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Brain Research Bulletin 76 (2008) 131–140

BRAIN RESEARCH BULLETIN

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Research report

Methylphenidate sensitization is prevented by prefrontal cortex lesion

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Received 26 October 2007; received in revised form 4 December 2007; accepted 7 December 2007
Available online 3 January 2008

Abstract

Methylphenidate (MPD), also known as Ritalin, is a widely used treatment for Attention Deficit Hyperactivity Disorder. Repeated administration of MPD causes dose-dependent sensitization. MPD binds to dopamine (DA) transporters, and DA, therefore, remain in the synaptic cleft for longer time, resulting in an indirect DA agonist effect. MPD affects neurotransmission in brain regions including the prefrontal cortex (PFC). The mechanisms of sensitization to MPD are not clear.

The aim of this study was to investigate the role of prefrontal cortex in effects of acute and chronic MPD administration, using the open field assay and male Sprague–Dawley rats with bilateral electrolytic lesions of PFC. After 1 day of control recording, following saline injection, the animals were divided randomly into three groups, (1) an intact control group, (2) a sham group, and (3) a lesion group. Then, groups 2 and 3 underwent surgery, followed by 5 days of recovery. Recordings were resumed following 1 day of saline injection and following six consecutive daily injections of 2.5 mg/kg MPD, 3 days of washout period, and another day of re-challenge injection of 2.5 mg/kg MPD.

Acute MPD elicited increases in locomotor activity, similar to those observed from intact animals, in both sham and lesion groups. The sham group was behaviorally sensitized while the PFC lesion group failed to exhibit behavioral sensitization.

These results suggest that the PFC does not interfere with the acute effects of MPD on locomotor activity but is required for development of behavioral sensitization to MPD.
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Keywords: Behavioral sensitization; Methylphenidate; Prefrontal cortex; Lesion; Acute and chronic treatment; Withdrawal

1. Introduction

The psychostimulant methylphenidate (MPD), also known as Ritalin, is the most prescribed drug used to treat children and adolescents with Attention Deficit Hyperactivity Disorder (ADHD) [2,9,36]. Chronic exposure of low to moderate dose of psychostimulants causes behavioral sensitization [14,31,45]. Behavioral sensitization is indicated by augmented locomotor activity to subsequent psychostimulant challenge.

The neuroanatomical circuit that is the target of these psychostimulant administrations is known as the motive circuit [28], which is responsible for turning biological stimuli into adaptive behavioral responses [22]. Long-term changes in neurotransmission within the motive circuit, which includes prefrontal cortex (PFC), are thought to be involved in the induction and expression of behavioral sensitization produced by chronic psychostimulant administration, like cocaine and amphetamine [8,28,34].

The fact that repeated injection of psychostimulants directly into the ventral tegmental area (VTA), but not PFC or nucleus accumbens (NAc), produces sensitization to these drugs [3,11,40] suggests that the VTA is the main site for the induction of behavioral sensitization. The expression of sensitization following chronic psychostimulant administration, on the other hand, is mainly contributed to NAc [10,27]. Major projections to VTA and NAc come from the PFC through excitatory amino acids (EAA) pathway [6,7,43] making PFC the vital part to the induction and expression of sensitization. Schenk and Snow [33] showed that daily electrical stimulation of medial PFC for about 32 days resulted in the much higher locomotor activity in response to the cocaine injections than the rats that received daily electrical stimulation of hippocampus or rats without any electrical stimulation. Repeated activation of prefrontal corti-

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0361-9230/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.brainresbull.2007.12.004

cal output by the electrical stimulation is suggested to be the cause of this heightened response to the effects of cocaine, which implies that PFC projection participates in the cocaine sensitization [33]. Furthermore, lesions of the PFC area were shown to inhibit the induction of sensitization to amphetamine [4,42] and to modulate sensitization to cocaine [38].

There are contradicting studies regarding the role of PFC in the expression of sensitization to psychostimulant. Li et al. [25] reported that expression of sensitization to cocaine is not affected by PFC lesion, while Pierce et al. [29] reported the opposite result. PFC lesions also failed to inhibit the expression of sensitization to amphetamine [24].

There is no report to our knowledge that investigates the role of PFC in the induction and expression of MPD sensitization. The present study was initiated to find out the role of PFC in MPD-induced behavioral sensitization since understanding the neuroanatomical site(s) of the psychostimulants action will yield better insight on how the drugs function and how to counteract their adverse effects. Intact Sprawgue–Dawley (SD) group, sham operated group, and PFC lesion groups were compared using the open field assay to record the animal behavior.

2. Materials and methods

2.1. Animals

Twenty-four adult male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) were housed in soundproof experimental room with four animals in each cage for 5 days. They were then individually housed in the same room in open field cages that became their home cage with free access to food and water and were allowed to acclimate for additional 2 days before the recordings began. The experiment room was kept at ambient temperature of $21 \pm 2\,^{\circ}C$ and relative humidity of 37–42% and maintained on a 12:12 light/dark cycle (light on at 06:00). At the beginning of the recordings (experimental day 1), the animals weighed between 220 g and 240 g.

2.2. Drugs

Methylphenidate hydrochloride (MPD), from Mallinckrot (Hazelwood, MO), was dissolved in 0.9 % isotonic saline solution to make 2.5 mg/ml MPD. Each animal was weighed every other day, and appropriate volume of 2.5 mg/ml MPD solution to make 2.5 mg/kg MPD dose was loaded into the syringe. Then saline was added to the syringe to make all the injections the same volume ($0.8\,cm^3$), i.e. all injections were of the same volume. Injections were made intra-peritoneally (i.p.) between 06:30 and 07:00 h. The recordings started immediately after injection. This dose and the time of injection were selected based on our previous dose response and different injection time experiments [14,16,45,46] showing that this dose in this injection time elicits behavioral and electrophysiological sensitization.

2.3. Surgeries

For the sham and lesion surgeries, rats were anesthetized with 50 mg/kg sodium pentobarbital, i.p., and placed in a stereotaxic apparatus. An incision was made in the scalp and cranial muscles, and two small holes were made above the PFC according to the atlas of Paxinos and Watson [26], at 3.2 mm anterior to bregma and 0.6 mm lateral to each side of midline. Electrode was made up of two twisted stainless steel wires, 80 μm in diameter. Bilateral electrolytic lesion was created by running 3 mA current through the electrode for a minute in three steps, first at 4.4 mm below the skull, next at 3.4 mm below the skull, and the last at 2.4 mm below the skull. After the surgery, the skin of the head was closed together using wound closing staples. For sham group, the electrodes were placed in identical locations for the same amount of time but without current.

2.4. Procedure

The first experimental day recording, following saline injection, was taken after 2 days of acclimation in home cages. The animals were then randomly divided into three groups, control group ($n = 8$), sham group ($n = 8$), and lesion group ($n = 8$). On experimental day 2, two groups underwent surgery, either sham or lesion surgery, and were allowed 5 days to recover (experimental days 3–7). On experimental day 8 the recordings were resumed post-saline injection followed by six consecutive days of 2.5 mg/kg MPD administrations (experimental days 9–14). Experimental days 15–17 were the washout period, and re-challenge dose of 2.5 mg/kg MPD injection was given on experimental day 18. Intact control group protocol was same as the sham and lesion groups but without the surgery (Table 1).

2.5. Apparatus

Open field cages (40.5 cm × 40.5 cm × 31.5 cm) were used to record locomotor activity. Computerized animal activity monitoring (CAAM; AccuScan Instruments, Inc., Columbus, OH) system, with two levels of 16 infrared beams and sensors, took continuous recording of subjects' activity. The interruptions of the beams by the animal movements were counted and compiled by AccuScan Analyzer and downloaded every 10 min into OASIS program, which organized these counts into several different locomotor indices.

2.6. Histology

After the completion of experiment, the animals were overdosed with sodium barbital and perfused intracardially with 10% formaldehyde containing about 3% potassium ferrocyanide. Their brains were removed and placed in 10% formaldehyde for at least 48 h. Then, the brains were sectioned in the coronal plane at 120 μm thickness and stained with Cresyl Violet. The atlas of Paxinos and Watson was used to determine the size and placement of the lesions (Fig. 1).

2.7. Data analysis

The 10 min bins of locomotor activity counts were used to produce two types of analysis. (1) Each bin was plotted sequentially to produce 2 h of temporal recording graph after the injection, and the standard error (S.E.) for each bins was used to calculate the significance of the change between the experimental days using ANOVA and Post hoc analysis with LSD test [13,16,45,48]. Sig-

Table 1
Schedule of MPD administration

Group	Experimental day						
	Day 1	Day 2	Days 3–7	Day 8	Days 9–14	Days 15–17	Day 18
Control	Saline	No treatment	No treatment	Saline	2.5 mg/kg MPD	Withdrawal/No treatment	2.5 mg/kg MPD
Sham		Surgery	No treatment	Saline	2.5 mg/kg MPD	Withdrawal/No treatment	2.5 mg/kg MPD
Lesion		Surgery	No treatment	Saline	2.5 mg/kg MPD	Withdrawal/No treatment	2.5 mg/kg MPD

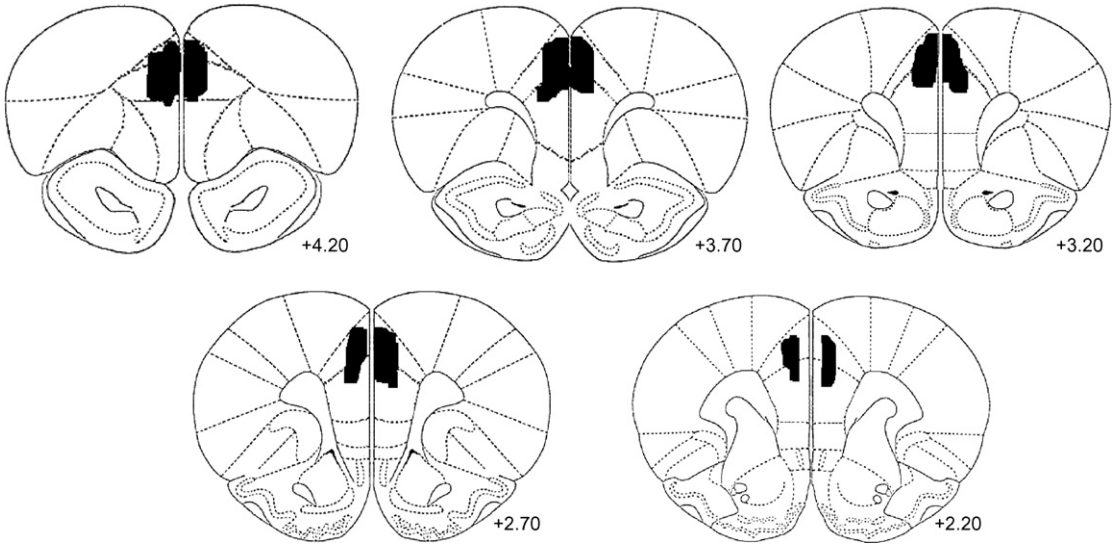

Fig. 1. The size and location of the PFC lesions.

nificant changes in at least two consecutive bins were considered as significant drug effect. (2)The sums of 2-h activity (12 bins) were used to get the average activity level during the initial 2 h after injection for each group (control, sham, and lesion). Comparisons between different groups were made with ANOVA, and Post hoc analysis was performed with LSD test. Significance was set at $P < 0.05$. Five comparisons were made. (1) Experimental day 1 was compared to experimental day 8 to find out whether the sham or lesion operations altered the baseline activity. (2) Experimental day 8 was compared to experimental day 9 to obtain the acute MPD effect. (3) Experimental day 9 was compared to experimental day 14 to find out whether the sensitization to MPD was induced. (4) Experimental day 8 was compared to experimental day 15 to observe the activity in the washout period. (5) Experimental day 9 (the initial MPD treatment to naïve animals) was compared to experimental day 18 (the last day of MPD administration) to see whether the sensitization to chronic treatment was expressed after 3 days of washout.

3. Results

3.1. Effect of saline on baseline activity

In previous studies, locomotor activities of saline control groups were recorded for 16–42 days, and it was observed that the activity following the saline injections during all the experimental days were about the same with non-significant minor fluctuation [13,14,48]. Therefore, the activity of experimental day 1 after saline injection was used as baseline control [13,16,44,47] in the intact control group to evaluate the acute and chronic drug effects (Table 1).

3.1.1. Effect of sham operation and PFC lesion on baseline activity

Fig. 2 summarizes the baseline activity level during the first 2 h following the saline injection before the surgery (experimental day 1) and after the sham surgery and PFC lesion surgery (experimental day 8), i.e. 5 days post-surgery, for each group. The locomotor baseline activity levels on experimental day 8 in all three groups were about the same as the levels observed on experimental day 1 (Fig. 2). Therefore, experimental day 8 activity was used as control recording to compare the drug effects in sham and lesion groups.

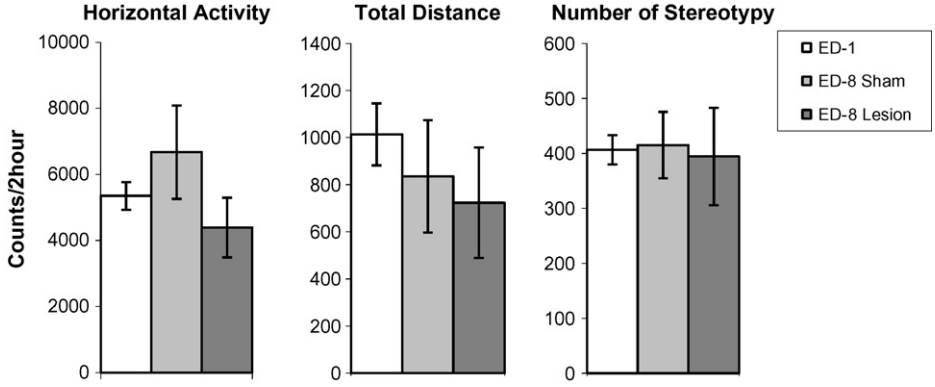

Fig. 2. Baseline activity before and after surgery of three locomotor indices of all three groups (each $N = 8$). HA: horizontal activity; TD: total distance traveled; NOS: number of stereotypic activity; experimental day 1: recordings were obtained from intact animals; experimental day 8: recordings after surgery. The locomotor baseline activity levels obtained on experimental day 8 in all three animal groups (intact control, sham, and PFC lesion) were about the same as the levels observed on experimental day 1.

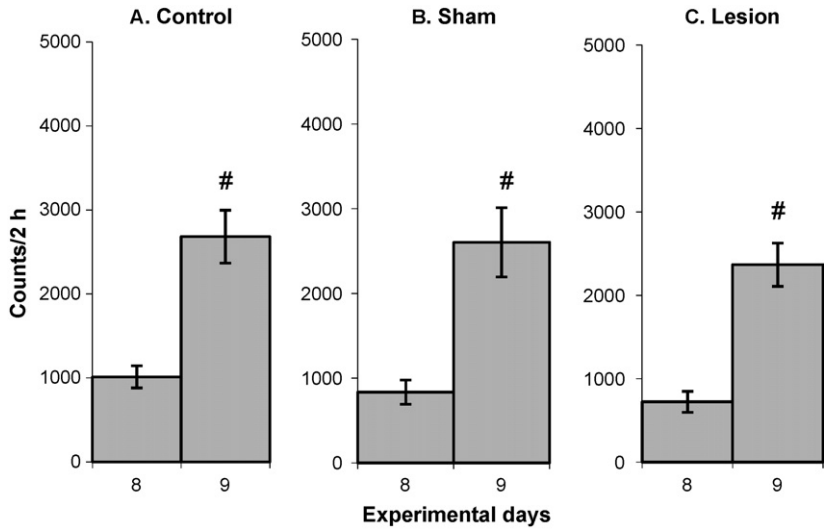

Fig. 3. The acute effect of MPD on TD traveled activity. '#' Mark indicates significant difference between the 2 h activities of experimental days 8 and 9 (Table 1). Significance was set at <0.05.

3.2. MPD acute effect: comparing experimental day 8 versus experimental day 9

Fig. 3 histograms compare the TD traveled activity levels of each group (control, sham, and lesion) during the first 2 h after MPD administration on experimental day 9 (initial 2.5 mg/kg MPD) with activity levels of experimental day 8 (activity post-saline injection). All three groups showed significant increases in TD traveled in response to a single injection of 2.5 mg/kg MPD. All the groups increased with the similar intensity, which suggests that sham surgery and PFC lesions did not interfere with the acute action of MPD (Fig. 3).

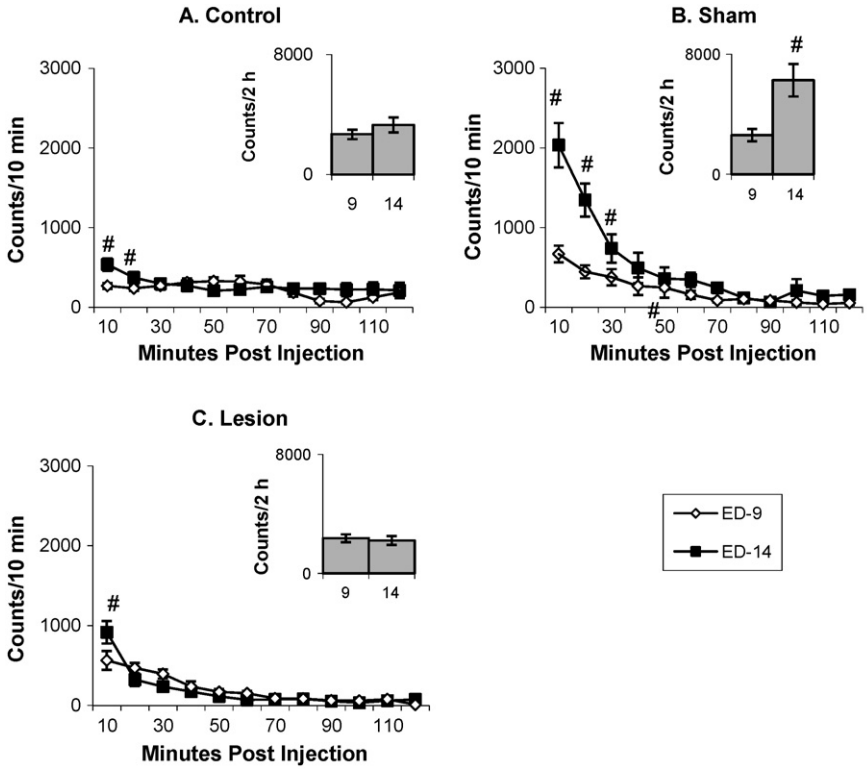

Fig. 4. The temporal graphs (activity counts/10 min) and 2 h histograms of TD traveled recordings on experimental days 9 and 14 (Table 1). '#' Mark indicates significant difference between corresponding points on the temporal graphs or significant difference between the 2 h activities of experimental days 9 and 14. Significance was set at <0.05.

3.3. Induction phase: comparing experimental day 9 versus experimental day 14

Fig. 4 summarizes the temporal graphs (activity counts/10 min) and the histograms, summation of the TD traveled under the curve (2 h of activity), of each group (control, sham, and lesion) comparing the activity levels of TD traveled in the first 2 h after MPD administration on experimental day 9 (the first day of MPD treatment) with activity recorded on experimental day 14 (the 6th day of MPD treatment). The control group exhibited significant increase in TD traveling in the initial 20 min post-injection on experimental day 14 compared to experimental day 9 in, as indicated by the LSD tests (Fig. 4A). This effect of the drug, i.e. increase in activity, for 20 min could also be seen in the 2 h histogram, but the difference between experimental day 9 and experimental day 14 in the histogram was not significant according to LSD test. This suggests that sometimes the 2 h histograms can skew the observation of short acting drug, so, in this case, temporal graphs are more suited to show the effect of MPD. In the sham group, the MPD treatment in experimental day 14 elicited increase in TD traveled for 30 min. This increase was expressed as significant change also in the 2 h histogram (Fig. 4B). The lesion group only showed increase in the initial 10 min after injection.

In previous study, it was observed that rats moved around the cage for several minutes after saline injection [15,45], which suggests that majority of the movement during the initial 10 min post-injection is due to handling and the injection. Therefore, only two consecutive time change (20 min) was considered as the drug effects. Hence, the PFC lesion prevented the induction of behavioral sensitization (Fig. 4C).

3.4. Activity during the washout phase: comparing experimental day 8 to experimental day 15

Fig. 5 summarizes the TD traveled temporal graphs and 2 h histograms of each group (control, sham, and lesion) comparing activity levels throughout the first 2 h after the saline injection on experimental day 8 (control recording) with activity levels throughout the first 2 h after the usual time of MPD administration on experimental day 15 (the first day after abrupt withdrawal of MPD administration; Table 1). The control, intact group (Fig. 5A) exhibited increased activities on experimental day 15 compared to experimental day 8 in first 40 min after 07:00, in which time the MPD injections were given in the previous 6 days (experimental days 9 through 14). The increase in activities was observed in the histogram as well. These increases in activities can be interpreted as expectation behaviors as well as withdrawal symptoms. The sham group showed increased activity during the first 20 min on experimental day 15 compared to experimental day 8 (Fig. 5B). The PFC lesion group failed to show increase in the activity levels on experimental day 15 compared to experimental day 8 (Fig. 5C).

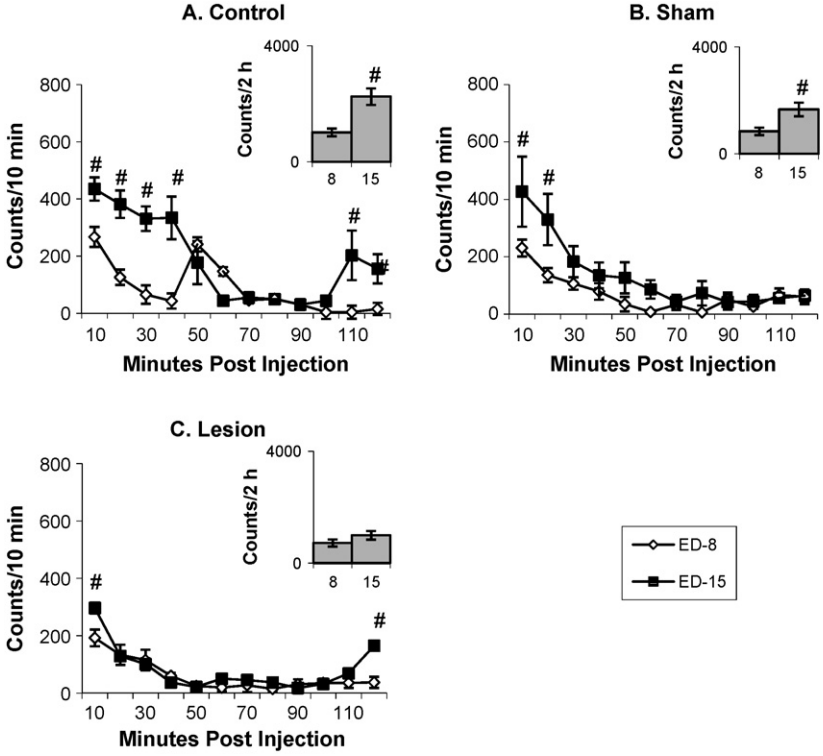

Fig. 5. The temporal graphs (activity counts/10 min) and 2 h histograms of TD traveled recordings on experimental days 8 and 15 (Table 1). '#' Mark indicates significant difference between corresponding points on the temporal graphs or significant difference between the 2 h activities of experimental days 8 and 15. Significance was set at <0.05.

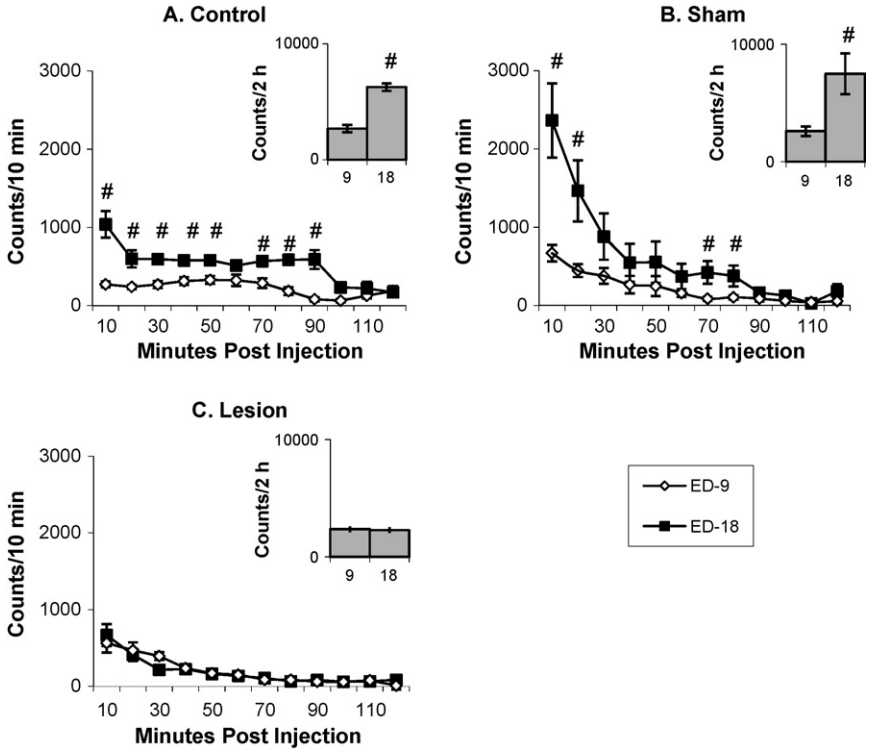

Fig. 6. The temporal graphs (activity counts/10 min) and 2 h histograms of TD traveled recordings on experimental days 9 and 18 (Table 1). '#' Mark indicates significant difference between corresponding points on the temporal graphs or significant difference between the 2 h activities of experimental days 9 and 18. Significance was set at <0.05.

3.5. Expression phase: comparing experimental day 9 versus experimental day 18

Fig. 6 summarizes the temporal graphs and 2 h histograms of each group (control, sham, and lesion) comparing the TD traveled levels throughout the first 2 h after MPD administration on experimental day 9 (the initial MPD injection) and experimental day 18 (MPD re-challenge). The control group expressed significant increase in TD traveling for about 90 min post-MPD injection, and this increase is also expressed in the 2 h histogram (Fig. 6A). The sham group also show similar effect but with different pattern (Fig. 6B). The lesion group did not exhibit significant increases after MPD injection on either in the temporal graph or 2 h histogram on experimental day 18 compared to experimental day 9 (Fig. 6C).

3.6. Other locomotor indices

Fig. 7 summarized the 2-h activity histograms of horizontal activity (HA), TD traveled, and number of stereotypy (NOS) activity for experimental day 8 (saline control), experimental day 9 (first day of MPD treatment), experimental day 14 (last day of MPD maintenance treatment), and experimental day 18 (MPD re-challenge). All the locomotor indices of all three groups, except for the NOS index of lesion group, exhibited increases in the activity following the initial MPD dose, similar to those observed in the TD traveled index. All the locomotor indices on experimental day 14 compared to experimental day 9 (induction phase) showed some increases in the activity levels. However, all these increases were non-significant, except for the TD traveled index of the sham group. Comparing the observation obtained on experimental day 9 with experimental day 18 (expression phase) shows that the control group and sham group exhibited significant increases in TD traveled and HA indices while their NOS activities exhibited similar activity to that obtained after the initial MPD injection. This suggests that in control and sham group, chronic MPD elicits the expression of behavioral sensitization. In the PFC lesion group, chronic MPD exert similar effect to the initial injection in all the locomotor indices (Fig. 7).

4. Discussion

The PFC has been associated with various functions like respiration, heart rate, blood pressure, decision making, goal directed behavior, working memory, and reward [1,12,17,23,30]. The PFC can participate in such diverse functions because of its efferent and afferent projections to numerous parts of the brain, including different cortical area, diencephalon nuclei, limbic structures, hypothalamus, midbrain, pons and medulla [17]. The PFC projects to motive circuit structures that play major roles in induction and expression of behavioral sensitization, VTA and NAc, respectively, to psychostimulant as well [8,28,34]. Although many studies involving PFC lesions have been conducted using cocaine and amphetamine to study the role of PFC in psychostimulant actions, there are no studies to our knowledge that studied the role of PFC in MPD action.

In this study, the bilateral electrolytic PFC lesions were created in SD adult male rats to study the role of PFC in MPD-

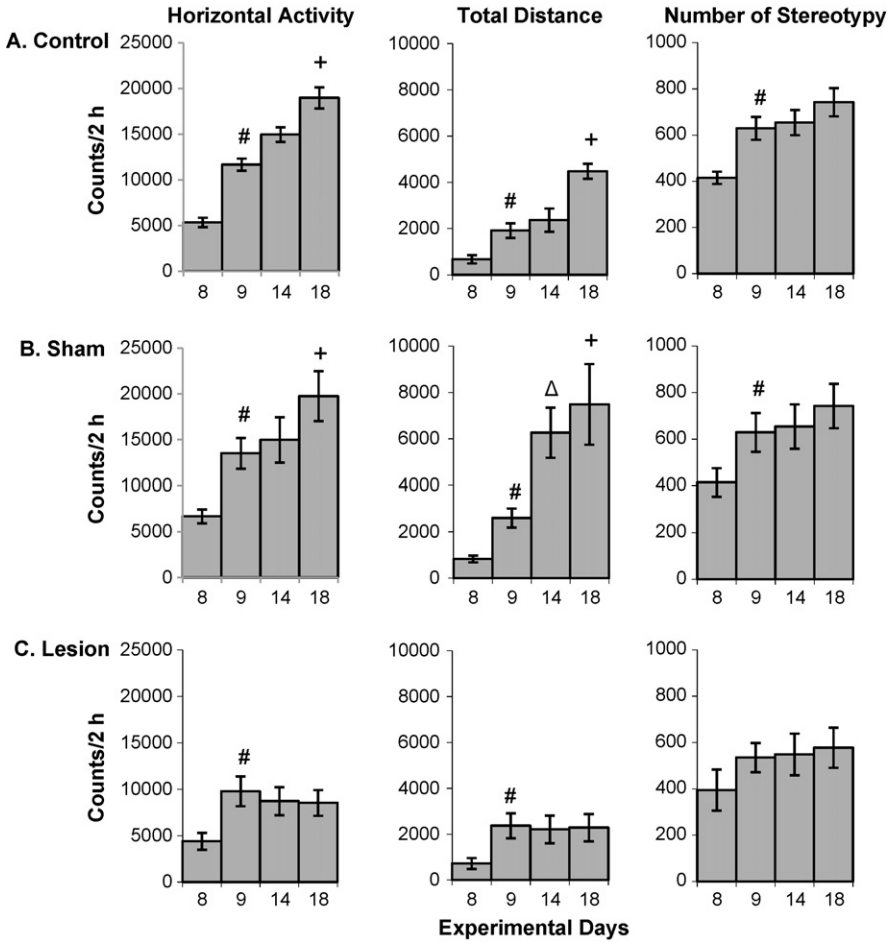

Fig. 7. The 2-h histograms of horizontal activity (HA), TD traveled, and number of stereotypy (NOS) recordings on experimental days 8, 9, 14, and 18. '#' Mark indicates significant difference between the 2 h activities of experimental days 8 and 9. 'Δ' Mark indicates significant difference between the 2 h activities of experimental days 9 and 14. '+' Mark indicates significant difference between the 2 h activities of experimental days 9 and 18. Significance was set at <0.05.

induced behavioral sensitization using the open field assay. The effect of acute and chronic MPD administration on locomotor activities was compared between intact animals, shame-operated animals, and the animals with PFC lesions.

The baseline activities (experimental day 8) in the three groups (two after the surgery) were not different from each other or from the activities obtained from the same animals before the surgery (experimental day 1), which indicate that sham and PFC lesion did not modify the baseline locomotor activity. The first MPD injection on experimental day 9 elicited similar level of increase in locomotor in all the three animal groups, suggesting that neither sham operation nor the PFC lesion affected the acute effect of MPD. This observation agrees with previous similar studies where chemical lesions produced by 6-hydroxydopamine [4] or ibotenic lesions [41] of PFC had no influence on acute amphetamine-induced locomotor activity. However, other authors have reported that ibotenic lesion of PFC caused an increased in acute response to amphetamine administration [18]. Their lesions were more extensive and resulted in heightened amphetamine-induced locomotion [5,18]. Therefore, more extensive damage to the PFC than the lesions created in the present study may be required to see the heightened MPD-induced locomotion in lesion group. Or, the electrolytic lesions may affect the locomotor activity differently than selective chemical lesions. Electrolytic lesions destroy all the neurons and the efferent and afferent pathways affecting numerous neuronal pathways while neurotoxins target specific types of neurons and create selective lesions.

After chronic MPD administration, the intact and sham-operated animals exhibited behavioral sensitization to 2.5 mg/kg MPD dose while the PFC lesion group failed to exhibit behavioral sensitization. This observation suggests that the PFC is an important CNS site involved in MPD-induced behavioral sensitization.

The MPD is an indirect dopamine (DA) agonist that binds to DA transporters (DAT) and, thereby, inhibits the re-uptake of DA which results in increased extracellular DA levels [19,35,41]. The increased extracellular DA stimulates the receptors, including D1 and D2 receptors. The stimulation of the DA receptors then initiates a cascade of reactions involving activation of G proteins, increase in cAMP concentration, and subsequent activation of protein kinase A (PKA) signaling pathways or reduction in PKA activation. This, then leads to activation of transcription factor cAMP response element-binding (CREB) protein, and the downstream products of CREB activity in VTA

are believed to be responsible for drug-induced reaction, like sensitization and expression [32].

The excitatory amino acids (EAAs) are important contributors to this cascade [28,43]. The increased DA, due to psychostimulant administration, binds to the D1 receptors on the cells projecting from PFC to VTA. This binding alters the EAA release [20,43], influences the EAA receptors on VTA, and eventually impacts the cellular cascade described above contributing to the induction and expression of behavioral sensitization to psychostimulants [20,32,43].

Many studies indeed suggest that EAA projections from PFC to VTA are necessary for induction of sensitization to take place. Li et al. [25] reported that both PFC lesion and *N*-methyl-D-aspartate (NMDA) antagonists prevented the induction of behavioral sensitization to cocaine as well as the cellular changes associated with cocaine sensitization, such as DA autoreceptor sensitivity in the VTA. Furthermore, both the bilateral 6-hydroxydopamine lesions [4] and bilateral ibotenic acid lesions [5] of PFC prevented the induction of sensitization to amphetamine, injected s.c. or directly into VTA. The observation that sensitization to amphetamine did not occur even when it was injected directly into VTA in the rats with PFC lesions again suggest that the connections between PFC and VTA are the essential to elicit behavioral sensitization [5]. However, contradictory result was also reported [39] where quinolinic acid, NMDA agonist, was used to lesion the PFC, i.e. this PFC did not prevent induction of sensitization to amphetamine. The electrolytic lesions in this study destroyed a big part of dorsal PFC and fibers and neural pathways that connect PFC to other parts of the brain, and behavioral sensitization was not observed. This suggests that the induction of MPD sensitization is prevented by the destruction of EAA projections from PFC to VTA.

On experimental day 18 (MPD re-challenge), compared to experimental day 9 (initial MPD administration), the intact control group and sham-operated group exhibited significant increases in locomotion, i.e. behavioral sensitization was expressed, while the lesion group did not. It is most likely that the reason the behavioral sensitization was not expressed on experimental day 18, because the lesion group was not sensitized to MPD effects. The PFC's influence on NAc dopamine levels [10,28], which is known to be responsible for expression of behavioral sensitization, seems to require also the intact connection between the PFC and VTA; the EAA receptor antagonists injected into VTA were shown to block the striatal dopamine regulation of the PFC [21,37]. Supporting this observation is the study that reported the changes in presynaptic and postsynaptic EAA transmission in the NAc were observed only in the rats that were sensitized to cocaine. The rats that did not show sensitization even with the cocaine injections did not show these changes in NAc EAA transmissions [27] either. Therefore, change in VTA EAA transmission, resulting in the behavioral sensitization, is an essential requirement before the expression of behavioral sensitization can take place.

However, other previous studies have indicated that when the sensitization has already taken place, NAc expresses the behavioral sensitization even when PFC is not intact. For example, ibotenic acid lesion in PFC failed to inhibit the expression of sensitization to amphetamine [24] and to cocaine [25] when the lesions were created after the sensitization had taken place. For cocaine, the opposite result has been reported as well, i.e. ibotenic PFC lesion prevented the expression of behavioral sensitization to cocaine [29].

In present study, lesions were created before the sensitization has taken place. Hence, further study has to be conducted where the lesion is created after the sensitization has been established to figure out the role of PFC in expression of MPD sensitization.

During the washout phase, the intact control group and the sham group exhibited significant increase in TD traveled on experimental day 15 compared to experimental day 8, while lesion group did not show any increase in TD traveled on experimental day 15 compared to experimental day 8. Just like the expression phase, the lack of anticipation or withdrawal effect in lesion group during the washout phase was probably because the sensitization had not taken place. Moreover, these observations suggest that behavioral sensitization and withdrawal may have some common mechanism.

Although both control and sham groups exhibited induction and expression of sensitization, their activity patterns during the first 2 h post-injection, as shown by the temporal graphs, were quite different. Sham group seems to react stronger, i.e. more active, to MPD treatment (Figs. 4 and 6). In the histology, however, brains of sham group rats did not show any visible damage in the PFC. Therefore, the surgery itself, or perhaps the remains of sodium pentobarbital given several days earlier, may have affected the MPD effect to some extent. Also, the control group and sham group have different baseline activity levels and patterns to begin with (experimental day 8), so it is likely that this disparity contributed to the differences between the control and sham groups' temporal graphs as well.

In conclusion, the bilateral electrolytic lesions of PFC in rats had no effect on the baseline locomotor activity or acute MPD-induced effect, but they prevented the induction of behavioral sensitization to MPD. This observation suggests that the MPD action involves similar brain structures and pathways as other, more studied psychostimulants, like amphetamine and cocaine. In order to determine the role of the PFC on expression of MPD sensitization, another study has to be performed with the PFC lesion being created after behavioral sensitization has taken place already.

Conflict of interest

None.

Acknowledgements

We wish to thank Mallinckrodt, Inc. for its gift of methylphenidate. This research was supported in part by the Pat Rutherford Chair in Psychiatry (ACS).

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