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Methylphenidate modulates the locus ceruleus neuronal activity in freely behaving rat

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Abstract

The electrophysiological properties of the locus coeruleus (LC) neurons in response to acute and chronic administration of methylphenidate (MPD) were investigated. The extracellular LC neuronal activities were recorded from non-anesthetized, freely behaving rats previously implanted bilaterally with permanent semi microelectrodes. The main findings were: (1) On experimental day 1 (ED1), 87% (94/108) of LC units significantly changed their firing rate after initial (acute) MPD (2.5 mg/kg, i.p.) administration. The majority of the responsive units (80%, 75/94) increased their firing rate; (2) Daily MPD (2.5mg/kg) injection was given on ED2 through ED6 followed by 3 washout days (ED7 to 9). On ED10, all LC units exhibited a significant change of their baseline activity compared to their baseline activity on ED1; (3) MPD rechallenge on ED10 elicits 94% (101/108) of LC units significantly changed their firing rate; the majority of them (78%, 79/101) increased their firing rate; (4) The effect of rechallenge MPD administration on ED10 were compared to the effect of initial MPD on ED1, 98% of the LC units exhibited a significant change in their firing rate. 41% (43/106) of them exhibited a significant increase in their firing rate while 59% (63/106) units significantly decreased their firing rate which can be interpreted as electrophysiological sensitization or tolerance respectively. In conclusion, the majority of LC neurons significantly increased their firing rate after acute and chronic MPD administration. This data demonstrated that enhanced LC neuronal activities play important role in the effect of MPD.

Keywords

Ritalin; Norepinephrine; Single unit activity; Sensitization; Tolerance

1. Introduction

Attention deficit/hyperactivity disorder (ADHD) is a behavioral disorder that affects millions of children and adults. It is characterized by symptoms of inattention, impulsivity, and/or hyperactivity. ADHD is a complex and heterogeneous disorder and its etiology is not

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yet completely understood. Dysregulated dopaminergic and noradrenergic neurotransmission has been widely implicated in the pathophysiology of ADHD (Arnsten, 2006; Biederman, 2005; Del et al., 2011). Methylphenidate (MPD) is one of the most widely prescribed drugs for the treatment of ADHD. The neurochemical mechanism whereby MPD produces its therapeutic effects is far from being completely revealed. It has been shown that MPD binds to dopamine and norepinephrine transporters and indirectly elevates dopamine and norepinephrine in the synaptic cleft that result to improve ADHD symptoms by increasing arousal and alertness of the central nervous system (Biederman and Spencer, 1999; Pliszka et al., 1996). Despite both dopamine and norepinephrine involvement in the action of MPD, the dopamine system has been the main focus to explain its pharmacological treatment of ADHD. Data examining the effect of MPD on the norepinephrine system is limited.

MPD treatment increases extracellular norepinephrine concentration (Kuczenski and Segal, 2002) and the increase in norepinephrine is known to profoundly affect performance of attention, especially the maintenance of arousal which is known to be deficient in ADHD (Biederman and Spencer, 1999; Drouin et al., 2006). The locus coeruleus (LC) is the principal site for brain synthesis of nor epinephrine. LC-Noradrenergic neurons project broadly throughout the central nervous system (Amaral and Sinnamon, 1977; Moore and Bloom, 1979). LC neurons display tonic and phasic firing pattern and the discharge activity of LC neurons is positively related to norepinephrine release (Brun et al., 1993; Florin-Lechner et al., 1996). LC- norepinephrine plays an important role in arousal-dependent and attention-induced modulation of sensory signal processing (Berridge and Waterhouse, 2003) and activation of LC neurons modulates the processing of incoming sensory information (Devilbiss and Waterhouse, 2004; Lecas, 2004). Several studies indicated that administration of MPD decreased the firing rate of spontaneous activity of LC neurons in anesthetized animals (Devilbiss and Berridge, 2006; Lacroix and Ferron, 1988; Olpe et al., 1985). While another report indicated that LC units show very different firing patterns in unanesthetized and anesthetized animals (Akaike, 1982). However, little is known about the effect of MPD on the LC neurons in freely behavior animals without the interference of anesthesia. The aim of the study was to investigate the effect of acute and chronic MPD on the LC neuronal activity recorded from non-anesthetized, freely behaving rats previously implanted with permanent semi microelectrodes using a new wireless (telemetric) recording technology system (Fan et al., 2011). This allows us to study the effect of MPD on the activities of LC neurons without the interference of anesthesia. This study may provide important insights useful for understanding the mechanism of MPD effect on LC- norepinephrine system.

2. Materials and methods

2.1 Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 150–175g upon arrival were housed two in a cage with free access to food and water for 3 to 5 days for adaptation prior to electrodes 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 electrodes implantation, animals were individually housed in clear acrylic cages which

became their home cage and test cage for electrophysiological recordings. On the first recording day the animals' weights were about 200 to 220g. 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.

2.2 Surgery

The 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 anesthesia. The animal was then placed into the stereotaxic instrument followed by a 2 cm incision on the scalp, the muscle was removed and the skull was exposed. Holes were drill above the LC in both hemispheres (9.3 mm posterior to bregma, 1 mm lateral to midline according to the atlas (Paxinos and Watson, 1986)) and an additional hole in the frontal sinus for reference electrode was created. Two twisted Nickel-Chromium electrodes (60 μ m in diameter; Diamel coated insulation except at the tip, each secured to a 1cm copper connector pin) were implanted in each hemisphere into the LC. In the vacant spot, six anchor screws were inserted to secure the skull cap with two pairs of electrodes with dental acrylic. During the placement of electrodes, unit activities were monitored. Electrodes were fixed to the skull only when spike activity exhibited at least a 3:1 signal to noise ratio. When the neuronal activity exhibited less than 3:1 signal to noise ratio, 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 7.4 mm below the skull (Chong et al., 2012; Claussen and Dafny, 2012; Dafny, 1980; 1982; Dafny et al., 1988; Dafny and Terkel, 1990; Salek et al., 2012). The copper pins of all the electrodes were inserted into one Amphenol plug which was fixed onto the skull with dental acrylic. Rats were allowed to recover from the surgical procedure for 4 to 7 days. During this recovery period, rats were placed with their home cage daily in the Faraday testing box and connected to the wireless (telemetric) head stage transmitter (Triangle BioSystems Inc., TBSI, Durham, NC, USA) to adapt and acclimate to the recording system.

2.3 Drug

Methylphenidate hydrochloride (MPD) was obtained from Mallinckrodt Inc. (St. Louis, MO, USA). The MPD was dissolved in 0.9 % isotonic saline (NaCl) solution. To prepare 2.5 mg/kg injection, MPD were calculated as a free base, control injection consisted of 0.8 ml isotonic saline solution. Previous dose response experiments using MPD from 0.1 to 40 mg/kg found that 2.5 mg/kg MPD (i.p.) elicited behavioral and neurophysiological sensitization (Gaytan et al., 1996; Lee et al., 2009; Podet et al., 2010; Yang et al., 2001; 2003b; 2006a; 2006c; 2007; 2010; 2011). Thus, the 2.5 mg/kg MPD dose was used in this experiment. The drug volume for each animal was calculated by animal weight and then added saline equaled to 0.8 ml, so that all injection (i.p.) volumes were the same for all animals.

2.4 Experimental Protocol and Data Acquisition

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 plug, 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 one hour followed by a saline (for the control group) or 2.5 mg/kg MPD injection with recording resumed for additional hour post MPD injection. The head stage sent neuronal activity signals through a transmitter to a receiver (TBSI) and to an analog-to-digital converter (Micro1401-3; Cambridge Electronic Design (CED)) that collected and stored the recorded data from each electrode in a separate file on a PC using the CED Spike2.7 software. On ED2 through ED6 animals received either daily saline (for the control group) or 2.5 mg/kg MPD injection in their home cage to initiate its chronic effect (Gaytan et al., 2000a; 2002; Yang et al., 2000; 2003a; 2010; 2011). On ED7 to ED9 the animals underwent washout period, in which no injections were given. On ED10, neuronal activity recordings were resumed for one hour post injection of saline and followed by either saline or rechallenge administration of 2.5 mg/kg MPD with neuronal activity recorded for additional hour, the same protocol as on ED1 (Table1). During all recording sessions, the rat was freely behaving in the home/test cage.

2.5 Spike sorting Data analysis

The recorded neuronal activity was replayed off-line for spike sorting and analysis with the CED Spike 2.7 software fixed template matching system (FTMS). The software sorts and discriminates spikes based on the spike waveform shape and amplitude by setting parameters for templates. 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. Thousand waveform data points were used to define a spike to create templates. 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. 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 the 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. Comparisons were made between: (1). Saline baseline and MPD administration on ED1; (2). Saline baseline of ED10 to baseline of ED1; (3). MPD administration on ED10 to saline baseline on ED10; and (4). Activity after MPD administration on ED10 to activity post MPD on ED1. Statistical significance between the above comparisons were based on 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., 2006a; 2006b; 2006c). $P < 0.05$ was considered significant.

2.6 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 5% 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 coordinates (Paxinos and Watson, 1986).

3. Results

Fifty two electrodes implanted in 19 animals were confirmed to be in the target (Figure1). One hundred and fifty six units exhibiting similar amplitude and pattern on ED1 and ED10 were evaluated for comparisons. The effects of saline and MPD administration were observed on 48 and 108 LC units respectively.

3.1 Effect of saline (control recording at ED1 and ED10)

Effects of saline injection were recorded in 48 LC units to obtain the control for animal handling and the injection volume. On ED1, LC units exhibited similar firing rate after the first and second saline injection (Fig 2. ED1). Daily saline injections were given on ED2 though ED 6 followed by 3 washout days (ED 7 to 9). On ED10, LC units exhibited similar firing rate after the first and second saline injection (Fig 2. ED10). The firing rate of LC units on ED10 and ED1 after saline injections was similar (Fig 2), i.e. saline administration did not change the firing rate of LC units. Thus any significant change following MPD exposure will be considered as the drug effects.

3.2 Acute effect of MPD on LC units

On ED1, following the initial (acute) MPD (2.5mg/kg) administration, 87% (94/108) of LC units exhibited a significant ($P<0.05$) response to the drug by changing (increase or decrease) their firing rate compared to their saline baseline activities. The majority of them (80%, 75/94) of the LC units exhibited a significant ($P<0.05$) increase in their firing rate and 20% (19/94) of them exhibited a significant ($P<0.05$) decrease in their activities (Table 2. Acute Response). Figure 2 shows two representative LC units that responded to 2.5 mg/kg MPD administration by significantly increasing its firing rate in A, and by decreasing its firing rate in B. In conclusion, three groups (increased, decreased and unresponsive units) of LC units could be identified based on their response to acute MPD administration.

3.3 Comparing ED10 baseline activities to ED1 baseline activities

After initial MPD administration and recording on ED1, the rats were injected daily with a single 2.5 mg/kg MPD dose for an additional 5 days (ED2 to 6), followed by 3 days washout (ED7 to 9) and recordings were resumed following saline administration on ED10. All LC units exhibited a significant change of their baseline activity on ED10 compared to ED1 baseline activity. 44% (47/108) of them showed a significant ($P<0.05$) increase in their ED10 baseline activities and 56% (61/108) 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 LC units to acute MPD on ED1 affected their baseline activity on ED10, the baseline activity on ED1 of those three groups of LC units (increased, decreased and unresponsive units to MPD as shown in Table 2) were compared with their baseline on ED10 (Table 3A). For the 75 LC units which exhibited a significant increase in their neuronal firing rate on ED1 following the initial MPD exposure, 45% (34/75) of these units exhibited a significant increase ($P<0.05$) in their baseline firing rate on ED10, while 55% (41/75) of these LC units exhibited a significant decrease ($P<0.05$) in baseline firing rate on ED10 compared to ED1 baseline.

19 LC units exhibited a decrease in their neuronal activity to MPD exposure on ED1, all of them exhibited a significant change ($P<0.05$) in their ED10 baseline activity compared to their ED1 baseline activity. The majority (74%, 14/19) of these units exhibited decreases ($P<0.05$) in their baseline activity on ED10, and 26% (5/19) of them exhibited an increase ($P<0.05$) in their baseline activity on ED10 compared to ED1 baseline.

Of the 14 unresponsive LC units to MPD exposure on ED1, 57% (8/14) of them showed a significant increase ($P<0.05$) in their baseline activity on ED10, and 43% (6/14) of them showed a significant decrease ($P<0.05$) in their baseline activity on ED10 compared to ED1 baseline activity. Figure 3 shows two LC units exhibiting a significant change in their ED10 baseline activities compared to their ED1 baseline activity.

3.4 Chronic effect of MPD on LC neurons - comparing the effect of MPD rechallenge on ED10 with ED10 baseline

The firing rate of LC units after 2.5 mg/kg MPD rechallenge on ED10 was compared to the saline baseline activity on ED10. 94% (101/108) of LC units exhibited a significant ($P<0.05$) change in their firing rate following MPD rechallenge on ED10. The majority (78%, 79/101) of them significantly ($P<0.05$) increased their activity, and 22% (22/101) of them significantly ($P<0.05$) decreased their activity (Table 2. Chronic Response).

In order to find out whether the acute response of LC units to MPD on ED1 affected their response to MPD on ED10, the three groups of LC units on ED1 (increase, decrease and unresponsive to the initial MPD as shown in Table 2) and their neuronal firing rate changes to MPD rechallenge on ED10 were compared and summarized in Table 3B. The table shows that of the 75 LC units which exhibited a significant ($P<0.05$) change in their firing rate on ED1 following the initial MPD exposure, 59 (79%, 59/75) of these LC units showed a significant ($P<0.05$) increase in their neuronal activity and 11 (15%, 11/75) of them showed a significant ($P<0.05$) decrease in their activity on ED10 following MPD exposure respectively.

Of the 19 LC units which exhibited a decrease in their activity on ED1 to MPD exposure, all of them exhibited a significant ($P<0.05$) change in their neuronal activity to MPD rechallenge on ED10. 12 (63%, 12/19) units significantly ($P<0.05$) increased their neuronal

activity and 7 (37%, 7/19) units significantly ($P<0.05$) decreased their activity on ED10 following MPD exposure, respectively (Table 3B).

Of the 14 unresponsive units to MPD exposure on ED1, 86% (12/14) of them exhibited a significant ($P<0.05$) change in their neuronal activity. 57% (8/14) of them significantly ($P<0.05$) increased their neuronal activity, while 29% (4/14) of them significantly ($P<0.05$) decreased their neuronal activities on ED10 following MPD exposure, respectively (Table 3B).

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

In order to find out whether chronic MPD exposure will elicit sensitization or tolerance in LC units, the effect of rechallenge MPD on ED10 was compared to the effect of initial MPD on ED1. The results were summarized in Table 3C. Of the 75 units which increased their firing rate on ED1 after initial MPD exposure, 43% (32/75) of them exhibited a significant ($P<0.05$) increase in neuronal activity. This increase in activity can indicate neurophysiological sensitization. 57% (43/75) of them exhibited a significant ($P<0.05$) decrease in their firing rate on ED10 following rechallenge MPD compared to initial MPD on ED1 (Table 3C). The opposite effect of MPD on these units can indicate neurophysiological tolerance. Figure 4 shows two representative LC units that exhibited an increase (upper panel) and decrease (lower panel) in activity to MPD rechallenge on ED10 compared to initial MPD exposure ED1.

Of the 19 units that exhibited a decrease in their firing rate on ED1 after initial MPD exposure, 95% (18/19) of them exhibited a significant ($P<0.05$) change in their neuronal activity. 21% (4/19) of them reversed their response direction and significantly ($p<0.05$) increased their neuronal activity and 74% (14/19) of them significantly ($P<0.05$) decreased their neuronal activity after MPD rechallenge on ED10 compared to initial MPD administration on ED1 respectively (Table 3C).

Of the 14 MPD unresponsive LC units on ED1, 93% (13/14) of them exhibited a significant ($P<0.05$) change in their neuronal activity following MPD rechallenge on ED10. 50% of them (7/14) exhibited a significant ($P<0.05$) increase their neuronal activities, while 43% (6/14) of them exhibited a significant ($P<0.05$) decrease in their neuronal activity on ED10 compared to initial MPD administration on ED1 respectively (Table 3C).

4. Discussion

The technique using permanent implanted electrodes for long period recording of extracellular neuronal activities has been established. For example, 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, we secured the electrodes onto the skull with screws and dental acrylic cement to avoid the migration of the electrodes. By sorting the electrical activity with Spike2 software (see methods), the spikes with a similar shape and amplitude recorded from the same electrode was considered as a single unit (Dafny and Gilman, 1973). Only such single units that exhibited nearly identical shape and amplitude on ED1 and ED10 were included in the analysis. The present

study investigated the effects of acute and chronic MPD exposure on LC neuronal activities. The main findings of this study are (1) 87% of recorded LC units significantly changed their firing rate after acute MPD administration. The majority of them (80%) increased their firing rate after acute MPD exposure. (2) All the recorded LC units significantly changed their baseline activity on ED10 after six daily repetitive exposure of MPD and three washout days. (3) 94% of recorded LC units exhibited a significant change in their firing rate after MPD rechallenge on ED10 compare to their baseline activity on ED10, the majority of them (78%) increased their firing rate. (4) Comparing the effect of MPD rechallenge on ED10 to the initial MPD exposure on ED1, 98% units significantly changed their firing rate. 41% (43/106) of the units exhibited a significant increase in their firing rate while 59% (63/106) of the units exhibited a significant decrease in their firing rate.

As a major source of norepinephrine in the mammalian brain, LC participates in various brain functions, including vigilance, attention and mediation of stress response (Aston-Jones et al., 1999; Foote et al., 1980; Olpe et al., 1985). Moreover, numerous studies have shown that application of norepinephrine (Devilbiss and Waterhouse, 2000; Manunta and Edeline, 1997; Waterhouse et al., 1980; 2000) or activation of the LC (Devilbiss and Waterhouse, 2004; Holdefer and Jacobs, 1994; Lecas, 2004; Waterhouse et al., 1998;) can modulate the processing of incoming sensory information in primary sensory circuits of mammalian brain. Considering this view, dysfunction in LC-noradrenergic system may be responsible for some of the symptoms (such as distractability and inattentiveness) observed in patients suffering from ADHD. Indeed, psychostimulants prescribed for the treatment of ADHD, such as MPD, enhance noradrenergic transmission by blocking norepinephrine reuptake via its transporter (Kuczenski and Segal, 1997). Several studies indicated that i.p. (0.25–10mg/kg) and i.v. (0.4–1.1mg/kg) MPD administration decreased the firing rate of spontaneous activity of LC neurons in anesthetized animals (Devilbiss and Berridge, 2006; Lacroix and Ferron, 1988; Olpe et al., 1985). In this experiment with freely moving rats, most of the LC neurons showed a significant increase in their firing rate after acute and chronic administration of MPD. Indeed, it was reported that LC units showed very different firing patterns in unanesthetized and anesthetized animals (Akaike, 1982). Thus, this different response of LC neurons to MPD may be caused by anesthetics. It has been reported that LC-noradrenergic neurons are involved in the mechanism of anesthesia, destruction of LC noradrenergic neurons by administration of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine significantly increased thiopental anesthesia duration (Kushikata et al., 2011). Similarly, the duration of thiopental, pentobarbital, methohexital, or hexobarbital anesthesia was markedly increased by depletion of brain norepinephrine in the LC projection system in rats (Mason et al., 1983). In contrast, destruction of LC noradrenergic neurons by N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine significantly decreased the duration of ketamine anesthesia (Kushikata et al., 2011). These observations suggested that LC noradrenergic neuronal activity plays a crucial role in the mechanism of anesthesia and its effect dependent on the type of anesthetics. Systemically administered morphine produced a significant increase in the spontaneous activity of norepinephrine neurons in the LC of freely moving cats. In addition, this same study found that when morphine was administered in an identical manner to cats anesthetized with chloral hydrate, a significant decrease in the activity of these neurons was then produced (Rasmussen and Jacobs, 1985).

These results suggest that LC unit activity affected by anesthesia. In order to exclude the interference of anesthesia in the response to MPD, the LC neuronal activity should be study in unanesthetized animals. Single-cell recording studies performed in freely moving mammals have revealed that LC neurons are tonically active during both quiet and active waking (Aston-Jones and Bloom, 1981; Foote et al., 1980; Hobson et al., 1975; Jacobs, 1986). LC neuronal activity contributes to the maintenance of muscle tone in waking, and that reduction in LC discharge plays a role in the loss of muscle tone in cataplexy and rapid-eye-movement sleep (Wu et al., 1999). The above reports indicate that LC neurons are electrophysiological quiet during low vigilance states such as sleep or in the lack of sensory input, while during active walking or exposed to a strong stimulus, the LC neurons markedly increase its firing rate. Consistent with the above studies, the present experiment found that after administration of MPD most of LC units significant increase their firing rate, since administration of MPD (2.5mg/kg or above) resulted in an increase in locomotor activity (Askenasy et al., 2007; Gaytan et al., 2000a; 2000b; 2002; Yang et al., 2003a; 2006a; 2006b; 2006c; 2007; 2010).

MPD administration elicits a decrease of LC neurons firing rate in anesthetized rats in previous studies, which was explained by the inhibitory feedback of presynaptic α_2 -adrenergic autoreceptors (Lacroix and Ferron, 1988). Obviously, this mechanism is not accountable for the increase of LC neurons firing rate in our freely moving rats, which, however, can be explained by other two mechanisms. Firstly, during waking, LC neurons are phasically activated by a variety of sensory stimuli (Aston-Jones and Bloom, 1981). The sensory feedback from behaviors elicited by MPD may attribute to the activation of LC neurons (Aston-Jones et al., 1991a), which is presumably absent under anesthetized condition. Secondly, the LC receives projections from many different brain regions, that release a wide spectrum of neurotransmitters, such as opiates, glutamate, GABA, serotonin, epinephrine (Aston-Jones et al., 1991b). While, general anesthetics affect the synaptic transmission in the central nervous system (Barker and Ransom, 1978; Nicoll and Madison, 1982; Richards, 1978; Scholfield, 1978; 1980). The interactions of these neurotransmitters with LC neurons may be the other possible explanation for the increase firing of LC neurons elicited by MPD in freely moving rats.

The 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). Chronic MPD 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). Chronic MPD exposure decrease dopamine transporter density (Moll et al., 2001; Izenwasser et al., 1999) and down-regulation of activity in the dopamine system (Chase et al., 2003; Hawken et al., 2004; Sadasivan et al., 2012; Vles et al., 2003). It is well known that both dopamine and norepinephrine system are involved effect of MPD and there is a reciprocal interaction between these two systems (Guiard et al., 2008a; 2008b). Selective loss of dopamine neurons in ventral tegmental area increased the firing activity of LC- norepinephrine neurons revealing the inhibitory effect of the dopamine input on LC- norepinephrine

neurons (Guiard et al., 2008b). Thus, the down-regulation of activity in the dopamine system may explain the different response of LC units to MPD between ED1 and ED10.

It has been reported that opiate withdrawal induced hyperactivity of LC neurons in morphine-dependent, halothane-anesthetized rats (Akaoka and Aston-Jones, 1991). In this experiment, the baseline activity of most LC units was significantly changed after six daily repetitive MPD administrations and three days of washout period; the majority of them increased their baseline firing rate. This change in baseline activity of LC units can be interpreted as neurophysiological withdrawal. In addition, the firing rate of LC units was altered by the rechallenge MPD administration on ED10 compared to initial MPD on ED1. Those LC units that showed an increase in their response to the initial MPD exposure on ED1 and further increase to rechallenge MPD administration on ED10 may be interpreted as neurophysiological sensitization. While those that showed a decrease in their neuronal activity to rechallenge MPD on ED10 compared to initial MPD on ED1 may be interpreted as neurophysiological tolerance. Histofluorescence and immunocytochemical studies have shown that most of LC neurons are noradrenergic (Swanson, 1976) although some are serotonergic (Sladek et al., 1977) and GABAergic neurons (Iijima et al., 1987). This heterogeneous composition of the LC neurocytology can explain the different response obtained in this study following MPD exposure. These neurophysiologic sensitization or tolerance of LC neurons may contribute to the behavioral sensitization or behavioral tolerance induced by chronic MPD administration. The correlation between them needs further elucidation.

In summary, using freely moving rats, we reported for the first time that acute and chronic administration of MPD induced an increase of the LC neuronal activity, which can probably interpret the enhancement effect of MPD on cognition and attention.

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Fig. 1.

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

Fig. 2.

Representative sequential frequency firing rate of a LC unit recorded before and after saline injection on ED1 and ED10. 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 ED10. Arrow indicates the time of saline administration. Insert waveforms show 20 spikes sorted in same template on ED1 and ED10 respectively.

Fig. 3.

A representative sequential frequency firing rate of two LC units on ED1. The first 60 min shows the baseline activity after saline injection followed by an additional 60 min after initial 2.5 mg/kg MPD administration. Arrow indicates the time of 2.5 mg/kg MPD administration. The unit in A exhibited an increase in firing rate after acute 2.5 mg/kg MPD administration. The unit in B exhibited a decrease in firing rate after acute 2.5 mg/kg MPD

Fig. 4.

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

Fig. 5.

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

Table 1

Experimental protocol

Experimental day	1	2–6	7–9	10
Control	Saline; Saline	Daily Saline	Washout	Saline; Saline
MPD 0	Saline; MPD	Daily MPD	Washout (no injection)	Saline; MPD

Table 2

The effect of MPD on the firing rate of 108 LC units

	Increase	Decrease	Unresponsive
(A) MPD acute Response	75	19	14
(B) ED10 BL compare to ED1 BL	47	61	—
(C) MPD rechallenger on ED10	79	22	7

(A) Summary the effect of MPD on ED1 compare to baseline activity (BL) on ED1. (B) Compares the ED10 BL after six daily MPD exposure and three washout days to ED1 BL. (C) Summary the effect of rechallenger MPD on ED10 compare to the ED10 BL. N=108

Table 3A

Comparing ED10 baseline activities to ED1 baseline activities

	<u>Change of ED10 baseline compare with ED1 baseline</u>	
	Increased	Decreased
75 units increased to MPD on ED1	34 (45%)	41 (55%)
19 units decreased to MPD on ED1	5 (26%)	14 (74%)
14 units unresponsive to MPD on ED1	8 (57%)	6 (43%)

N=108

Table 3B

Comparing the effect of rechallenge MPD to baseline activities on ED10

	Number (%) of LC units response to MPD on ED10		
	Increased	Decreased	No change
75 units increased to MPD on ED1	59 (79%)	11 (15%)	5 (6%)
19 units decreased to MPD on ED1	12 (63%)	7 (37%)	—
14 units unresponsive to MPD on ED1	8 (57%)	4 (29%)	2 (14%)

N=108

Table 3C

Comparing the effect of rechallenge MPD on ED10 to initial MPD on ED1

	Effect of rechallenge MPD on ED10 compare to MPD on ED1		
	Increased	Decreased	No change
75 units increased to MPD on ED1	32 (43%)	43 (57%)	—
19 units decreased to MPD on ED1	4 (21%)	14 (74%)	1 (5%)
14 units unresponsive to MPD on ED1	7 (50%)	6 (43%)	1 (7%)

N=108