity on ED10 compared to ED1 baseline activity (Table 3A). Figure 4 shows two DR units exhibiting a significant change in their ED10 baseline activities compared to their ED1 baseline activity.

Effect of repetitive MPH administration on DR neurons: comparing the effect of MPH rechallenged on ED10 with ED10 baseline

The neuronal activity following 2.5 mg/kg MPH rechallenge on ED10 was compared to the neuronal activity following saline administration on ED10. 63 % (75/119) of DR units exhibited a significant ($p < 0.05$) change in their firing rate following MPH rechallenge on ED10. The majority (76 %, 57/75) of the responsive DR units exhibited a significant ($p < 0.05$) increase of their activity, only 24 % (18/75) of them exhibited a significant ($p < 0.05$) decrease of their activity (Table 2, effect of MPH on ED10).

In order to find out whether the acute response of DR units to MPH on ED1 affected their response to MPH on ED10, the three groups of DR units on ED1 (increase, decrease and unresponsive to the initial MPH as shown in Table 2) and their neuronal firing rate changes to MPH on ED10 were compared and summarized in Table 3B. The table shows that for the 59 DR units which exhibited a significant increase in their firing rate following the initial MPH exposure on ED1, 59 % (35/59) of them showed a significant ($p < 0.05$) increase in their neuronal activity and 7 (12 %, 7/59) of them showed a significant ($p < 0.05$) decrease in their neuronal activity following rechallenge MPH administration on ED10, respectively.

Of the eight DR units which exhibited a decrease in their activity on ED1 to MPH exposure, four (50 %, 4/8) units exhibited a significant ($p < 0.05$) increase in their neuronal activity and three (38 %, 3/8) units exhibited a significant ($p < 0.05$) decrease in their activity on ED10 following MPH exposure, respectively (Table 3B).

Of the 52 unresponsive units to MPH exposure on ED1, 35 % (18/52) of them exhibited a significant ($p < 0.05$) increase in their neuronal activity and 15 % (8/52) of them exhibited a significant ($p < 0.05$) decrease in their neuronal activities following MPH exposure on ED10, respectively (Table 3B).

The effect of rechallenge MPH on ED10 compared to the initial MPH exposure on ED1

In order to find out whether repetitive MPH exposure elicited electrophysiological sensitization or tolerance to DR units, the effect of rechallenge MPH on ED10 was compared to the effect of initial MPH on ED1. The results are summarized in Table 3C. For the 59 units which increased their firing rate on ED1 after initial MPH exposure, 42 % (25/59) of them exhibited a significant ($p < 0.05$) further increase in neuronal activity. This further increase in neuronal activities can be interpreted as neurophysiological sensitization. 49 % (29/59) of them exhibited a significant ($p < 0.05$) decrease in their firing rate on ED10 following rechallenge MPH exposure compared to initial MPH on ED1. This opposite effect of MPH on these units can indicate neurophysiological tolerance. The remaining 9 % (5/59) units did not exhibit any change in their firing rate following MPH rechallenge on ED10 compared to initial MPH administration on ED1. This unresponsiveness to the drug can be interpreted also as expression of tolerance. Figure 5 shows two representative DR units that exhibited a further increase (upper panel) and decrease (lower panel) in their activities to MPH rechallenge on ED10 compared to initial MPH exposure on ED1.

Of the eight units that exhibited a decrease in their firing rate on ED1 after initial MPH exposure, 75 % (6/8) of them reversed their response direction and significantly ($p < 0.05$) responded by increasing their neuronal activity, and one DR unit (12.5 %, 1/8) exhibited a further significant ($p < 0.05$) decrease in its neuronal activity after MPH rechallenge on ED10 compared to initial MPH administration on ED1, respectively (Table 3C).

For the 52 unresponsive DR units on ED1, 48 % (25/52) of them exhibited a significant ($p < 0.05$) increase in their neuronal activities, 33 % (17/52) of them exhibited a significant ($p < 0.05$) decrease in their neuronal activities after MPH rechallenge on ED10 compared to initial MPH administration on ED1, respectively (Table 3C).

Discussion

The DR nucleus is a major source of 5-HT in the mammalian brain and plays an important role in the regulation of many physiological processes, such as sleep/arousal, food intake and mood (Jacobs and Azmitia 1992). The serotonergic system has been implicated in the etiology of ADHD (Clark et al. 2004; Sinzig and Lehmkuhl 2007; Zepf et al. 2008). The DR neurons display firing rates correlated with diverse behavioral events, including rewards, conditioned cues, and impulsive behaviors (Miyazaki et al., 2011; Nakamura et al. 2008; Ranade and Mainen 2009). It has been reported that cocaine inhibits the DR 5-HT neurons

firing rate in rats anesthetized with chloral hydrate and that amphetamine increases the spontaneous firing rate of DR neurons in urethane anesthetized rats (Cunningham and Lakoski 1988, 1990; Heidenreich et al. 1987). The different responses of DR neurons to psychostimulants in those experiments may be caused by the different anesthetics. In order to exclude the interference of anesthesia, the present study investigated the effects of acute and repetitive MPH exposure on DR neuronal units in freely moving rats previously implanted bilaterally with permanent semi microelectrodes. The technique using implanted electrodes for recording of extracellular neuronal activities has been successfully established during a variety of behavioral paradigms in several species of laboratory animals. Stable neuronal activities have been obtained in monkeys for 15 days and in rats for up to 153 days (Dickey et al. 2009; Thompson and Best 1990). In order to obtain stable single unit activity for a prolonged period of time, we secured the electrodes onto the skull with screws and dental acrylic cement to avoid the migration of the electrodes. The same parameters of spike sorting (see "Materials and methods") used on ED1 for a particular electrode were used on ED10. We assume that the recording from the same electrode on ED1 and ED10 that exhibited similar amplitude and pattern and sorted by our sophisticated software is obtained from the same unit (i.e. only such single units that exhibit nearly identical shape and amplitude between ED1 and ED10 were included in the analysis).

The main findings of this study were as follows: (1) about half of DR units exhibited a significant change in their firing rates after the initial 2.5 mg/kg MPH administration (acute effect). The majority of the DR units responded to acute MPH by significantly increasing their firing rates, while only a few of the DR units exhibited a significant decrease in their firing rates; (2) 83 % of the DR units showed a significant change in baseline on ED10 compared to their baseline on ED1, majority of them increased their baseline activity; (3) 63 % of the DR units significantly changed their firing rate following repetitive MPH administration (ED10) compared to their ED10 baseline, with the majority showing an increase in their firing rate; (4) acute and repetitive MPH evoked different effects on the DR unit; (5) when the effect of rechallenge MPH administration on ED10 was compared to the effect of initial MPH on ED1, 47 % of the DR units exhibited a significant increase in their firing rate, while 40 % of the units exhibited a significant decrease in their firing rate, which may be interpreted as neurophysiological sensitization and tolerance, respectively.

MPH is one of the most commonly prescribed psychostimulants for the treatment of ADHD, but the electrophysiological mechanism whereby MPH produces its psychopharmacology effects is not completely revealed. In particular, the effects of MPH on 5-HT systems have not been well investigated. 5-HT has been reported to inhibit the motor activity (Geyer 1996) and impulsive behavior (Baumgarten and Grozdanovic 1995; LeMarquand et al. 1998; Robbins et al. 1998), which suggested that DR participates in the modulation of behaviors. Indeed, increasing scientific literature indicates that central serotonergic and dopaminergic systems interact with each other and play a critical role in the regulation of normal and abnormal behaviors, the dysfunction of the DA and 5-HT neurotransmitter systems contribute to various mental disorders (Di 2008). It was reported that the activation of 5-HT_{1A} receptors reduced the expression of amphetamine-induced sensitization in mice (Przegalinski et al. 2000), and that 5-HT₃ receptors have been found to be involved in the development and expression of cocaine sensitization in rats (King et al. 1997, 2000).

Agonists of 5-HT_{1B} receptors enhance cocaine reinforcement (Parsons et al. 1996, 1998). All these findings suggest that 5-HT systems participant in the mechanisms of psychostimulant (amphetamine and cocaine) action. Currently, the data regarding the effect of MPH on 5-HT system are limited. Some investigators believe that MPH predominately works on the mesoaccumbens and mesocorticolimbic dopaminergic system with little or no effect on the 5-HT system, since MPH has very low affinity to 5-HT transporter (Gatley et al. 1996; Pan et al. 1994). Unlike amphetamine and cocaine, MPH does not increase the extracellular 5-HT concentration in the brain (Kuczenski and Segal 1997; Segal and Kuczenski 1999). However, it has been reported that pretreatment with 5-HT uptake blocker fluoxetine (10 mg/kg, i.p.) potentiated the motor activation induced by MPH, and a 5-HT_{1B} receptor agonist (CP 94253; 1–10 mg/kg, i.p.) in a dose-dependent protocol potentiated MPH-induced locomotor activation in rat (Borycz et al. 2008). In addition, MPH induced Fos-like immunoreactivity in the dorsal raphe in wild-type mice but not in dopamine transporter knockout mice (Trinh et al. 2003). Fos upregulation is a reliable indicator of neuronal activation (Bullitt 1990), this data demonstrated that MPH could increase the neural activities in DR neurons. The above report is consistent with the results in this experiment that most DR units increased their firing rates after acute and repetitive MPH administration. Our data shows that MPH modulates the DR neuronal activity, suggesting that the DR participates at least in part in the MPH acute effect, and taking a more active role in the effect of repetitive MPH exposure. MPH potentially acts directly or indirectly on DR neurons by activation of other remote CNS sites that project to DR. Based on our observation, most DR units altered their neuronal baseline activity on ED10 after repetitive MPH exposure compared to their ED1 baseline activity and more DR units responded to rechallenge MPH on ED10 compared to the number of DR units responding to initial MPH exposure on ED1. It is possible to assume that DR neurons contain silent receptors that are activated following repetitive MPH. DR contains both serotonergic and non-serotonergic neurons (Halberstadt and Balaban 2006, 2007). Considering the intimate relationship between psychostimulants and the 5-HT system, it is reasonable to assume that the neurons exhibiting an increase in their firing rate after MPH administration are at least in part serotonergic neurons, and those exhibiting inhibitory effects and unresponsive neurons are likely non-serotonergic neurons. Further studies are needed in order to identify the specific neuronal types in DR units that respond to MPH.

Repeated exposure to psychostimulants causes adaptive changes in the central nervous system which may play a pivotal role in the process of establishing dependence/addiction (Dafny and Yang 2006; Fernandez-Espejo and Rodriguez-Espinosa 2011). Repetitive MPH administration may produce long-lasting molecular and cellular changes, such as protein synthesis and gene alteration in dopaminergic signaling systems and increase dendritic spine populations (Carrey and Wilkinson 2011; Kim et al. 2009), which may explain why more DR units responded to repetitive MPH exposure compared to acute MPH exposure. It has been reported that repetitive cocaine can increase the number of 5-HT uptake sites in dorsal raphe regions which in turn can alter the ability of cocaine to inhibit the serotonergic activity in dorsal raphe neurons (Cunningham et al. 1992). There are no reports concerning MPH-induced neuroadaptive changes in the DR thus far. In this experiment, the baseline activity of most DR units was changed after repetitive MPH administration; the majority of them

increased their baseline firing rate. In addition, the firing rate of DR units was altered by the rechallenge MPH administration on ED10 compared to initial MPH on ED1. Those DR units that further increased their response to the rechallenge MPH administration on ED10 compared to ED1 may be interpreted as neurophysiological sensitization. And those DR units that decreased or unchanged their neuronal activity to rechallenge MPH on ED10 compared to initial MPH on ED1 may be interpreted as neurophysiological tolerance. This data suggests that repetitive MPH administration may induce some neuroadaptive changes in the DR, which in turn regulates the electrical activity of remote neurons. Further experiments are needed to clarify the underlying mechanism of the DR neuronal activity and behavioral changes elicited by MPH.

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Fig. 1.

Summarizes the location of the dorsal raphe (DR) recorded electrode. The *lower photograph* shows a representative histological section and the location of two electrode tips within the DR (*white arrow* indicates the electrode location). The others are reconstructs histologically sketched and summarize the electrode tip placements. The rat atlas plates represent the DR in serial coronal sections. The number next to each section represents the anterior/posterior distance (mm) from bregma. *Black dots* are designated to represent electrode placement of those that were found in the DR. Twenty-four pair electrodes (i.e. 48 electrodes) were securely placed in the DR and the recordings from these electrodes were evaluated

Fig. 2.

Representative sequential frequency firing rate of a DR unit recorded before and after saline injection on ED1 (**a**) and on ED10 (**b**). The first 60 min shows the baseline activity after the first saline injection, followed by an additional 60 min recording after the second saline injection on ED1 and on ED10. *Arrow* indicates the time of saline administration. *Insert* waveforms show 20 spikes sorted in same template on ED1 and on ED10, respectively. *ED1* experimental day1, *ED10* experimental day10

Fig. 3.

A representative sequential frequency firing rate of two DR units on experimental day1. The first 60 min shows the baseline activity after saline injection followed by an additional 60 min after initial 2.5 mg/kg MPH administration. *Arrow* indicates the time of 2.5 mg/kg MPH administration. The unit in **a** exhibited an increase in firing rate after acute 2.5 mg/kg MPH administration. The unit in **b** exhibited a decrease in firing rate after acute 2.5 mg/kg MPH. MPH methylphenidate

Fig. 4.

The baseline activity of two representative DR units recorded at ED1 and at ED10. The *upper DR unit* exhibited an increase in activity on ED10 baseline compared to ED1 baseline activity. The *lower figure* shows a DR unit that exhibited a decrease in baseline on ED10 compared to baseline activity on ED1 baseline. *Insert* waveforms show 20 spikes sorted in same template on ED1 and ED10, respectively. *ED1* experimental day1, *ED10* experimental day10

Fig. 5.

A representative sequential frequency firing rate of two DR units recorded at ED1 and again at ED10. The first 60 min shows the baseline activity after saline injection, followed by an additional 60 min after 2.5 mg/kg MPH administration. *Arrow* indicates the time of 2.5 mg/kg MPH administration. The *upper panel* shows a DR unit that exhibited further significant increases in its activity at ED10 compared to the initial MPH exposure on ED1. The *lower panel* shows a DR unit that exhibited significant increases in its activities after initial MPH on ED1 but less increase its activity after MPH rechallenge on ED10. *Insert* waveforms show 20 spikes sorted in same template on ED1 and ED10, respectively. *MPH* methylphenidate, *ED1* experimental day1, *ED10* experimental day10

Table 1

Experimental protocol

Experimental day	1	2–6	7–9	10
Control	Saline; saline	Daily saline	Washout	Saline; saline
MPH	Saline; MPH	Daily MPH	Washout (no injection)	Saline; MPH

The table describes the experimental protocol of the two groups used in this study
MPH methylphenidate

Table 2

The effect of saline and MPH on the firing rate DR units

	Increase	Decrease	Unresponsive
Acute MPD effect			
Saline	0	0	24 (100 %)
MPH	59 (49 %)	8 (7 %)	52 (44 %) *
Effect of MPD on ED10			
Saline	0	0	24 (100 %)
MPH	57 (48 %)	18 (15 %)	44 (37 %) *
ED10 BL: ED1 BL			
Saline	0	0	24 (100 %)
MPH	63 (53 %)	36 (30 %)	20 (17 %) *

MPH methylphenidate, *ED1* experimental day1, *ED10* experimental day10, *BL* baseline activity

* Significantly different from saline group, $p < 0.0001$

Table 3

The table summarizes the acute and chronic effect of MPH on DR units providing three different comparisons (A, B and C)

A. Comparing ED10 baseline activities to ED1 baseline activities			
	Change of ED10 baseline compared with ED1 baseline		
	Increased	Decreased	No change
59 U increased to MPH on ED1	32 (54 %)	23 (39 %)	4 (7 %)
8 U decreased to MPH on ED1	5 (63 %)	3 (37 %)	–
52 U unresponsive to MPH on ED1	26 (50 %)	10 (19 %)	16 (31 %)

B. Comparing the effect of rechallenge MPH to baseline activities on ED10			
	Number (%) of DR units response to MPH on ED10		
	Increased	Decreased	No change
59 U increased to MPH on ED1	35 (59 %)	7 (12 %)	17 (29 %)
8 U decreased to MPH on ED1	4 (50 %)	3 (38 %)	1 (12 %)
52 U unresponsive to MPH on ED1	18 (35 %)	8 (15 %)	26 (50 %)

C. Comparing the effect of rechallenge MPH on ED10 to the initial MPH on ED1			
	Effect of rechallenge MPH on ED10 compared to MPH on ED1		
	Increased	Decreased	No change
59 U increased to MPH on ED1	25 (42 %)	29 (49 %)	5 (9 %)
8 U decreased to MPH on ED1	6 (75 %)	1 (12.5 %)	1 (12.5 %)
52 U unresponsive to MPH on ED1	25 (48 %)	17 (33 %)	10 (19 %)

ED1 experimental day1, ED10 experimental day10, MPH methylphenidate, DR dorsal raphe