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A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI

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ABSTRACT

Transcranial direct current stimulation (tDCS) is one of the noteworthy noninvasive brain stimulation techniques, but the mechanism of its action remains unclear. With the aim of clarifying the mechanism, we developed a rat model and measured its effectiveness using fMRI. Carbon fiber electrodes were placed on the top of the head over the frontal cortex as the anode and on the neck as the cathode. The stimulus was 400- or 40-μA current applied for 10 min after a baseline recording under an anesthetized condition. The 400-μA stimulation significantly increased signal intensities in the frontal cortex and nucleus accumbens. This suggests anodal tDCS over the frontal cortex induces neuronal activation in the frontal cortex and in its connected brain region.

Noninvasive brain stimulation techniques are receiving attention for clinical applications and as new tools for brain research [7,19]. Transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are mainly studied now, but the mechanisms of their action in the brain are still unclear.

With regard to basic studies of clinical applications and functional mechanisms, animal models, especially rodent models, are needed for TMS and tDCS in order to evaluate the safety of the stimulation for clinical treatment and to improve the effectiveness of experimental animal studies. Rodent models of TMS have shown effectiveness in the treatment of depression-like behavior [10,22], epileptic seizures [18] and Parkinson's disease-like symptoms [23]. Moreover, basic research of the neuronal mechanisms of TMS's action has shown that TMS increases dopamine release in the ventral striatum in rats [12]. In these ways, studies of rodent models for TMS are showing TMS's potential for future clinical applications and revealing its action mechanism in the brain.

For tDCS, a rodent model in which a stimulus electrode is embedded in the skull has recently been proposed. Liebetanz et al. examined the safety limit of tDCS and showed that a current density should be between 142.9 and 285.7 A/m^2 to avoid lesion formation in the cortex beneath stimulus electrode [14]. Cambiaghi et al. applied the same stimulus method to the brain of mice and showed that the size of motor-evoked potentials increases after anodal tDCS and decreases after cathodal tDCS [5]. That is, it is possible to examine the neuronal activity in the cortex directly beneath the stimulus point.

In human tDCS, safety requires a contact area of a certain size. Moreover, localization of the effects of tDCS is important. In previous studies, the electrode for tDCS in animal models was much smaller than that used for humans. The small electrode ensures that the effects of the tDCS are local, making it possible to examine the effects just beneath the electrode. However, no animal models of tDCS have used electrodes with a large contact area like the ones used in human tDCS. In an animal model with such a large electrode, it would be necessary to examine the tDCS's effects on the whole brain and ascertain whether the stimulus activates neurons in the brain region just beneath the electrode or not.

The purpose of this study was to develop a rat model of tDCS similar to the method used for humans and to evaluate the local and global effects of tDCS by a large electrode. For this purpose, we examined the effectiveness of tDCS using functional magnetic resonance imaging (fMRI). We evaluated the validity of this method by examining the tDCS-induced change rate in the fMRI signal intensities in the brain regions.

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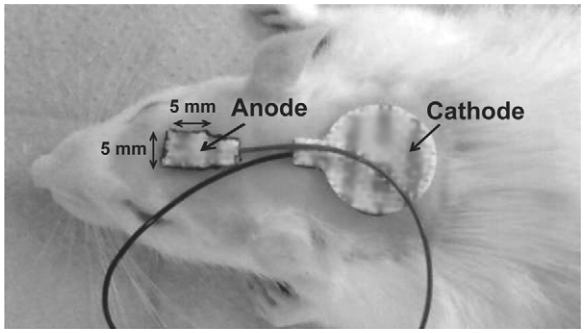

Fig. 1. Positioning of stimulus electrods for rat tDCS. The center of one side edge of the anodal stimulus electrode is positioned at the midpoint of the lateral angle of the eyes. The prefrontal cortex in rats is under the anodal electrode. The cathodal electrode is positioned from the neck to shoulder areas.

Twelve male Sprague–Dawley rats (Sankyo Labo Service Co., Inc.) with mean weight of 288 at the time of the experiment (SD = 34) were housed individually in a temperature-controlled (22–23 °C) animal room under a 12-h light/dark cycle (light from 8:00 to 20:00). Prior to the experiments, the rats had free access to laboratory chow and tap water in their home cages. The experimental protocols were approved by the Japan Science and Technology Agency Ethical Board for Experiments on Animals, and the experiments were conducted in accordance with the "Official Notification on Animal Experiments" (JST notification no. 32, revised 2004).

Rats were anesthetized with urethane (1.0 g/kg, i.p.), sheared around their head and neck, and placed in a stereotaxic apparatus, with their body temperature maintained at around 37 °C by a heat mat. Carbon fiber electrodes coated with conductive gel were placed on the top of the head over the frontal cortex (5 mm × 5 mm) and neck (20 mm in diameter). By placing the end of anodal electrode at the midpoint of the lateral angle of the eyes, we tried to fix the stimulation point over the frontal cortex of the rats (Fig. 1).

All fMRI experiments were performed on a 4.7-T, 33-cm bore with a 6.5-G/cm gradient set MR imaging system (Oxford Magnet; console by Varian Inc., Palo Alto, CA, U.S.A.). After the animal had been placed in a cradle, a surface coil (2-cm diameter) was positioned on top of the animal's head. A three-plane scout image, including axial, coronal, and sagittal slices, was used to select fMRI planes and anatomical images according to a brain atlas [17]. Prior to the fMRI experiments, to improve the homogeneity of the magnetic field, we adjusted the first- and second-order shims in the volume containing the fMRI slices. This localized shimming procedure constantly resulted in a half width for water of about 0.2 ppm. Anatomical images were obtained by using a gradient-echo technique (data matrix = 256 × 128, FOV = 4 cm^2, TR/TE = 0.02/0.008 s, flip angle = 23°, slice thickness = 2 mm). From these images, eleven 1-mm-thick planes were selected for transverse imaging. In all scans T_2*-weighted functional MR images were obtained by gradient-echo imaging (data matrix = 128 × 64, FOV = 4 cm^2, TR/TE = 0.0178/0.007 s, flip angle = 7°, slice thickness = 1 mm, NEX = 2). To minimize the inflow effect, we set the flip angle of a radio-frequency pulse at approximately 7°, which is much smaller than the Ernst flip angle. Additionally, two slices were added before and after nine measurement slices to suppress inflow of fresh spins into them. It took 30 s to obtain one volume of images (11 slices), including a rest state (5 s). The total volume of T_2*-weighted images during the experiment (40 min) was 80. Twenty volumes (10 min) just before stimulation and 20 volumes after it were used for the statistical analysis.

After the baseline recording (15 min), we stimulated the top of the animal's head with 400- (n = 6) or 40-μA (n = 6) current for 10 min (the electrode on the top of the head was positive) (STG1002, Multi Channel Systems, Germany). Current density in the 400-μA condition was 16.0 A/m^2, a value small enough not to damage brain tissue, considering the safety limits reported in a previous study that stimulated the skull of rats [14]. Current density in the 40-μA condition was 1.6 A/m^2, which was the control in this experiment. We selected 40-μA because it is a sufficiently smaller current than 400-μA and because electrical noises produced when the stimulator is switched off and on are equalized with 400- and 40-μA current. These current densities were higher than that for humans [15,11,21]. The recording of fMRI after tDCS was performed for 15 min.

The averaged fMRI signal intensity of each region of interest (ROI) was calculated before and after stimulation and compared between 400- and 40-μA. The signal intensities of the brain regions, whose size and borders were determined after evaluation of the brain atlas [17] and anatomical images of each animal, were measured, analyzed, and output after co-registration with the anatomical images, spacial smoothing, and DC correction by Stimulate software [20]. The ROIs were the frontal cortex composed of pre-limbic cortex and infra-limbic cortex of rat (frontal), cortex in the left hemisphere (cortex L), cortex in the right hemisphere (cortex R), orbitofrontal cortex (orbital), nucleus accumbens (NAcc), striatum, thalamus, and hypothalamus (Fig. 2). We drew these ROI borderlines with straight lines using Image J software by evaluating the cortex, fiber, and cerebral ventride of anatomical images and referring to the brain atlas [17].

We used the fMRI data obtained 10 min before tDCS as a baseline, and used the data obtained 10 min after tDCS as tDCS effects. For the data before and after tDCS, the average signal intensities were calculated by the ROIs, with the average after tDCS divided

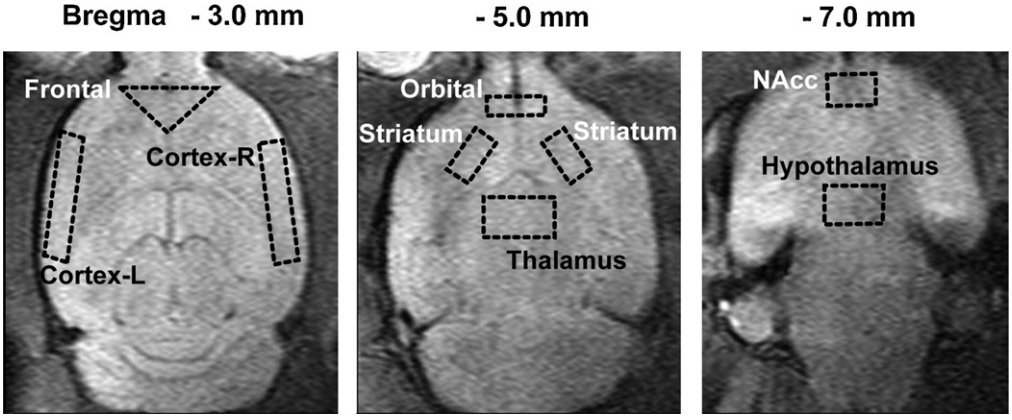

Fig. 2. ROIs in this study. Black lines are regional borderlines with the brain-region name.

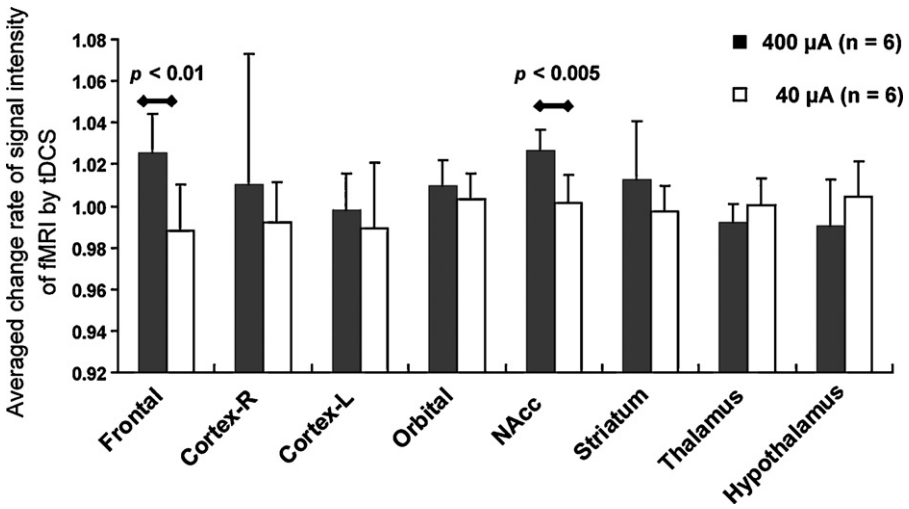

Fig. 3. Activations of signal intensities of fMRI by anodal tDCS to the top of the head of rats. The error bars show standard deviations. Compared between 400- and 40-μA. The difference in signal intensities in the frontal cortex and nucleus accumbens (NAcc) were significant.

by that before tDCS, and the increase rate of signal intensity was calculated. We compared the increase rates for 400-μA tDCS and 40-μA tDCS in a *t*-test using SPSS 12.0 for Windows.

As shown in Fig. 3, the signal intensities in the frontal cortex and NAcc were significantly increased by 400-μA stimulation compared to those with 40-μA stimulation (frontal: $t(10) = 3.207$, $p < 0.01$; NAcc: $t(10) = 3.743$, $p < 0.005$). In the other brain regions, these values were not significantly changed by tDCS (cortex R: $t(10) = 0.668$, *n.s.*; cortex L: $t(10) = 0.577$, *n.s.*; orbital: $t(10) = 0.876$, *n.s.*; striatum: $t(10) = 1.244$, *n.s.*; thalamus: $t(10) = -1.323$, *n.s.*; hypothalamus: $t(10) = -1.231$, *n.s.*).

In the present study, increased activations of the frontal cortex and NAcc of rats were observed after the anodal tDCS to the top of the head over the frontal cortex. The rat model we developed is valid because the brain region beneath the anodal electrode, the frontal cortex, was activated by anodal tDCS. In human tDCS, brain regions beneath the anodal electrode are activated. Nitsche and Paulus showed that anodal tDCS to the motor cortex enhances motor-evoked potentials in the motor cortex [15]. Jang et al. have shown that anodal tDCS targeting the somatosensory cortex in humans induces activation of this area during hand gripping tasks after anodal tDCS [11].

The present study showed that tDCS to the head over the frontal cortex induces activations of the frontal cortex and NAcc, which receives strong projections from the frontal cortex [8,1]. These results suggest that simulation of the frontal cortex from the top of the head induces neural activities in the proximate area and its connected brain region, especially in the NAcc. In a human study, Boros et al. showed that premotor tDCS selectively influences intracortical excitability of ipsilateral primaly motor cortex and suggested a connectivity driven effect of tDCS on remote cortical areas [4]. This indicates the possibility that tDCS affects the global network in the brain, not just the cortex beneath the electrode.

In human tDCS experiments, it has been shown that the spatial selectivity of tDCS effects can stimulate more functional specific areas. Nitsche and Paulus showed that an electrode arrangement over the motor cortex and on the forehead affects motor-evoked potentials, whereas other arrangements did not, which indicates the spatial specificity of the effect of tDCS [15]. Tanaka et al. showed that anodal tDCS over the leg motor cortex transiently induces an increase in maximal leg pinch force without affecting hand function, again suggesting that tDCS can affect functionally and spatially specific areas [21]. As rodent studies have yet to examine the effects of behaviors, the required level of spatial selectivity will be the same level as for humans. In this context, our finding of selective activation under an electrode with a large contact area is important. A future challenge is to reveal what specific brain region or behavioral function is stimulated by the arrangement of electrodes in the method we developed.

The spatial selectivity would be in conflict with the influence of tDCS on the brain's global network. The larger the activation the tDCS induces in the whole brain, the smaller would be the spatial selectivity of tDCS. In the present study, the tDSC to the frontal cortex induced activation of the NAcc, but the frontal cortex also has other connections. A future study should attempt to clarify the selection mechanism that determines which connection is activated.

In the present study, the electrode was put on the skin, as it is in human tDCS. Then, the anodal tDCS had effectiveness. In this point, our results are critical for connecting animal model studies with human tDCS. These results are mediated through stimulation of skin, skull, and meningeal receptors. At this time, it is unknown how skin, skull, and meningeal receptors are related to the action mechanism of tDCS in the brain.

Deep brain stimulation in the frontal cortex has shown effectiveness in the treatment of depression-like behavior in a rodent model [9] and that in the NAcc has shown antidepressant effects in human depression patients [2]. Moreover, human tDCS has been examined for depression treatment [3,6]. Therefore, if rat tDCS can be shown to decrease depression-like behaviors, the present method is expected to be useful for basic studies of the treatment of depression by non-invasive brain stimulation. In the present study, only anodal stimulation to the top of the head was examined, and the cathode position was the neck, not the head. In contrast, the cathodal electrode is often placed on the contralateral frontal area in human tDCS studies, including in depression treatment [13,16]. Therefore, it is necessary to examine the polarity effect for the present method.

The signal intensities of fMRI in the frontal cortex and the nucleus accumbens were significantly increased by anodal tDCS in rats. This suggests anodal tDCS over the frontal cortex induces neuronal activation in the frontal cortex and its connected brain region. The rat model of tDCS we developed is useful for investigating the mechanisms and clinical effectiveness of tDCS in more detail.

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