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Anodal Transcranial Direct Current Stimulation Relieves the Unilateral Bias of a Rat Model of Parkinson's Disease

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Abstract- **The unilaterally lesioned rat model of Parkinson's disease which fails to orient to the food stimuli presented on the contralateral side of its preferential side of body could be induced by the injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB). We employed transcranial direct current stimulation (tDCS, current intensity: 80 μA, and 40 μA; anodal electrode area: 3.14 mm^2; stimulation time: 30 minutes) over the M1 area to relieve the ipsilateral bias in the rat model. A corridor test was set to count the ipsilateral bias of the rats. In this experiment, 30 Sprague-Dawley rats (80 μA: n = 8, 40 μA: n = 8, sham: n = 7, healthy control: n = 7) were chosen for the corridor test and the tDCS session. The lesioned rats exhibited increased ipsilateral bias 4 weeks after the lesion surgery ($P < 0.01$), and the anodal tDCS with the active electrode on the lesioned side relieved the ipsilateral bias significantly ($P < 0.01$) immediately after the surgery and the improvement lasted for nearly 1 day. The rats in the group of 80 μA exhibited more significant changes than the 40 μA group after one day. After all the experiments, the histological process showed no neurotrauma led by the tDCS. In conclusion, the modulatory function of the cortical excitability of the tDCS may awaken the compensatory mechanisms and the response mechanisms which modulate the loss of the brain function. Further studies should be done to provide more evidence about the assumption.**

I. INTRODUCTION

BEHAVIORAL tests such as drug-induced rotation, contralateral sensorimotor neglect, and contralateral akinesia on the unilateral lesioned rat model of Parkinson's disease (PD) are screens of the impairment level and the tyrosine hydroxylase positive (TH^+) cell loss of the lesioned brain region [1]. The improvement of the behavioral performance is essential for the assessment of the therapeutic effects of embryonic dopamine neuron grafts [2], deep brain stimulation (DBS) [3], and non-invasive brain stimulation [4].

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Grafts, DBS, and transcranial magnetic stimulation (TMS) have been applied clinically and maintain less side-effects than pharmacotherapy. As a kind of non-invasive brain stimulation, repetitive TMS (rTMS) has been proved to relieve behavioral symptoms [4] in UPDRS, modulate the cortical-subcortical circuitry activity [5],[6], and also induce dopamine release in basal ganglia [7]-[11] of humans and animals. Recent years, transcranial direct current stimulation (tDCS) is considered to be another promising tool as adjunctive therapies for PD patients. tDCS is a neural modulatory intervention that acts similarly with TMS but has lots of advantages over TMS, such as low-cost, high security, and more reliable sham-stimulation conditions. Effects of tDCS are based on the experimental protocol, such as the polarity of the electrodes, the stimulus position, the stimulus duration and intensity. However, there are only two studies about tDCS and repetitive tDCS on PD patients [12], [13], and no studies on PD animals until now. Compared to other behavioral tests on the rodent model of PD, the behavior of contralateral neglect is a simpler, a more objective, and drug-free behavioral screens of the therapeutic effects. Inspired by the reduction of the neglect of food in contralateral space of the unilateral lesioned rats by transplants [14], we assumed that anodal tDCS could relieve the ipsilateral bias of PD rats by the compensatory mechanisms [13] which make up for the loss of modulatory function of dopamine. On the other hand, anodal tDCS could enhance the excitability of the target cortex [15].

However, to our best knowledge, there is not any standard experimental protocol for tDCS on PD patients and PD animals. Surface electrodes are easily attached on humans but difficult on freely moving rodent animals. So, lacking of animal experiments is an obstacle for exploring the mechanisms and the best stimulus protocol of tDCS. tDCS has been conducted to induce functional and histological changes of anesthetic rat models of stroke [16] and to induce neuronal activation in the frontal cortex of the rat models by a fMRI study [6]. But, the anesthetic will affect the accuracy of the experimental result.

Liebetanz et al. developed the skull-surface electrodes in freely moving rats [17] which make it possible to observe the effects of tDCS on not anesthetized rodent animals. So in order to observe the behavioral changes, skull-surface electrodes must be applied to avoid the anesthetic affects and the interruption by paws during the tDCS.

The aim of this study is three-fold: first of all, to design a new experimental protocol for tDCS on freely moving PD rats; furthermore, to validate the reduction of the ipsilateral bias by anodal tDCS; thirdly, to check whether the intensity we used could induce some neurotrauma.

II. MATERIALS AND METHODS

A. The Rat Model of PD

6-OHDA (Sigma, dissolved in 0.01% ascorbate saline) was injected unilaterally into the medial forebrain bundle (MFB) (4 µl of 4 µg/µl, AP - 4.4 mm, ML ± 1.0 mm, DV -7.8 mm), the injection was at the rate of 0.5 µl/min and we waited for 5 minutes before the withdrawal of the pinhead to make the tissue completely absorb the toxin. The retraction of the needle was at a low speed about 1mm/min. 5 weeks later, 23 female Sprague-Dawley rats that rotated more than 7 loops per minute induced by Apomorphine (0.5 mg/kg) and 7 healthy rats were selected for the further study (successful PD model: n = 23; healthy control: n = 7). Rats were divided into 4 groups: 40 µA group (n = 8), 80 µA group (n = 8), sham group (n = 7), and control group (n = 7).

Fig. 1. Anodal electrode used for transcranial direct current stimulation in rats. The electrode was inserted in a plastic tube which was fixed onto skull by dental ionomer cement.

B. Transcranial Direct Current Stimulation

Dental glass ionomer cement was used to fix a plastic tube onto the skull. A saline saturated sponge and an active electrode (inner diameter: 2 mm) were inserted into the plastic tube during tDCS (Fig. 1.). Constant currents of 40 µA (charge density: 22930 C/m2) and 80 µA (charge density: 45859 C/m2) were delivered to the rats of the two groups for 30 minutes separately. According to the earlier study [16], this charge density will not cause brain lesions. We had the rat wear a jacket with the reference electrode (surface area: 10 cm2) attached to it (when the rat wear the jacket, the reference electrode is on the thorax of the rat). Electrode were also sticked to the skulls of the rats from the sham group, but they did not receive tDCS. Rats from the control group did not receive any surgery. All rats were supplied water and food ad libitum. We observed acute changes of the ipsilateral bias immediately after a single stimulus session, and then tested the after-effects on the 1st day, the 3rd day, the 5th day, the 7th day, and the 9th day.

C. Corridor Test

We set a corridor (two boards, length: 205 cm, width: 10 cm) (see Fig 2) with 28 plastic pellet containers (diameter: 2.5 cm, depth: 1.5 cm) put adjacently in two rows [15]. Each rat has been trained to adapt to the test environment for 2 days, and the baseline was recorded preceding the first test. Rats have unilateral bias innately, in order to separate the effect of the innate bias, 6-OHDA was injected into the side opposite to the innately biased side. 5 weeks after the surgery, all the rats preferentially poked their nose from the side ipsilateral to the lesions. Poking nose into a container will be counted as a "retrieval" despite of whether the content was eaten or not.

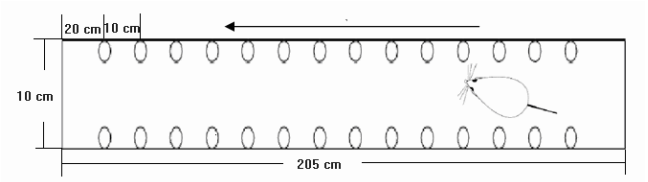

Fig. 2. Schematic diagram of corridor test. 28 containers were put in two rows in a corridor with intervals of 10cm. The rat was put in one end of the corridor, the number of the pellet retrievals from the left or the right side of its body were counted.

D. Histological Processing and Analysis

Surgery was conducted after all of the behavioral experiments. The rats were anesthetized before the surgery. We perfused 0.9% saline (NaCl) transcardially, then followed up with a fixative (4% paraformaldehyde in 0.1 M phosphate buffer) until severe convulsions manifested. After that, brains were removed and immersed into a 30% sucrose solution.

Coronal sections of the brains were cut by a paraffin slicing machine (Leica) with a thickness of 10 µm. Sections were serially cut and stained by H&E for histological examination. A Light microscope was used to examine whether the tDCS had generated any electrical neurotrauma.

III. RESULTS

Data was subjected to one-factor analysis of variance (ANOVA). The significance level was $P < 0.05$. There were no significant differences ($P = 0.12$) between the four groups before the 6-OHDA lesion. Unilateral 6-OHDA lesion increased the ipsilateral bias on the lesioned rats when compared to the control group ($P < 0.01$) (Fig 3). The preferential side for the lesioned rats to retrieve changed after the lesion. Anodal tDCS reduced the bias significantly ($P < 0.01$) (Fig 4), and this effect lasted for about 1 day. During the interval (2 days) of every test session, no food provided to the rats. There are significant differences between the active groups and the sham group (rats from the 80 µA group compared to the sham: $P < 0.01$; rats from the 40 µA group compared to the sham: $P < 0.01$). The

tDCS with 80 μA led to more significant changes than the 40 μA tDCS 1 day after the tDCS session.

There are no significant pathological changes such as oedema, haematoma and electrical neurotrauma to be seen on the brain sections (Fig. 5).

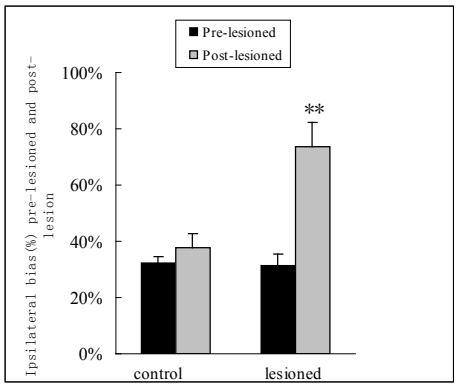

Fig. 3. Ipsilateral bias (%) of the pre-lesioned and the post-lesioned rats, the ipsilateral bias changed significantly after the unilateral lesion of the brian contralateral to the preferential side. * P<0.05, ** P<0.01. Error bars mean SD.

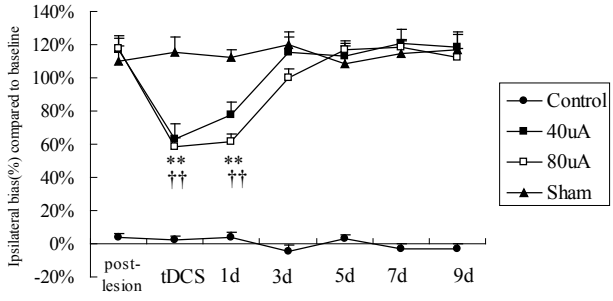

Fig. 4. Ipsilateral bias compared to the baseline. Anodal tDCS reduced the ipsilateral bias significantly. This effect lasted for about 1 day. 1d-9d means the days after the tDCS session. *, †: P < 0.05; **, ††: P < 0.01. Error bars mean SD.

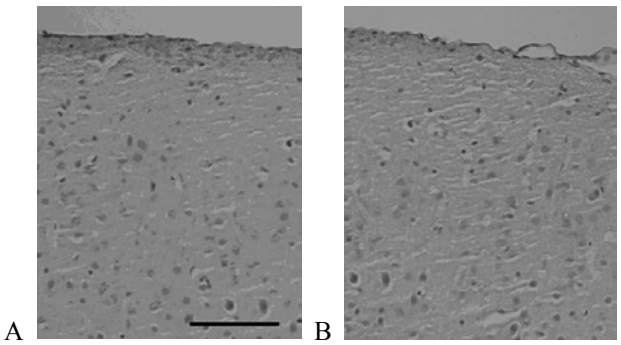

Fig. 5. Paraffin sections of rats from sham (A), and active stimulation group (B). Haematoxylin and Eosin (H&E) stained the sections and there are no obvious neurotrauma on these brain sections. Scale bar=150 μm.

IV. DISCUSSION

To our best knowledge, this is the first study to explore behavioral effects of tDCS on the rat models of PD. We chose corridor test in this study because it is the most sensitive marker to the brain lesion severity [1]. Unilateral neglect of the PD rats has been discussed in the earlier studies [19]-[21]. Rats' MFB were lesioned by 6-OHDA unilaterally to cause the food neglect from one side of its body. MFB lesion led to almost complete loss of TH+ cells and the deafferentation of striatum [14]. The Postsynaptic supersensitivity which contributed to rotational asymmetry occurs only after most of the dopaminergic neurons in the SNpc have been eliminated [22], so we selected this target for injection, which will improve the success rate of PD rat modeling.

In our previous study it has been proved that the cathodal stimulation could reduce Apomorphine-induced rotation even more significantly than the anodal stimulation. We assumed the cathodal polarity effect inhibitted the activity of M1 neurons, and the modulatory effects also suppressed the excitability of cortical-subcortical circuitry and the postsynaptic supersensitivity. As we know, anodal stimulation increases the motor excitability in humans [23], and the dysfunctional basal ganglia may be compensated indirectly by enhancing the corticospinal excitability, because the increase of the MEPs present a significant correlation with the UPDRS ratings in PD humans [13]. The effect of the single stimulus session may be caused by modification of the membrane function [24] and long-lasting after-effects are associated with the modification of NMDA receptor efficacy [15]. Depending on neuroplastic modulations, N-methyl-D-aspartate (NMDA) receptor-dependent cortical activity and activity shifts were induced by tDCS [25], so membrane-stabilizing substances and ketamine might be problematic in animal experiments with tDCS, that is because the anesthetic will confuse the effects on the polarized cortex, especially in the behavioral studies. The electrode montage in this study is available for freely moving rats with no anesthetic.

Benninger [12] applied repetitive anodal tDCS on M1 of PD patients during "on" and "off" medication status. The performance of the Gait Test was improved for a short time, but bradykinesia was improved for more than 3 months, and no changes were discovered in the UPDRS and the reaction time. However, Fregni [13] reported that single anodal tDCS session on M1 improved the PD patients' UPDRS performance, and elevated MEPs size. The only two studies in tDCS for PD maintain a little inconsistence. In addition, tDCS may cause dopamine release in the caudate nucleus or in the striatum as rTMS does [10],[11]. Because the dopaminergic action maybe reflected by the prolonged cortical silent period, and tDCS on M1 could prolong the cortical silent period, which is associated with the excitability of the motor cortex [12]. The compensatory mechanisms and the response mechanisms of the cortical-subcortical circuit activated by tDCS relieved the behavioral deficits of the PD rat model, but further studies are still required to reveal the best therapeutic protocol for PD patients.

In conclusion, anodal stimulation may improve the motor funtion of PD patients and the rat model. Even though tDCS

in PD patients provided positive effects of improving the behavioral performance, a study of large, and repetitive cathodal and anodal stimulation regarding to montage the two polarities in PD patients are still needed to solve both akinesia, rigidity, and gait problems as well as tremor before clinical application of tDCS.

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