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The different performance among motor tasks during the increasing current intensity of deep brain stimulation of the subthalamic nucleus in rats with different degrees of the unilateral striatal lesion

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ABSTRACT

Of all the parameters in the deep brain stimulation (DBS) of the subthalamic nucleus (STN) in the Parkinson disease (PD) animal models, the selection of the stimulation current intensity is alterable and argumentative to affect the stimulation charge or charge density. In order to observe the different performances among several motor tasks during the STN-DBS in rats, we observed the behavioral performance during the stimulation with 0, 100, 150 and 200 μA currents. We found that the DBS efficacy reached the climax during the 200 μA stimulation at the methamphetamine-induced rotational behavioral test, however at the stepping test and rotarod test, the critical current were 150 μA to reach the best improvements. Such findings suggest that the stimulation parameters to reach the climax efficacy among the different symptoms are different during the STN-DBS experiments in rats. The appropriate stimulation parameters should be selected by the symptoms separately according to the aim of each study.

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been widely reported as an effective surgical therapeutics both in the patients [1] and the animals [4,20]. The charge and charge density play important roles in the efficacy and safety of DBS, which are decided by the stimulation parameters, material and shape of the electrode [7,12]. The aim of the surgical management for DBS is to adjust an optimal combination of frequency, current intensity and pulse width to get an ideal charge density for reaching a balance of efficacy and safety. In the previous studies in rodent models, short duration (60–90 μs) high frequency (130–160 Hz) stimulation has been widely adopted during the STN-DBS in animal studies [2,4,13,20] as well as in clinical applications. We selected the stimulation current intensity as an alterable and manageable index as in our previous study [4].

Motor impairments in Parkinson disease (PD) rat models caused by different mechanisms are imitated by several behavioral tests: defective motor abilities such as akinesia/bradykinesia were evaluated by stepping test and rotarod test [17,19]; defective motor coordination and asymmetry were evaluated by methamphetamine/apomorphine-induced rotational behavioral test [4,5,11,20]. In the most of the previous studies, only responses of one motor task during different DBS parameters (mainly stimulation current) were observed. The "effective" current decided by one task was always adopted for the other tasks directly [2,4]. Similar to the clinical treatment, an ideal current intensity in PD animals is also considered as "as low as possible to reach the best improvements" to avoid the secondary dyskinesia and other side effects. It is still unclear if the lowest "effective" DBS parameters are similar among all the different behavioral tasks to improve the different symptoms. In the present study, we observed the performance of the two types of behavioral tasks during the STN-DBS with various stimulation current intensities of 0, 100, 150 and 200 μA to find the lowest "effective" current intensity among these tasks.

Twelve adult male Sprague–Dawley rats (SLC Hamamatsu, Japan) weighting 350 g on average were used in this study. All rats were housed individually in cages with free access to food and water in 23 °C air-conditioned room. All the experiments were performed 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 were approved by the Animal Experimental Committees at Hamamatsu University School of Medicine.

Rats received one (n = 6) or four lesions (n = 6) with unilateral stereotactic injections of 6-OHDA (Sigma, USA) in 0.9% saline (1 μg/μl) into the lateral sector of the striatum. Each lesion was made by injecting 1 μl/min for 7 min (7 μg) of 6-OHDA using a microinjector. The stereotactic coordinates for the one-lesion were AP = 1.0 mm rostral to the bregma, L = 3.0 mm right of the midline, V = 5.0 mm ventral to the dural surface and those for the four-lesion were (1) AP = 1.3 mm, L = 2.6 mm, V = 5.0 mm, (2) AP = 0.4 mm,

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L = 3.0 mm, V = 5.0 mm, (3) AP = −0.4 mm, L = 4.2 mm, V = 5.0 mm, (4) AP = −1.3 mm, L = 4.5 mm, V = 5.0 mm. Details of this surgical process were described in our previous studies [4,5].

A bipolar stimulating electrode guided by an extracellular recording system [4,13,14] was inserted into the medial part of the right STN (targeting AP = −3.6 mm, L = 2.6 mm from bregma [18]) by the procedure described in the previous study [4] 2 days after the first behavioral evaluation. We used a double-cored polyurethane-insulated stainless steel electrode. The diameter of each electrode was 0.2 mm, and 0.1 mm of the electrode tip was non-insulated. Two electrodes were attached side by side, with the tip of one electrode placed 0.1 mm above the tip of the other. The positions of the electrodes were confirmed by the cresyl violet staining in all of the rats after completion of the behavioral experiments the rats with the electrode outside STN were excluded.

The behavioral evaluation was including the methamphetamine-induced rotational behavioral test, stepping test and rotarod test which were described in detail in our previous studies [4,5]. All training and testing were started at 5:00 pm according to the rhythm of the rats [15,16]. We performed the behavioral tests with the sequence of amphetamine-induced rotational behavioral test, stepping test and rotarod test with 1 day interval to exclude the possible mutual influence.

The first behavioral tests were performed at 3 weeks after 6-OHDA administration to confirm the effects of 6-OHDA administration. Methamphetamine-induced rotational movements were monitored with a high-speed video camera over 60 min by intraperitoneal injection of 3 mg/kg of methamphetamine. The turning rates were recorded as turns/min [4,5,10]. Stepping test was performed to measure the initiation time, stepping length and adjusting steps in forward and backward directions [4,5]. Rotarod test was performed 24 h after stepping test to evaluate the motor ability of rats in a rotating rotarod. The duration times of each rat at different speed (5, 10, 15, 20, 25, 30, 35, and 40 rpm) were recorded and calculated the "overall rod performance" (ORP) [4,5,19].

One week after electrode implantation, we started DBS. The other stimulating parameters of STN-DBS were 130 Hz frequency and 60 μs pulse width, which were same as the clinical parameters and the values used in previously experimental studies [2,4,5]. We performed the behavioral tests during the stimulation with the current intensities of 0, 100, 150, 200 and 250 μA. In order to exclude the possible influence of the previous stimulation with different current intensities, we changed the current strength with an interval of 1 h rest time. We started stimulation 5 min before the behavioral tests and continued until the end of the tests. The continuous stimulation time was limited no more than 1 h to prevent possible lesions caused by stainless electrode [8]. The 250 μA current stimulation caused serious epilepsy in 4 of 6 one-lesion rats and 5 of 6 four-lesion rats, and therefore we gave up the stimulation above 200 μA. We found the extremely large individual difference of performance of the rotarod test, we also calculated the "improvement index" as $OPR_{100\,\mu A}$–$OPR_{0\,\mu A}$, $OPR_{150\,\mu A}$–$OPR_{0\,\mu A}$ and $OPR_{200\,\mu A}$–$OPR_{0\,\mu A}$.

After finishing all behavioral tests, rats were deeply anesthetized with intraperitoneal injection of an overdose of pentobarbital (Kyoritsu Seiyaku, Japan) and perfused through the ascending aorta with 50 ml of 1% PBS (pH 7.4) followed 50 ml of 10% formalin solution. The brains were removed and dehydrated in 10% sucrose solution for 1 day and then 30% sucrose solution for 2–3 days. Serial frozen coronal sections (30 μm in thickness) were made through areas that contained the electrode track, SN, and the 6-OHDA injection points. Adjacent sections were stained with cresyl violet or immunostained with tyrosine hydroxylase (TH). TH staining was performed as our previous studies [4,5]. Anti-TH rabbit polyclonal antibody (Protos Biotech Co., USA) diluted 1:100 was used as the primary antibody. We counted all TH-positive neurons in the SN pars compacta in these adjacent slices in normal and lesion side of the one-lesion and four-lesion rats to calculate the depletion rate. The locations of the electrodes within the STN were confirmed with cresyl violet staining of sections guided by the rat brain atlas [18].

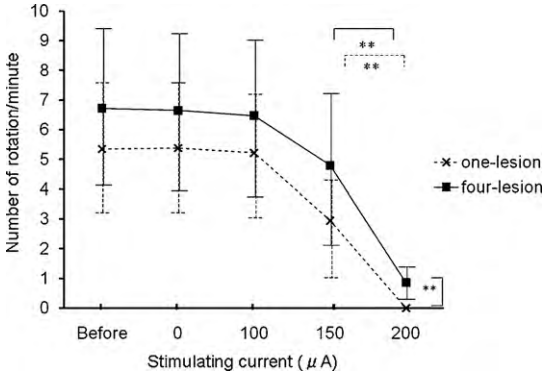

Fig. 1. Numbers of rotations induced by intraperitoneal injection of 3 mg/kg of methamphetamine 3 weeks after the 6-OHDA lesion before electrode implantation, and then 1 week after electrode implantation during STN-DBS with the current intensities of 0, 100, 150 and 200 μA (means ± SEM). There were no significant differences in numbers of rotation between the one-lesion group and four-lesion group during the stimulation before electrode implantation. During DBS with 0, 100, 150 μA, the numbers of rotation did not significantly decrease, but during DBS with 200 μA, the number of rotation significantly decreased and rotation stopped in all one-lesion rats and all but one of the four-lesion rats. Differences in the number of rotation between DBS with 150 μA and DBS with 200 μA were statistically significant both in one-lesion and four-lesion rats (**$p < 0.01$). At 200 μA STN-DBS, the numbers of rotations was significantly smaller in the one-lesion rats than those in the four-lesion rats (**$p < 0.01$), while there were no such differences between the two groups during STN-DBS below 200 μA.

All the data were recorded as the mean ± SEM. The t-test was used to compare differences between two groups, and one-way analysis of variance (ANOVA) was used to analyzed difference among groups. All of the data were analyzed with SPSS software.

Several TH-positive neurons were observed in the SN pars compacta of the one-lesion rats, while TH-positive neurons were hardly seen in the SN pars compacta of the four-lesion rats. The average depletion rates of the TH-positive neurons were 26% for the one-lesion rats and 90% for the four-lesion rats. The results are in accordance with our previous studies [4,5] and that by Kirik et al. [11].

We observed the turning rates induced by intraperitoneal injection of 3 mg/kg of methamphetamine 3 weeks after the lesion before electrode implantation, and then 1 week after electrode implantation during stimulations with the current intensities of 0, 100, 150 and 200 μA (Fig. 1). There were no significant differences in number of rotation among before electrode implantation and during stimulation with current intensities of 0, 100, and 150 μA in both one-lesion rats and four-lesion rats. When the current intensity increased to 200 μA, significant reductions in numbers of rotation were observed in both one-lesion and four-lesion rats (Fig. 1, $p < 0.01$) as compared to those of 150 μA. The rotation was completely stopped in all rats in one-lesion group and all but one in four-lesion group. The current intensity of 200 μA was considered as the "effective current" to improve the methamphetamine-induced rotational behaviors. Moreover, at the stimulation current intensity of 200 μA, the numbers of rotations was significantly smaller in the one-lesion rats than those in the four-lesion rats ($p < 0.01$), while there were no differences between the two groups during the stimulation at the current intensities below 200 μA.

There were no differences in all the indices of the stepping test among before electrode implantation and during stimulations with