Effects of Behavioral and Neurochemical Changes on Adult Excitotoxic Lesion of the Ventral Hippocampus

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Abstract: ***Backgrounds:*** The postmortem and magnetic resonance imaging studies for schizophrenic patients showed neuropathological abnormalities including neuron loss and volume reduction in ventral hippocampus (VH), some longitudinal studies suggest these changes may be a neurodegenerative process. ***Objectives:*** The present study examined the effects of adult bilateral VH lesions on a dopaminergic stimulant, methamphetamine (METH)-induced and an *N*-methyl-$_D$-aspartate (NMDA) receptor antagonist, dizocilpine (MK-801)-induced behavioral and neurochemical changes in rats, in order to evaluate a potential of adult VH lesion animals for a model of schizophrenia. ***Methods:*** To study the behavioral effects after bilateral VH lesions in adult rats, locomotor activity was measured individually by an infra-red sensor. Extracellular concentrations of dopamine in the nucleus accumbens (NAc) were measured using *in vivo* brain microdialysis. ***Results:*** The bilateral adult VH lesion rats showed a significant enhanced hyperlocomotion in response to METH but no changes to MK-801 and phencyclidine; while bilateral adult VH lesion enhanced METH-induced increasing dopamine levels in the NAc. ***Conclusions:*** The bilateral adult VH lesions enhanced locomotor activity, which related to increased dopamine releases in the NAc, induced by a dopaminergic stimulant; these findings may suggest a potential of adult VH lesion animal for a model reflecting dopamine D2 receptor antagonist–responsive pathophysiology of schizophrenia by way of neurodegenerative processes.

Key Words: Dizocilpine (MK-801), dopamine, excitotoxic lesion, locomotion, methamphetamine (METH), rat, ventral hippocampus (VH).

INTRODUCTION

Recently, a lot of postmortem studies of schizophrenia, cell loss and other neuropathological changes are shown in the patients [1,2]. A serial of imaging studies have revealed morphological abnormalities, such as the volume reduction in the hippocampus of patients with schizophrenia [3-6]. Longitudinal magnetic resonance imaging (MRI) studies show that neuropathological and morphological abnormalities are progressive. Although, most of these investigators believe the behavioral abnormalities in schizophrenics owing to regional neuropathological changes in their early neurodevelopmental process [7,8], but some findings suggest that these neuropathology and morphological abnormalities in the hippocampus are not only due to a first-hit, neurodevelopmental process, but also a second-hit, ongoing neurodegenerative process as well [9].

In animal model of schizophrenia, the use of neonatal hippocampal lesioned rats was established by Weinberger and colleagues [10-12]. Those rats showed a delayed behavioral abnormality expressed as a hyperresponsiveness to stress and amphetamine (AMPH) [10]. Therefore, we consider that adult ventral hippocampus (VH)- lesioned animals can be useful for an animal model of the hypofunction of the VH induced by some neurodegenerative processes. Previous report has already shown that bilateral larger lesions of the VH than those in present study in adult animals enhance AMPH-induced hyperlocomotion and increases in dopamine releases in the nucleus accumbens (NAc) [13].

Neurotransmitter inputs in the prefrontal cortex (PFC) arise mainly from the efferent projection of the mediodorsal thalamus, the hippocampus and the amygdale [14-16]. Studies have shown previously that rats with a neonatal excitotoxic lesion of the VH display in adulthood a variety of abnormalities reminiscent of schizophrenia and can be used as an animal model of this disorder [17-19]. Not a few studies showed enhanced AMPH-induced hyperlocomotion [13, 10, 18, 20], however, Wan *et al.* [20] reported that AMPH-induced release in NAc is not enhanced in animals that show increased hyperlocomotion. The present study examined the effects of bilateral adult excitotoxic lesion of VH on methamphetamine (METH)-induced hyperlocomotion and dopamine releases in NAc.

AMPH/METH-induced abnormal behavior is considered to be a model for dopamine D2 receptor antagonist-responsive pathophysiology of schizophrenia [21-23]. On the other hand, Abnormal behavior induced by *N*-methyl-$_D$-aspartate (NMDA) receptor antagonists, such as dizocilpine (MK-801) and phencyclidine (PCP), is a model for treatment-resistant schizophrenia, as haloperidol, a dopamine D2 receptor antagonist, weakly blocks PCP-induced abnormal behavior; meanwhile, clozapine, an atypical antipsychotic, can well block this abnormal behavior [24-26].

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Taken together, we hypothesized that adult rats' VH excitotoxic lesion, which was recommended for only destroying neurons while leaving fibers of passage intact [27], will significant interrupt the mainly neurotransmission by way of neurodegenerative processes. The purpose of present study was to examine the effects of bilateral ibotenic acid (IBO) lesions of the VH on behavioral and neurochemical changes induced by different psychostimulants, METH or MK-801.

METHODS AND MATERIALS

Animals

Male Sprague-Dawley rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan), 6 weeks old, weighing about 160g when arrived, were housed in groups of 4 in one standard breeding plastic cage (30×25×18 cm, with a wire mesh top and with bedding of sawdust) with food and water available *ad libitum*. The rats were placed in a rearing room at constant temperature (24±1 °C), humidity (50%) and maintained for one week on a 12-h/12-h light-dark cycle (light phase: 06:30-18:30). All the procedures were in strict accordance with a Guide for the Care and Use of Laboratory Animals regulated by Hokkaido University School of Medicine as well as NIH guidelines on animal care.

Drugs

Ibotenic acid (IBO) solution was prepared from 1 mg of IBO (Tocris Cookson Inc., Ballwin, MO, USA) in 0.1 ml of phosphate buffer solution (PBS, 0.1 M, pH 7.4). Methamphetamine (METH, Dainippon Seiyaku Pharmaceutical Co. Ltd., Japan), dizocilpine [MK-801, (+)-5-methyl-10,11-dihydroxy-5H-dibenzo (a,d)-cycloheptan-5,10-imine, dizocilpine, Bnayu, DRL, Japan] and phencyclidine hydrochloride (PCP, synthesized in the laboratory of Hokkaido University, Japan) were dissolved in saline. The concentration of the solutions of METH, MK-801, and PCP was 0.3 mg/kg, 0.2 mg/kg, 7.5 mg/kg respectively. The injection of METH was given subcutaneously (s.c.), while injections of MK-801 and PCP were given intraperitoneally (i.p.). All drugs were administered at the volume of 1 ml/kg.

Experimental Protocol

In experiment 1, we examined the behavioral effects of bilateral adult VH IBO lesion on METH, MK-801, and PCP -induced locomotor activity.

In experiment 2, by using *in vivo* brain microdialysis, we examined effects of bilateral adult VH IBO lesion on MK-801-induced changes in extracellular concentrations of dopamine levels in NAc.

Surgery

VH Ibotenic Acid Lesion

One week after arrivals, anesthetized rats [pentobarbital (40 mg/kg), i.p, and the rats weighing 220-230g at the time of surgery] were individually immobilized by taping onto a steel platform, which was positioned in ear bars of the stereotaxic frame (Kopf, USA). An incision was made in the skin and two small holes were made over lesion sites by a dental drill. All the animals were stereotaxically implanted with bilateral 26-gauge stainless steel guide canulae directed toward their VH at the coordinates AP -6.3 mm, ML ±5.5 mm, and DV -6.5 mm, relative to bregma according to Paxinos and Watson [28]. Bilateral infusions were given with two 33-gauge needles, using a Hamilton syringe with a Harvard microinfusion pump, to complete ibotenic acids or microinfusion-injections. For the lesion group, 2.0 μg IBO (dissolved in 0.2 μl PBS) was infused bilaterally into the each hemisphere of the MDT at a rate of 0.2 μl/min. The needle was withdrawn 4 min after completion of the injection to allow diffusion of solution from the needle tip. For the sham group, 0.2 μl PBS was infused at the same rate into the each hemisphere of VH and done as same procedure.

Sixteen male Sprague-Dawley rats (lesion=8, sham=8) were used for behavioral study. Before removal from the stereotaxic frame, rats' wounded skin was closed with a suture clip, injected with 0.3 ml gentamycin (10mg/ml, i.m), and then housed individually.

Implante Microdialysis Guide Canula

After the microinjection of IBO or PBS (for lesion or sham group respectively) into their bilateral VH, rats were immediately implanted stereotaxically with G-8 guide canula (Eicom, Kyoto, Japan) leading to the surface of the NAc (AP: +1.7 mm, ML +1.1 mm, and DV -6.0 mm). The coordinate was with respect to the bregma according to the atlas of Paxinos and Watson [28]. The guide cannula was secured in place with dental cement, and occluded with an obturator made of 33-gauge stainless steel wire. Before removal from the stereotaxic frame, each rat was injected with 0.3 ml gentamycin (10mg/ml, i.m), and then housed individually. Another sixteen male Sprague-Dawley rats (lesion=8, sham=8) were used for the VH lesion *in vivo* brain microdialysis study.

One day before *in vivo* brain microdialysis (13 days after surgery), a dialysis probe made of regenerated cellulose with an outer diameter of 220 μm (AG8-02, Eicom, Kyoto, Japan) was inserted into the guide canula so that 2 mm of the probe was exposed to the tissue of the NAc.

Behavioral Testing

Testing Procedure

2 weeks later after surgery, the rats were moved into an activity chamber and placed in plastic cages individually to observe their behaviors respectively by an apparatus equipped with an infra-red sensor that detects thermal radiation from animal (Supermex: Muromachi Kikai, Tokyo, Japan). The testing was operated in 3 separate days and each stimulant-induced behavioral test was parted for a five-day period to avoid the drug after-effect. On the first testing day, all the rats were injected saline (s.c.) firstly and observed for 90 minutes, then administered methamphetamine (0.3mg/kg, s.c.) and observed for the following 90 minutes. On the second testing day, the experiment was done as same procedures, except given saline (i.p.) at first and then MK-801 (0.2 mg/kg, i.p.) administration at 90 minutes later. On the last testing day, the procedure was the same as before while the drug was changed to PCP (7.5 mg/kg, i.p.). The entire tests were operated between 7:30 am and 12:00 am on each testing day.

Measurement of Locomotion

Locomotor activity was automatically monitored and measured as previously described by Ohmori *et al.* [29]. This measurement was begun after at least one night habituation, and was continued for 180 min after the first injection. Horizontal movements of the rats were digitized and fed into a computer every 10 min.

Microdialysis

14 days after lesioning surgery, in freely moving rats, perfusion was started using artificial cerebrospinal fluid (CSF) (147 mM NaCl, 2.4 mM KCl, 1.2 mM $CaCl_2$ and 1.0 mM $MgCl_2$, pH7. 4) at the flow rate of 2 μl/min. Following initial perfusion for 1.5 hr, baseline samples were obtained every 20 min for 80 min. Dialysis samples were collected every 20 min for 240 min following the last baseline collection, and each sample was automatically collected in a microtube containing 40 μl of 50 mM acetic acid with 20 mg/l L-cystein. A 25 μl sample of the dialysate was used for quantify dopamine.

Measurement of Dopamine

The high-performance liquid chromatograph (HPLC) system consisted of a liquid chromatograph pump (EP-300; Eicom, Japan), a degasser (DG-300; Eicom, Japan), an electrochemical detector (EDC-100; Eicom, Japan), and a column oven (CTC-100; Eicom, Japan). Eicompak CA-5 ODS 2.1 × 150 mm (Eicom, Japan) was used for measuring concentration of dopamine. The mobile phase consisted of 0.06 M Na_2HPO_4, 0.01 M Na_2HPO_4, 5 mg/l Na_2-EDTA (PH 3.2) and 15% (v/v) methanol. A 25 μl of dialysate was injected into the HPLC system. Flow rate was 0.23 ml/min. The voltage of the electrochemical detector was set at 450 mV and separation was conducted isocratically at 30°C.

Histology

Rats were sacrificed by decapitation at the end of the behavioral testing and the *in vivo* brain microdialysis process; brains were removed after 0.1M PBS and 4% PFA perfusion (5 min and 20 min respectively) and stored in 4% PFA solution. Portions containing the VH were sectioned into 30μm slices on a cryostat (Leica, USA) and slices were Cresyl violet-stained. Each section was examined under a light microscope to determine the location and extents of lesions and the placement of dialysis probe.

Statistical Analysis

Data from locomotor activity, cumulated counts of locomotion, and extracellular concentrations of dopamine were analyzed by unpaired *t*-test (defined as $P<0.05$), then a post-hoc Duncan test was used to determine which group significantly differed from another one (defined as $P<0.05$).

RESULTS

Histological Analysis

Only animals with correct cannulae placement were included in the statistical analysis. In this study, histological analyses indicate that the injection sites, either in the sham or lesion group, were equally distributed in the VH. Bilateral microinfusion of IBO injections into the VH resulted in a clearly defined region of complete neural cells loss (Fig. (**1**)). In addition, all dialysis probe placements were situated on the NAc, which was surrounded by core regions of the NAc (Fig. (**2**)) as defined in the atlas of Paxinos and Watson [28].

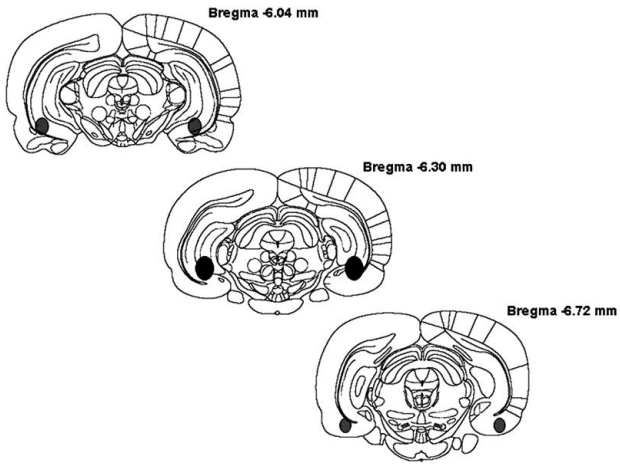

Fig. (1). Illustration of coronal sections adapted from the rat atlas of Paxions and Watson representing the site of the ventral hippocampus (AP: -6.3 mm, ML: ±5.5 mm, DV: -6.5 mm) lesion.

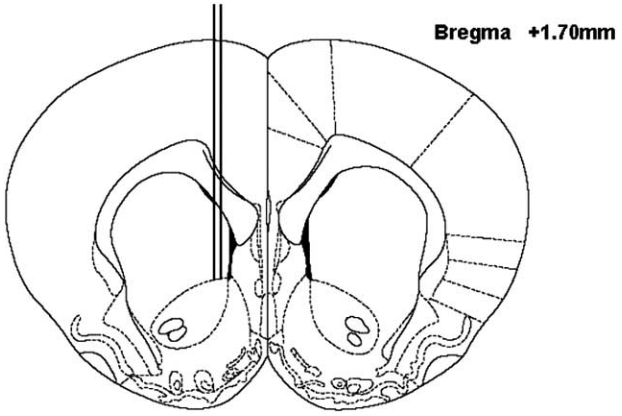

Fig. (2). Illustration of coronal sections adapted from the rat atlas of Paxions and Watson representing the site of implanting microdialysis guide canula to the surface of the nucleus accumbens (AP: +1.7 mm, ML +1.1 mm, and DV -6.0 mm).

Effect of Bilateral VH Lesion on METH-, MK-801-, and PCP-induced Hyperlocomotion

METH-induced hyperlocomotion of the VH IBO lesion group was larger than that of the sham lesion group at 140 min ($P<0.01$) (Fig. (**3A**)), but there was neither difference of MK-801-induced hyperlocomotion nor PCP-induced hyperlocomotion between the VH lesion and sham lesion groups (Fig. (**3B**, **3C**)).

In addition, the cumulated counts of METH-induced hyperlocomotion of the VH IBO lesion group was significantly larger than that of the sham lesion group ($P<0.05$); but there

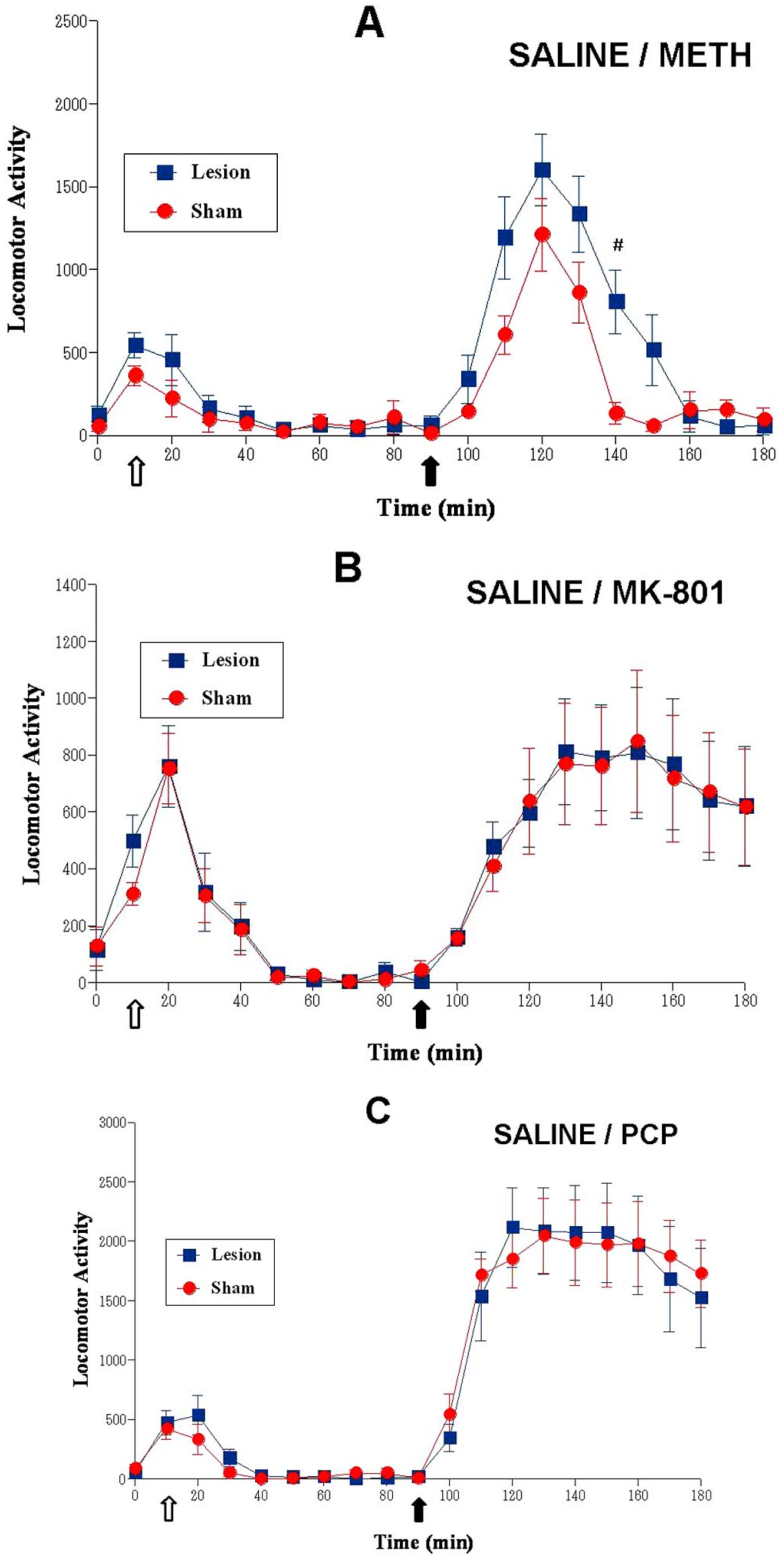

Fig. (3). Hyperlocomotion induced by a single injection of METH (0.3 mg/kg) (A), MK-801 (0.2 mg/kg) (B), PCP (7.5 mg/kg) (C) after VH IBO and sham lesions.

#: $P<0.01$; IBO lesion group *vs.* sham lesion group.

Saline was injected at 10 min, and then METH (0.3 mg/kg), MK-801 (0.2 mg/kg), and PCP (7.5 mg/kg), each was injected at 90 min.

was no difference of the cumulated counts of MK-801- or PCP-induced hyperlocomotion between the VH lesion group and the sham lesion group (P<0.05) (Fig. (**4**)).

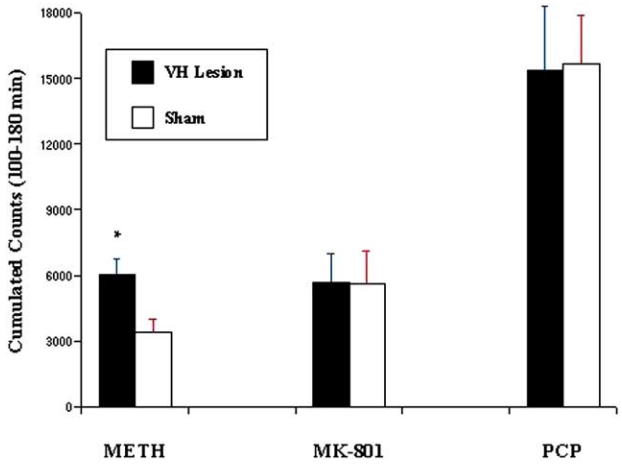

Fig. (4). Cumulated counts of locomotion (100-180 min) induced by a single injection of METH (0.3 mg/kg), MK-801 (0.2 mg/kg) and PCP (7.5 mg/kg) after VH IBO and sham lesions.

*: *P*<0.05; IBO lesion group *vs.* sham lesion group.

Effect of Bilateral VH Lesion on METH-Induced Increases in Dopamine in the NAc

Extracellular concentrations of dopamine of the VH IBO lesion group were higher than those of the sham lesion group, at 160 min (P<0.05), 180 min (P<0.01), 220 min (P<0.05), and 240 min (P<0.05) (unpaired *t*-test). Basal level of dopamine (fmol/μl) of VH IBO lesion group and sham lesion group was 0.44±0.04 and 0.44±0.07, respectively (Fig. (**5**)).

DISCUSSION

The adult VH lesions enhanced hyperlocomotion induced by a psychostimulant METH, but not NMDA receptor antagonists, MK-801 and PCP. Furthermore, in the adult VH lesioned animals, METH-induced dopamine release in the NAc was enhanced. In most of lesion studies of VH [10, 18-20], lesion surgery were made in the stage of neonatal period. Neonatal lesions of VH enhance AMPH-induced hyperlocomotion in the adult rats; these results are consistent with the findings of the present study. However, our results are partially inconsistent with the finding of Wan *et al.* [20], in which they showed that neonatal VH lesions enhanced AMPH-induced hyperlocomotion but had no effect on AMPH-induced dopamine release in the NAc in adult animals. This partially inconsistency may reflect the difference in ages when the lesions were made and the neurodevelopmental factors in neonatal lesioned animals. Wilkinson *et al.* [13] showed that adult hippocampal lesions enhanced AMPH-induced hyperlocomotion and dopamine releases in the NAc, these findings are consistent with those in the present study. Although the hippocampal lesions in the study of Wilkinson *et al.* [13] are larger than the VH-located lesions of our present study, there is no difference of dopamine stimulant-induced hyperlocomotion and increases in dopamine releases in the NAc between these studies.

Adult VH lesions had no effect on MK-801- or phencyclidine- induced hyperlocomotion. In the report of Lipska *et al.* [18], temporary inactivation of the neonatal ventral hippocampus induced enhancement of MK-801-induced hyper-

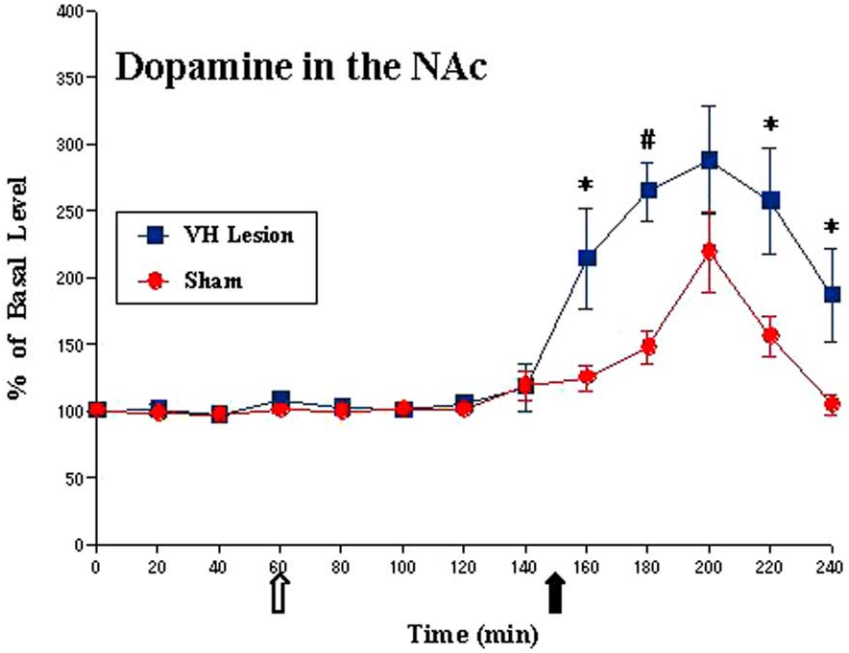

Fig. (5). Effect of the bilateral VH lesion on METH-induced increases in dopamine in the nucleus accumbens.

*: *P*<0.05, #: *P*<0.01; IBO lesion group *vs.* sham lesion group.

Saline was injected at 60 min, and then METH (0.3 mg/kg) was injected at 150 min.

locomotion in adult rats. The inconsistency of findings between Lipska *et al.* [18] and the present study may reflect the difference in ages when the lesions were made and the neurodevelopmental processes in neonatal lesioned animals. So far, we could find few any reports that show the effect of adult VH lesion on MK-801-induced behavioral changes.

AMPH psychosis and positive symptoms of schizophrenia respond well to typical antipsychotics; the main pharmacological action of these drugs is to block dopamine D2 receptors [30,31]. Therefore, psychosis induced by dopaminergic stimulants, AMPH/METH could be a model for haloperidol-responsive symptoms of schizophrenia. Taken together, we can hypothesize that the adult VH lesions model would reflect dopamine D2 receptor antagonist–responsive pathophysiology of schizophrenia.

In summary, adult excitotoxic lesions of bilateral VH enhanced METH-induced hyperlocomotion and METH-induced increases in dopamine levels in the NAc, but had no effect on MK-801-induced hyperlocomotion. Adult VH lesions model may have a potential for a model for dopamine D2 receptor antagonist–responsive pathophysiology of schizophrenia reflecting the way of ongoing neurodegenerative process, since the adult lesions enhanced behavioral and neurochemical changes induced by a dopaminergic stimulant but not a NMDA receptor antagonist.

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