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Striatal glutamate release during novelty exposure-induced hyperactivity in olfactory bulbectomized rats

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Abstract

Striatal glutamate release during novelty exposure-induced hyperactivity was studied by microdialysis in freely-moving olfactory bulbectomized (OBX) rats. After collecting three 10 min basal striatal dialysate samples, the animals were transferred to an open-field apparatus (novelty) and locomotor activity recorded for 60 min. OBX rats showed significantly more locomotor activity (1210 ± 270 cm) than sham-operated rats (420 ± 70 cm), but only in the first 10 min after exposure to the novel environment. During the same period, striatal glutamate levels increased to 163 ± 21% of the basal value in OBX rats, while no changes were seen in the striatum of sham-operated controls. These findings suggest that olfactory bulbectomy results in an increased response of the striatal glutamatergic system to novelty stress, and may consequently cause hyperactivity. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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When exposed to a novel environment, animals demonstrate increased behavioral activation. In rats, clear physiological responses, such as increases in plasma corticosterone, norepinephrine, epinephrine, and dihydroxyphenylglycol, are elicited following novelty stress [19]. Neurochemical findings suggest that stress is associated with an increase in excitatory amino acid neurotransmission in the brain. For example, restraint stress increases the rate of glutamate release and uptake [7], and tail pinch, forced swimming, and foot shock have been shown to increase glutamate efflux in vivo [1,10,12,20]. Heavy glutamatergic projections from the cortex to the striatum are known to exist [6], and to be involved in motor functions [13].

McNish and Davis [11] have reported that olfactory bulbectomized (OBX) rats are more susceptible to stress than sham-operated controls. Obvious hyperactivity is a characteristic behavior of OBX rats subjected to novelty stress, but the neuronal mechanisms involved are not clear. For example, it is not known whether the striatal glutamatergic system is involved. The aim of the present study was to use microdialysis to investigate the relationship between hyperactivity and striatal glutamate release in freely-moving OBX rats after novelty exposure while concurrently monitoring locomotor activity.

Male Wistar rats weighing 350 ± 50 g were anesthetized using sodium pentobarbital (10 mg/kg, i.p.) plus ketamine (45 mg/kg, i.p.). For olfactory bulbectomy, the rats were placed in a stereotaxic instrument, then two 2 mm diameter holes were drilled in the skull (5.0 mm rostral to the bregma and ±1.5 mm lateral to the midline) and the olfactory bulbs removed by suction. After surgery, the rats were kept individually in plastic cages for recovery. The sham-operated group underwent the same surgical procedure except for the removal of the olfactory bulbs. All rats were housed in an animal room with a 12 h light–dark cycle (lights on at 06:00 h). Food and water were provided ad libitum. All experimental procedures involving laboratory rats in the present study conformed to the NIH Guide for the Care and Use of Laboratory Animals.

At 15:00 h on the 14th day after surgery, two homemade microdialysis probes (4.0 mm tip length) were implanted

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bilaterally in the striatum (coordinates: AP: +1.0; L: ±2.8; DV: −7.5 mm from the bregma and dura) of each rat, as previously described [8]. The animals were then placed in an observational bowl to recover and allowed to move freely. The probes were continuously perfused with artificial cerebrospinal fluid (140 mM NaCl, 1.2 mM $CaCl_2$, 3.0 mM KCl, 1.0 mM $MgCl_2$, pH 6.5) at a flow rate of 1.15 μl/min. At 09:00 h the next day, three consecutive 10 min dialysate samples were collected to measure basal glutamate levels, then the animals were placed in an automated Digiscan-16 Animal Activity Monitor System (model RXYZCM, Omnitech Electronics Columbus, OH) to assess their locomotor activity in the novel environment. Every 10 min for a period of 60 min, the total horizontal movement of the rats was recorded and a dialysate sample (11.5 μl) collected. The microdialysis probes used were calibrated using 10 μM glutamate and exhibited an in vitro recovery rate of 12.2 ± 2.1%. After completion of the experiment, histological sections of the brain were used to confirm the accuracy of probe placement. If both probes were found to be in the striatum, data from only one, selected randomly, was used for analysis.

The concentration of glutamate in the dialysate was measured using HPLC. Twenty microliters of the reagent (400 mM borate, 40 mM *o*-phthaldialdehyde, 400 mM 2-mercaptoethanol, pH 10.4) was added to a 5 μl sample of the dialysate for 60 s at 4°C, then, using a CMA auto-sample injector, 20 μl of the derivatized aliquot was injected onto the HPLC column (5 μm C18 particles; 250 mm long and 4 mm i.d.; Merck). The elution buffer, consisting of 88% mobile phase A (20 mM sodium acetate, (pH 7.2); 0.6% tetrahydrofuran; 0.02% triethylamine) and 12% mobile phase B (20% 100 mM sodium acetate, (pH 7.2), 35% acetonitrile, 45% methanol), was applied at a flow rate of 1 ml/min using a pump (126 solvent module; Beckman). The detector was a model 125 fluorometer (Gilson) with excitation and emission wave lengths of 370 and 450 nm, respectively.

The statistical comparison of changes in locomotion and glutamate concentration (μM) between the OBX and sham-operated groups was carried out by two-way repeated measures analysis of variance (surgery × time), and the comparison within each group was by one-way repeated measures analysis of variance, following Scheffé's multiple comparison test. *P*-values less than 0.05 were taken as statistically significant. The results for glutamate release were expressed as percentage changes (mean ± SEM) of the basal level.

The total horizontal distance that the OBX rats moved in the 60 min experiment was 1750 ± 390 cm, while the corresponding value for the sham-operated controls was 520 ± 200 cm. The most significant effects of novelty exposure on locomotor activity were seen in the first 10 min in both OBX and sham-operated animals. In the first 10 min after novelty exposure, the distances that the OBX and sham-operated rats moved were 1210 ± 270 cm and 420 ± 70 cm, respectively, the values being significantly different ($F(1,11) = 4.84$, $P < 0.05$). In contrast, no difference in locomotor activity was seen between the groups over the rest of the observational period (Fig. 1A).

The baseline glutamate level was taken as the mean value for the three 10 min basal samples collected immediately prior to novelty exposure. As shown in Fig. 1B, the baseline glutamate level in OBX rats (0.86 ± 0.13 μM) was not significantly different from that in sham-operated rats (0.66 ± 0.14 μM). Novelty exposure resulted in a significant increase in glutamate levels in OBX rats to 163 ± 21% of the baseline value in the first 10 min ($F(1,7) = 14.28$, $P < 0.01$, compared with baseline, and $F(1,11) = 5.84$, $P < 0.05$, compared with the sham-operated group at the same time point); thereafter, glutamate levels did not differ significantly from the baseline value. In contrast, no changes in glutamate levels were seen in sham-operated rats at any time in the observational period (Fig. 1B).

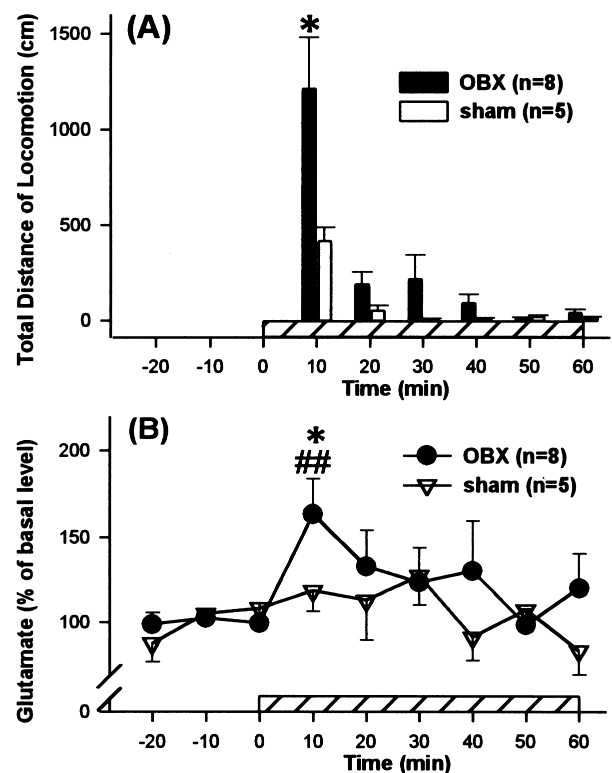

Fig. 1. Effects of novelty exposure on locomotor activity (A) and extracellular striatal glutamate levels (B). The first 10 min of novelty exposure produced a significant increase in locomotion in OBX rats compared with that in sham-operated controls (*$P < 0.05$). No significant difference in novelty exposure-induced locomotor activity was seen between the two groups in the rest of the observational period. A significant elevation in striatal glutamate levels was also seen in OBX rats during the first 10 min following novelty exposure compared with baseline values (##$P < 0.01$) and to that seen in sham-operated controls at the same time (*$P < 0.05$). Glutamate release was unchanged in the sham-operated group throughout the novelty exposure period. The data are the mean ± SEM. The hatched box indicates the novelty exposure period.

The increase in striatal glutamate release in the OBX rats in response to novelty exposure was similar to that previously reported in intact rats with novelty stress [16], tail pinch [1], and restraint stress [10], which are associated with an increased extracellular glutamate concentration in the brain. These results suggest that the glutamatergic system is involved in stress responses. Our findings that this increase in the striatal glutamate level is only seen in OBX rats in the first 10 min after novelty exposure are similar to results from previous studies in which extracellular glutamate levels in the rat hippocampus increased only during the first 10 min of a 1-h restraint stress period [10]. In addition, increased glutamate levels are seen in the nucleus accumbens in the first 5 min of novelty stress [16]. Moreover, Bagley and Moghaddam [1], by collecting dialysates at minute intervals, found that tail pinch almost immediately increases extracellular glutamate levels in the prefrontal cortex and hippocampus. These results show that the pattern of glutamate release in response to stress generally has a fast onset and short duration.

On the other hand, tolerance of the prefrontal cortex glutamatergic response to a repeated tail pinch stress lasting 10 min has been reported in rats [1]. Although the novelty exposure of 60 min used in the present study is not a repeated nociceptive stressor, adaptation to novelty stress occurs rapidly in both the behavioral and neurochemical response. Both OBX and sham-operated rats showed progressively reduced locomotor responses after novelty exposure for 10 min and neurochemical adaptation was also seen in OBX rats. This finding is similar to the previous report that hippocampal glutamate release induced by 1 h of restraint stress is only seen in the first 20 min [10].

Since OBX rats showed both marked behavioral and neurochemical responses to novelty stress in the present study, it seems that the striatal glutamatergic function of OBX rats is more sensitive to stress than that of controls. This result supports the view in previous reports that olfactory bulbectomy results in increased vulnerability to stressors [2,11]. The enhanced glutamate release in the striatum of OBX rats after novelty stress may be partially attributed to elevation of plasma corticosterone levels by olfactory bulbectomy, since several lines of evidence have shown that corticosterone affects stress-induced glutamate levels in the brain [10] and a significantly higher basal plasma corticosterone level is seen in OBX rats than in sham-operated controls [2].

Another possible mechanism underlying the increase in glutamate levels in the striatum of OBX rats during novelty exposure may be functional changes in the amygdala. The olfactory bulbs heavily project glutamatergic fibers to the amygdala [5]; therefore olfactory bulbectomy may cause functional changes in the amygdala. Changes of the amygdala electroencephalogram (EEG) to arousal pattern are seen immediately following olfactory bulbectomy [18]. Furthermore, the neuronal activity of the amygdala shows hyperexcitability 10 days after olfactory bulbectomy [14]. It is well documented that the amygdala is responsible for the stress response and that there are wide projections from the amygdala to the striatum, thalamus, and nucleus accumbens [3,9,15]. Thus, novelty stress possibly results in a greater response in amygdaloid neurons in OBX rats than in sham-operated controls via the amygdalostriatal connection. In addition, the thalamus is thought to act as a filter protecting the cortex from sensory information overload [4]. The amygdalothalamal projection provides a pathway through which the amygdala can influence the activity of the thalamus and cortex. Since the neocortical EEG gradually shows an arousal pattern after olfactory bulbectomy [18], functional changes in the amygdala may be expected to affect cortical functions and consequently the response of the corticostriatal glutamatergic system to stress. However, the role of the amygdala in behavioral expression in OBX rats in stress conditions requires further study.

Increased exploratory behavior and glutamate release in the nucleus accumbens has been shown to occur in rats 10 min after novelty exposure [16]. In contrast, although the present study showed increased locomotor activity in sham-operated rats during the first 10 min following novelty exposure, no increase in striatal glutamate levels was seen. Such a regional difference between the nucleus accumbens and striatum in glutamate response to novelty stress is in agreement with previous studies [7,12,17]. These previous results and our own support the view that some major neurotransmitter systems in the striatum, such as the dopaminergic [17] and glutamatergic [7,12] systems, are relatively resistant to stress-induced changes compared with the limbic system.

The striatum is known to play an important role in the regulation of locomotion. Wheeler et al. [20] found that a 5-min tail pinch increases both striatal glutamate levels and motor activity. Both responses show a coincident time course, i.e. a fast onset and short duration. Our results are consistent with theirs. These findings imply that the striatal glutamatergic system directly modulates motor function under stress. The striatal glutamatergic system demonstrates phasic facilitation of locomotor activity of OBX rats exposed to novelty stress, while, in sham-operated rats, novelty exposure is not accompanied by obvious changes in striatal glutamate levels. Further experiments should be performed to investigate the mechanisms underlying striatal glutamate release and locomotor activity in sham-operated rats during novelty exposure.

In the present study, novelty exposure did not influence glutamate release in the striatum of sham-operated rats, but resulted in an increase in striatal glutamate release in OBX rats. In contrast, Moghaddam [12] reported that both restraint and forced swimming stress result in increased glutamate release in the striatum. These conflicting results might be due to the different stressors used.

In summary, the present study provides direct evidence that novelty exposure transiently increases extracellular glutamate levels in the striatum of OBX rats, but not in sham-operated rats. This increase occurs coincidentally with an increase in

behavioral hyperactivity of OBX rats exposed to novelty stress, suggesting that novelty exposure induces activation of the striatal glutamatergic system in OBX rats and may consequently cause behavioral hyperactivity.

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