Behavioral Performance at Early (4 Weeks) and Later (6 Months) Stages in Rats With Unilateral Medial Forebrain Bundle and Striatal 6-Hydroxydopamine Lesions

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Abstract

Our previous studies showed differences in striatal D₂ receptor functional activity between two different rat parkinsonian models, with lesions induced by 6-hydroxydopamine injection in the striatum and in the medial forebrain bundle (MFB) at both early (4 weeks) and later (6 months) stages after lesioning. The present study compared behavioral changes, including rotational movements induced by methamphetamine and bromocriptine, and the stepping test, in both models at both stages. No differences in behavioral performance were observed between the early and later stages in both striatal and MFB lesion models, whereas simultaneous D₂ receptor study showed dynamic change in D₂ receptors in MFB lesion rats. Behavioral characteristics might be controlled by comprehensive effects of the whole dopaminergic system, instead of variation in a few parameters of the dopaminergic system. More behavioral tests of different mechanisms with simultaneous molecular studies are needed for evaluation of parkinsonian animal models and the efficacy of treatments.

Key words: Parkinson’s disease, behavioral test, dopaminergic system, D₂ receptor, rat

Introduction

Behavioral tests have been employed in evaluating the rodent model of Parkinson’s disease (PD). There are two types of behavioral tests used in PD rats: One is based on the unbalance of the dopamine (DA) receptors in the hemi-PD rats, such as amphetamine- or apomorphine-induced rotation movements; the other is based on the observation of the bradykinesia and/or stiffness, such as the stepping test or rotarod test. In our previous studies using rat striatal PD models, we found no differences in the rotational behavior and stepping test of rats with increasing degrees of lesions in the striatum, although the differences in DA neuronal loss were highly significant. A later study further demonstrated no differences in the stepping tests between rats with single and four striatal lesions and rats with medial forebrain bundle (MFB) lesions. Such behavioral results contradict the conventional belief that motor performances are always positively correlated with the loss of DA neurons in the substantia nigra (SN) of PD. All these data were observed at an early stage after lesioning (3–4 weeks after 6-hydroxydopamine [6-OHDA] injection).

The present study investigated the time course of behavior changes in the single and four striatal and unilateral MFB PD rat models at the advanced stage (6 months after 6-OHDA injection), based on the significant decrease of D₂ receptor binding within 6 months after lesioning in MFB lesion rats.

Materials and Methods

Behavioral tests were performed on the same animals used for the D₂ receptor study previously reported. Briefly, 59 adult male Sprague-Dawley rats (270–290 g) were divided at random into four groups: single striatal lesion model (n = 17), four striatal lesion model (n = 17), MFB lesion model (n = 17), and sham-operated controls (n = 8). All rats were investigated for behavioral changes in the early stages after lesioning (3–4 weeks after 6-OHDA injection).

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stage (4 weeks) and late stage (6 months) after lesioning. The surgical processes were as described previously. Briefly, under anesthesia with intraperitoneal injection of Equithesin (0.3 ml/100 g; Kyoritsu Seiyaku, Tokyo), rats received unilateral stereotaxic injections of 6-OHDA (Sigma-Aldrich, St. Louis, Missouri, USA) into one or four sites in the striatum or into the MFB. 6-OHDA (7 mg) was dissolved in 0.9% saline (7 ml) to form a solution of 1 µg/µl concentration. Lesions were induced by injecting 1 µl/min for 7 minutes of 6-OHDA (7 µg) using a microinjector. The three-dimensional coordinates of the 6-OHDA injections for the single striatal lesion were anteroposterior (AP) = 1.0 mm rostral to the bregma, lateral (L) = 3.0 mm right of the midline, ventral (V) = 5.0 mm ventral to the dural surface, tooth bar (TB) = 0.0 mm (horizontal); coordinates for the four striatal lesions were (1) AP = 1.3 mm, L = 2.6 mm, V = 5.0 mm, TB = 0.0 mm, (2) AP = 0.4 mm, L = 3.0 mm, V = 5.0 mm, TB = 0.0 mm, (3) AP = −0.4 mm, L = 4.2 mm, V = 5.0 mm, TB = 0.0 mm, and (4) AP = −1.3 mm, L = 4.5 mm, V = 5.0 mm, TB = 0.0 mm; and coordinates for the MFB lesion were (1) AP = −4.4 mm, L = 1.2 mm, V = 7.8 mm, TB = 2.4 mm (up to the horizon), and (2) AP = −4.0 mm, L = 0.8 mm, V = 8.0 mm, TB = −3.4 mm (down to the horizon). All rats were treated in accordance with the Rules of Animal Experimentation and the Guide for the Care and Use of Laboratory Animals of Hamamatsu University School of Medicine, and all experiments were approved by the Animal Experimental Committees at Hamamatsu University School of Medicine.

The behavioral tests were performed in the sequence of methamphetamine-induced rotations, bromocriptine-induced rotations, and the stepping test at 4 weeks and 6 months after lesioning. Methamphetamine-induced rotational movements were monitored over 60 minutes after intraperitoneal injection of 3 mg/kg of D-methamphetamine. Bromocriptine-induced rotations were induced by intraperitoneal injection of 5 mg/kg of bromocriptine. Net rotations were assessed for 2 hours. The details of these behavioral tasks were described in our previous studies.1,2,13)

Finally, unbiased stereological methods were used to estimate the number of dopaminergic cells in the SN of the rats. All rats were deeply anesthetized with chloral hydrate (30 mg/100 g, intraperitoneal injection; Mylan Seiyaku, Tokyo) and the brains were removed for tyrosine hydroxylase immunohistochemistry as in our previous studies.1,2,12,13) The tyrosine hydroxylase-positive neurons of all slices in the SN pars compacta were counted in each group of 6-OHDA lesioned rats, and the depletion rate was calculated as in the previous studies.2,12,13)

All data were recorded as the mean ± standard error of the means. The t-test was used to compare differences between two groups and one-way analysis of variance was used to analyze differences between multiple groups. All statistical procedures were performed with SPSS® software (IBM Corp., Armonk, New York, USA).

**Results**

Depletion rates of tyrosine hydroxylase-positive cells in the SN were about 20% in the single striatal, 85% in the four striatal, and >95% in the MFB lesion models, as found previously.13) The depletion rates did not differ at 4 weeks and 6 months after lesioning between these models (Table 1). The differences in DA transport and D₂ receptor binding assays at two different time points are summarized in Table 2.12)

Intraperitoneal injection of 3 mg/kg methamphetamine caused ipsilateral rotations in both the striatal and MFB lesion rats. No significant differences in

**Table 1** Percentages of dopaminergic cell loss in substantia nigra

<table>
<thead>
<tr>
<th>Stage</th>
<th>Striatal lesion model</th>
<th>MFB lesion model</th>
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<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Four</td>
</tr>
<tr>
<td>4 weeks after lesioning</td>
<td>19.5 ± 7.6</td>
<td>85.2 ± 4.7</td>
</tr>
<tr>
<td>6 months after lesioning</td>
<td>22.7 ± 2.9</td>
<td>84.6 ± 4.0</td>
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MFB: medial forebrain bundle.

**Table 2** Difference of dopamine (DA) transport and D₂ receptor binding at different time points

<table>
<thead>
<tr>
<th>Stage</th>
<th>DA transport binding (%)</th>
<th>D₂ receptor binding (%)</th>
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</thead>
<tbody>
<tr>
<td>Four striatal lesion model</td>
<td>4 weeks after lesioning</td>
<td>6 weeks after lesioning</td>
</tr>
<tr>
<td></td>
<td>−23.7 ± 5.0</td>
<td>−23.7 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>−26.5 ± 2.8</td>
<td>−26.5 ± 2.8</td>
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MFB: medial forebrain bundle.
methamphetamine-induced rotational behavior were found between the single striatal, four striatal, and MFB lesion models, at either 4 weeks or 6 months after lesioning (Fig. 1A). Intraperitoneal injection of 5 mg/kg bromocriptine caused contralateral rotations in MFB lesion rats, but ipsilateral rotations in striatal lesion rats, both at 4 weeks and 6 months after lesioning (Fig. 1B), in accordance with our previous observations.13) No rotations were observed in the sham-operated control rats at 4 weeks and 6 months after lesioning (Fig. 1B).

Initiation time of the stepping test was significantly longer in the lesion side compared with the normal side (p < 0.001). However, no differences were observed between the single striatal, four striatal,
and MFB lesion models, at 4 weeks and 6 months after lesioning (Fig. 2A). Stepping length was significantly shorter, and adjusting steps measured in the forward and backward directions were significantly reduced in the lesion side compared with the normal side (p < 0.001). However, no differences were observed between the single striatal, four striatal, and MFB lesion models, at 4 weeks and 6 months after lesioning (Fig. 2B, C).

**Discussion**

The present study found no behavioral changes between the early and later stages after lesioning in the striatal and MFB lesion rats, even in the most sensitive indicator of initiation of the stepping test. This is very interesting because there were obvious differences in the loss of DA neurons in the SN between the groups, and dynamic change was observed in D₂ receptor binding in the MFB lesion rats between 4 weeks and 6 months after lesioning.

Behavioral impairments are correlated with the loss of DA neurons in SN and reduction of the striatal DA level. In rats, amphetamine-induced rotation starts at 40–50% reduction in total striatal DA level and 30–50% loss of tyrosine hydroxylase-positive neurons in the SN, and impairments in motor tasks such as the stepping test and rotarod tests can be observed at 80–90% reduction in the striatal DA level and 60–80% loss of tyrosine hydroxylase-positive neurons in the SN. Impairments in the motor performance became more severe with increasing degrees of lesioning in the striatum using an analogous task in rats. In our series of studies, we only checked the loss of DA neurons in the SN, and did not check the status of damaged areas in the striatum in the striatal PD models. This is a potential limitation of our studies, which makes it difficult to directly compare our results with those of the previous studies.

The causes of this discordance are mostly unknown but we speculate as follows. We did not find differences in the behavioral performance in the MFB lesion model between the early and advanced stages during which dynamic change of D₂ receptor binding occurred, suggesting again that the final motor performance was not decided by a few factors in the dopaminergic system. Some unknown processes may have occurred after lesioning to sustain behavioral performance, because the dopaminergic system is important for survival in animals. However, more evidence regarding the relationship between behavioral performances and variations in the functions of the dopaminergic system should be obtained to validate our hypothesis in the future.

We selected several behavioral tests with different mechanisms for behavioral evaluation of these disorder models. In our previous studies, we selected rotational movements for unbalance of DA receptors, the stepping test for bradykinesia, and the rotarod test for stiffness. Currently, static tremor has not been investigated in rodent PD models. By using tasks with different mechanisms, observation bias for evaluation of a certain PD model may be reduced.

In our previous study, we found a dynamic change in D₂ receptor binding, drastic increase at 4 weeks and normalization at 6 months after lesioning. However, we still do not know the precise time course of interactions of the factors in the dopaminergic system in PD. We do not know whether the peak increase in D₂ receptor binding occurs at 4 weeks after lesioning or later, and whether D₂ receptor binding continues to decrease after 6 months. We are planning to sequentially measure D₂ receptor binding using C-11 compounds and positron emission tomography in the same rat. Simultaneous evaluation of D₂ receptor binding and other molecules with behavioral performance tests in certain intervals may reveal the turning points of the dopaminergic system, which could then explain the background molecular events controlling behavioral changes and possibly suggest a new index of the general functional state of the dopaminergic system in PD rat models.

**Conflicts of Interest Disclosure**

The authors have no conflict of interest or any financial disclosures to make. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.
References


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