

Research report

Effects of olfactory bulbectomy on NMDA receptor density in the rat brain: [³H] MK-801 binding assay

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Abstract

Olfactory bulbectomy (OBX) transects the glutamatergic efferents from the olfactory bulbs, and the changes of glutamatergic N-methyl-D-aspartate (NMDA) receptor-mediated function are thought to be involved in the behavioral deficits seen in OBX rats. In the present study, irritability scores in OBX male Wistar rats were correlated with discrete regional effects on NMDA receptor function measured using a [³H] MK-801 binding assay. Irritability scores, measured before and for 2 weeks after OBX, showed a gradual increase in irritability after OBX. A reduction of the NMDA receptor density was observed in the cerebral cortex and amygdala 16 days after OBX, but not in the striatum, olfactory tubercle, entorhinal cortex, and hippocampus. These results demonstrate that OBX causes changes in the NMDA receptor system in certain brain regions and suggest that these changes may be responsible for the behavioral deficits of OBX rats. © 2001 Elsevier Science B.V. All rights reserved.

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Topic: Excitatory amino acid receptors: physiology pharmacology and modulation

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1. Introduction

The olfactory bulbs project neuronal fibers widely to the olfactory cortex, including the olfactory tubercle, amygdala (AMG), and entorhinal cortex [14,22]. Olfactory bulbectomy (OBX) induces various behavioral and neurotransmitter changes. The behavioral changes in OBX rats are reported to be similar to those seen in depressed patients [18], and OBX has been used as an animal model of depression for clinical antidepressant screening [40]. The glutamatergic system is known to be involved in the pathogenesis of depression (reviewed in [15,21]). The

behavioral changes seen in some depression animal models, such as the alterations of the responsiveness to rewarding stimuli and motor activity in open field, are inhibited by N-methyl-D-aspartate (NMDA) receptor antagonists [24,30,31,39].

After OBX, a reduction of the glutamate content is seen in the olfactory cortex [10,35]. Moreover, a decreasing of the NMDA receptor binding affinity for glycine has also been reported in the rat cortex [27]. In contrast, Nakanishi et al. [26] found an increased NMDA receptor-mediated function in the medial AMG after OBX. In terms of behavioral alterations, the OBX-induced hyperactivity is suppressed by NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5 H-ibenzo[a,d]cyclohepten-5,10-imine (MK-801) [33]. Moreover, our preliminary data also showed that the OBX-induced muricidal behavior could be suppressed by MK-801. Impairment of the stress responses and memory is seen in OBX rats [6,25]. These behavioral

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deficits are related to NMDA receptor-mediated dysfunction in the hippocampus and AMG [1]. It seems that glutamatergic dysfunction, especially the NMDA receptors, may play a role in the behavioral changes in OBX rats. However, few studies have been performed to evaluate the NMDA receptor binding activity in the brain of OBX rats [27]. In the present study, we examined the effects of OBX on NMDA receptor binding characteristics in discrete rat brain regions by using a [^3H] MK-801 binding assay and tried to correlate with the behavioral changes following OBX.

2. Materials and methods

All the experiment procedures involved in the present study conformed to the NIH Guide for the Care and Use of Laboratory Animals. Male Wistar rats weighing 350 ± 50 g were used and housed individually in plastic cages ($20 \times 26 \times 47$ cm) in an animal room with a 12 h light–dark cycle (lights-on at 22:00 h) with food and water provided ad libitum. After habituation to the home cage for 1 day, basal scores for irritability were recorded over 3 consecutive days. Irritability was rated using a 6-category scale with ‘1’ being a startle response to air puffed on the rat’s back and to gentle touching of the dorsal lumbar region with a rod; ‘2’ a biting reaction to a gloved hand placed in the cage 1–3 cm in front of the rat’s snout; ‘3’ a biting reaction to a gloved hand pushing the rat backward against the cage wall, ‘4’ resistance to capture by the gloved hand; ‘5’ resistance to holding; and ‘6’ vocalization during the test. A score of 0 (no response) to 3 (intense response) was given for each category, and the irritability score for a given day was the sum of the scores for the six categories [7]. OBX and sham operation were performed on day 4, as described previously [16], and the rats were kept individually in plastic cages for recovery. Irritability scores were measured every other day for 2 weeks after surgery. All the behavioral observations were made 2 h after lights-off.

Sixteen days after OBX or sham operation, the rats were sacrificed by exposure to CO_2 vapors and their brains were removed immediately, and the cerebral cortex, AMG, hippocampus, striatum, olfactory tubercle and entorhinal cortex were dissected out on an ice-bath plate. Localization and dissection of the bilateral AMG was performed as previously described [3,12]. Briefly, two coronal cuts were made along the brain, firstly at the caudal end of the optic chiasma and then caudal to the mammillary bodies. This central coronal section was next turned on its rostral surface, and a horizontal section was made from the left to the right rhinal fissure. Two oblique sagittal sections were made along each internal capsule to expose the left and right amygdaloid areas, which were then removed. Pooled brain tissue from three rats was used for one saturation curve assay for the AMG, striatum, olfactory tubercle, and

entorhinal cortex; the tissue was homogenized as previously described [42]. The final pellets were re-suspended in 4 ml of 50 mM Tris–acetate buffer (pH=7.4) and stored at -70°C until use.

For the binding assay, the membrane suspension was thawed at room temperature, then centrifuged at $48\,000 \times g$ for 20 min at 4°C and re-suspended in assay buffer (5 mM Tris–acetate buffer, pH=7.4). All the assays were performed in the presence of 10 μM glycine and 100 μM NMDA. One-hundred microliters of membrane suspension (150–250 μg of protein) was incubated at room temperature ($25\text{--}28^\circ\text{C}$) for 2 h with eight concentrations of [^3H] MK-801, ranging from 0.3 to 60 nM. The binding assay was terminated by rapid filtration through filters presoaked in 0.1% polyethyleneimine, using a cell harvester (model 11021; Skatron Instruments, Sterling, VA, USA). Filters were washed with ice-cold assay buffer (5 ml \times 2 times) and then soaked overnight in liquid scintillation cocktail (Ready Safe; Beckman, Fullerton, CA, USA). The radioactivity was measured by liquid scintillation spectrometry with 60% counting efficiency. All the measurements were performed in duplicate. Nonspecific binding was determined by addition of a 500-fold molar excess of unlabelled MK-801 relative to the [^3H] MK-801. The B_{max} and K_d values were calculated from Scatchard plots. Protein concentrations were determined according to the method of Lowry et al. [23]. [^3H] MK-801, specific activity 22.5 Ci/mmol, was purchased from DuPont/New England Nuclear (Boston, MA, USA). MK-801, NMDA, and glycine were purchased from Research Biochemicals International (Natick, MA, USA).

The data were expressed as the mean \pm S.E.M. Comparisons of the irritability scores on a given day were performed using the Mann–Whitney U -test. Scatchard analysis was performed by the linear regression function of the EBDA/LIGAND program. Statistical differences in B_{max} and K_d were evaluated using Student’s t -test. P -values less than 0.05 were taken as statistically significant.

3. Results

Sham operation did not affect the irritability of rats. In contrast, between days 6 and 14 after operation, the irritability scores of OBX rats were significantly higher than those of the sham-operated rats ($z > 3.28$; $P < 0.001$) (Fig. 1). For the [^3H] MK-801 binding assays, the B_{max} of all brain regions of the sham-operated group did not differ from those of naïve rats (data not shown). In the cerebral cortex, the B_{max} of the OBX group (1.4 ± 0.1 pmol/mg protein) was significantly lower than that of sham-operated rats (1.9 ± 0.2 pmol/mg protein) ($t_{(23)} = 3.03$; $P < 0.01$). In the AMG, the B_{max} of the OBX rats (0.6 ± 0.2 pmol/mg protein) was also significantly lower than that of sham-operated rats (1.3 ± 0.1 pmol/mg protein) ($t_{(5)} = 4.25$; $P < 0.01$) (Table 1). In the striatum, the K_d value of the OBX

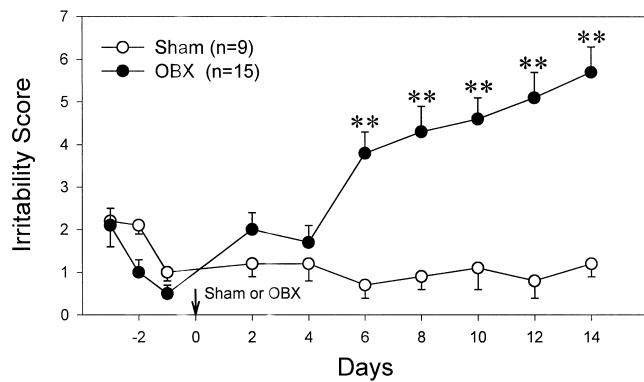


Fig. 1. Time course of irritability changes after olfactory bulbectomy (OBX). The basal irritability scores were the average of scores from 3 consecutive days before assignment to undergo OBX (●) or sham operation (○). The irritability scoring was measured every other day during the 2 weeks following surgery. ** $P < 0.001$ compared with the sham-operated group at the same observation time.

group (24.1 ± 0.7 nM) was significantly higher than that of the sham-operated group (14.1 ± 3.6 nM) ($t_{(4)} = 3.52$; $P < 0.05$). No other differences were observed in B_{\max} and K_d values between the two groups (Table 1).

4. Discussion

This is the first report demonstrating the changes in the NMDA receptor binding activity following OBX. In control group, the values of B_{\max} and K_d of [^3H] MK-801 binding are in the same range as previous study [36]. Sixteen days after OBX, the NMDA receptor density was significantly reduced in the cerebral cortex and AMG, but not in the hippocampus, striatum, olfactory tubercle, and entorhinal cortex. NMDA receptors are known to be

Table 1
Effects of olfactory bulbectomy on [^3H] MK-801 binding activity in various brain regions^a

| | B_{\max} (pmol/mg protein) | | K_d (nM) | |
|--------------------|------------------------------|--------------------|----------------|------------------|
| | Sham | OBX | Sham | OBX |
| Cerebral cortex | 1.9 ± 0.2 | $1.4 \pm 0.1^{**}$ | 19.2 ± 2.2 | 14.7 ± 0.8 |
| Hippocampus | 1.7 ± 0.2 | 1.5 ± 0.2 | 18.0 ± 1.9 | 12.2 ± 0.9 |
| Striatum | 0.6 ± 0.1 | 0.8 ± 0.2 | 14.1 ± 3.6 | $24.1 \pm 0.7^*$ |
| Entorhinal cortex | 2.0 ± 0.3 | 1.2 ± 0.4 | 22.0 ± 3.3 | 12.1 ± 3.6 |
| Olfactory tubercle | 1.0 ± 0.1 | 0.9 ± 0.1 | 14.7 ± 1.5 | 17.9 ± 1.4 |
| Amygdala | 1.3 ± 0.1 | $0.6 \pm 0.2^{**}$ | 14.9 ± 1.4 | 13.3 ± 1.5 |

^a Sixteen days after OBX or sham operation, the rat brains were removed and prepared for the [^3H] MK-801 binding assays. All the assays were performed in duplicate in the presence of 10 μM glycine and 100 μM NMDA. Eight concentrations of [^3H] MK-801 ranging from 0.3 to 60 nM were used for saturation binding. Nonspecific binding was determined by addition of unlabelled MK-801 (500-fold molar excess of the added [^3H] MK-801). The data are the average of at least four experiments and are expressed as the mean \pm S.E.M. * $P < 0.05$ and ** $P < 0.01$ compared to sham-operated control.

involved in the neuronal activity of the olfactory cortex [11,26]. OBX is reported to induce robust changes in the glutamatergic system in the olfactory cortex. Reductions of the level of glutamate and the glutamate synthesizing enzyme glutaminase are observed in the olfactory cortex following OBX [10,34,35]. Moreover, Kinzie et al. [19] found a decrease of the metabotropic glutamate receptor in the piriform cortex following OBX. The reduction in NMDA receptor density in the cerebral cortex and AMG of OBX rats, seen in the present study, provides further evidence for the role of the glutamatergic system in the behavioral changes seen in OBX rats.

Previous studies have shown that apparent hyperactivity [5] and learning impairment [6] are observed 2 weeks after OBX in rats. In the present study, the irritability score of rats was increased during days 6–14 after OBX. Some studies found that stress can up-regulate NMDA receptors in the brain; Akinci and Johnston [2] demonstrated an increase of the NMDA receptor density in the forebrains of swim-stressed mice. The stress-induced NMDA receptor activation only lasted for 24 h [4,20]. Therefore, in the present study, we sacrificed the rats 48 h after the last behavioral observation for avoiding the effect from the handling stress.

Redmond et al. [33] observed a behavioral hyperactivity in a stressful open field in OBX rats. McNish and Davis [25] also found that the threshold of shock-induced sensitization was reduced in OBX rats. Several studies showed that NMDA receptors in the AMG were involved in many aspects of stress responses. Adamec et al. [1] found that predator-induced long-lasting anxiety-like activity in rats was inhibited by microinjection of the NMDA receptor antagonists MK-801, (\pm)-2-amino-7-phosphonohepanoic acid (AP7), or (\pm)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), into the AMG 30 min before stress treatment. In contrast, microinjection of glutamate into the AMG resulted in an increased release of corticotropin-releasing factor from the hypothalamus and enhanced the plasma corticosterone level to an unpredictable stressor in rats [13]. Shors and Mathew [38] found that the stress-induced learning facilitation of the eye-blink was blocked by injection of the NMDA antagonist (\pm)-2-amino-5-phosphonopentanoic acid (AP5) into the basolateral AMG. Furthermore, antagonism of NMDA receptor function can prevent the stress-induced reduction of the basolateral AMG spontaneous activity [37]. The OBX-induced behaviors are known related to the change of NMDA receptor function in AMG. Nakanishi et al. [26] observed burst discharges after local stimulation of the medial AMG neurons in OBX rat brain slices, and suggested that this neuronal hyperexcitability was due to enhancement of the NMDA receptor function. Our preliminary study showed that MK-801 perfusion into the medial AMG could suppress the muricidal behavior of OBX rats. Moreover, in the AMG kindling model, Cain and Corcoran [8] also found that OBX potentiated seizure development. How-

ever, the local injection of NMDA receptor antagonist AP5 into the AMG suppressed kindling development [17]. Thus, the reduction of the NMDA receptor density in the AMG seen in the present study may be responsible for the abnormal stress responses seen in the OBX rats.

The olfactory bulbs have been shown to project nerve fibers to the AMG [22], and Heimer [14] observed nerve degeneration in the AMG of OBX rats. This may be responsible for the NMDA receptor down-regulation seen in the AMG in the present study. There has no report on a direct connection between the olfactory bulbs and cerebral cortex; however, complex reciprocal innervations between the AMG and cerebral cortex were demonstrated [9,32,41]. O'Neill and Liebman [29] showed that local injection of the NMDA receptor antagonist CPP into the medial prefrontal cortex induced OBX-like hyperactivity and darting behavior, and suggested that such behaviors might be due to antagonism of the NMDA receptor function in the cerebral cortex. This hypothesis was supported by Nowak's report, a reduction of the glycine binding affinity of NMDA receptors in the cerebral cortex of OBX rats [27]. Similarly, decrease of the NMDA receptor density was observed in the cerebral cortex of OBX rats in our present study.

Although it has been found that OBX rats show the learning deficits [43] and that the NMDA receptors in the hippocampus are important in learning and memory, no change of NMDA receptor density in the hippocampus was found in the present study. The behaviors seen in OBX rats, such as hyperirritability to handling, hyperactivity in a new environment, deficit in acquisition of the avoidance condition, and enhanced sensitivity of the startle reflex, have been interpreted as an increase of the vulnerability and a decrease of the adaptation to a stressful environment [25,40]. The experimental handling and apparatus used in the passive avoidance learning task may also act as stressors to OBX rats. Shors and Mathew [38] suggested that the stress-induced learning facilitation depended on the NMDA receptor function in the AMG. Thus, NMDA receptor down-regulation in the AMG of OBX rats may make these rats loss of the learning facilitation and cause the learning deficits in passive avoidance learning task. In our recent study, we found that behavioral hyperactivity in OBX rats was accompanied by an increase of glutamate release in the striatum [16]. Moreover, Redmond et al. [33] also found that the behavior was inhibited by NMDA receptor antagonist MK-801. These results suggest that the striatal glutamatergic system may be involved in the behavioral changes observed in OBX rats. In the present study, we did not find any difference in striatal NMDA receptor density between OBX and sham-operated rats. However, an increase in the K_d of [3 H] MK-801 binding was observed.

The OBX rat is widely used as an animal model of depression [18]. Antagonism of NMDA receptors can improve the behavioral deficits seen in OBX rats [33] and

other depression animal models [24,31,39]. A growing body of evidence indicates that glutamatergic NMDA receptors are involved in the pathophysiology of depression and the therapeutic action of antidepressants (reviewed in [15,21]). A reduction of the NMDA receptor binding activity in the frontal cortex in depressed suicide victims is observed by Nowak et al. [28]. Our present study provides further evidence that the substantial changes in glutamatergic NMDA receptors in discrete brain regions may play a role in the expression of the behaviors observed in OBX rats.

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