J. Phys. Ther. Sci.
26: 247–249, 2014

The Effect of tDCS on Cognition and Neurologic Recovery of Rats with Alzheimer's Disease

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Abstract. [Purpose] This study examined the effect of the application of transcranial direct current stimulation (tDCS) on neurologic recovery and cognitive function of rats with Alzheimer-like dementia induced by scopolamine injections. [Subjects] To create a cognition dysfunction model, intraperitoneal injection of scopolamine was given to Sprague-Dawley rats that subsequently received tDCS for 4 weeks. [Methods] Changes in motor behavior were evaluated by conducting an open field test. Acetylcholine content in the cerebral cortex and hippocampus was examined for a biochemical assessment. [Results] With respect to changes in motor behavior, group II showed the most meaningful difference after scopolamine injection, followed by group III. In the biochemical assessment, the results of the examination of acetylcholine content in the tissue of the cerebral cortex and the hippocampus on the 14th and 28th days, respectively, showed the most significant increase in group II, followed by group III. [Conclusion] The above findings confirm that tDCS application after the onset of cognitive dysfunction caused by Alzheimer's disease leads to a positive effect on motor behavior and biochemical changes, and this effect is maintained over a specific period of time.
Key words: tDCS, Neurologic recovery, Alzheimer's disease

(This article was submitted Jul. 24, 2013, and was accepted Sep. 5, 2013)

INTRODUCTION

The most severe problem of Alzheimer's disease is damage to the cognitive function caused by nerve degeneration. Cognitive impairment may have started before it is diagnosed, and cognition dysfunction is usually found in 25% of newly diagnosed patients[1)]. With Alzheimer's disease, cognition dysfunction can cause challenges in completing tasks of daily living, making them more difficult than before the disease onset[2, 3)]. Important research currently under way is examining invasive and non-invasive innervation research. As part of this research, the effects of transcranial direct current stimulation (tDCS) on movement, sense, cognitive ability, exercise rehabilitation, and change brain action potential are being investigated[4, 5)]. It is known that acetylcholine in the hippocampus usually works as a neurotransmitter and plays an important role in cognitive abilities related to learning and memory[6)]. At present, most interventional treatment focuses on the origin of the disease and on regenerating the activity of the central nervous system. However, it is nigh impossible to completely re-activate the central nervous system and regeneration is also very limited[7)]. The body of existing research about the effects of tDCS use on Alzheimer's disease is rather small. Accordingly, this research is intended to provide basic data for further study by seeking observable improvement induced by the administration of tDCS in central nervous system activity and indices of cognitive ability. Additionally, the effects on the body, potential changes in behavioral reaction to certain stimuli, and biochemical aspects were also studied.

SUBJECTS AND METHODS

In this research, Sprague-Dawley white rats weighing 230 ± 20 g were selected for intraperitoneal injection of scopolamine, and 16 rats each were randomly allocated to 3 experimental groups to conduct the experiment. All animal experiment protocols were performed in accordance with the guidelines of the Dongshin University Animal Care and Use Committee. Group I (n=16) was the non-treatment group; Group II (n=16) was the tacrine group; and Group III (n=16) was the tDCS group. The recovery of cognitive function was evaluated by the animals' behavioral reaction in the open-field test, which was conducted right after induction of Alzheimer's disease and 4 more times at week-long intervals (7, 14, 21, 28 days). Biochemical evaluation of acetylcholine was also conducted on the last day. Eight rats from each group were euthanized for this purpose. To induce cognitive dysfunction in normal rats, scopolamine (Sigma, S1875, St Louis, Missouri, USA) 1 mg/kg, that was

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lysed and diluted in physiological saline at 0.9%, was injected into the abdominal cavity once daily over a period of 30 days. In addition, to provide a comparison group for the experimental group that received scopolamine, tacrine (Sigma, A3773, St. Louis, Missouri, USA) 10 mg/kg once daily, that was lysed and diluted in physiological saline at 0.9%, was injected over a 4-week period after injection of scopolamine, to detoxificy the Group II as well as Group I. According to Kim's method[8], tDCS in this experiment was delivered used a direct current machine (Cyber Medic Co. Jeonju, Korea) that can be controlled at 0.1 mA. The strength of the current was set at 0.1 mA for that lasted 20 minutes. Applications were performed 2 times a day, 5 times a week, for 4 weeks at the same time every day.

Open-field tests were conducted before and 7, 14, 21 days after induction of Alzheimer's disease to evaluate the behavior of the rats with cognitive impairment. A lamp was placed in the center of a black box that was 78 cm wide, 78 cm long, and 30 cm in height, and had a transparent acrylic panel lid. After a rat entered the black box, the time to first movement, and the time it took from this first movement to pass a point where food and the numbers 15, 16, 21, and 22 were placed were measured. Regardless of the distance moved, this time was recorded as 300 seconds, if more than 300 seconds had passed. The measurement of acetylcholine was based on the reaction of an o-acyl inducer, alkaline hydroxylamine, according to the method of Galgani et al[9]. A homogenate, 50-μL of the hippocampus and cerebral cortex (12.5 mM sodium phosphate buffer pH 7.0, 400 mM NaCl) was mixed with 50-μL of hydroxylamine (Sigma, USA) and adjusted to a pH of 1.2±0.2. After being mixed with an additional 500 μL $FeCl^3$ (10% in 0.1 N HCl), the acetylcholine content was measured by checking the optical density. The statistical analysis of this study was done using SPSS ver. 18.0 for Windows. The values are presented as averages with standard deviations. The behavioral comparison among the groups was performed for each measurement time using one-way analysis of variance. Tukey's multiple range test was used as a post hoc test. To check changes among each group according to time period, the paired t-test was conducted. The significance level was chosen as α=0.05.

RESULTS

The comparison among the three groups in terms of which rats reached the food pallets during the open-field tests revealed differences in the decline of cognitive function between the groups at every measured point in time from the 7th day onward. Results of the post hoc test from day 7 revealed greater evident change in cognitive function in experimental group I than in Group II ($p<0.01$). On day 14, experimental Group II showed the greatest decrease in cognitive function ($p<0.001$); there was also a decrease in cognitive function in experimental Group III ($p<0.05$). On day 21, there were greater decreases in cognitive function in experimental Groups II and III than in Group I ($p<0.001$). Experimental Groups III and II showed evident decreases in cognitive function ($p<0.05$). The results of the post hoc test on day 28 were the same as the day 21.

The paired-t test was used to check the changes of the results according to the time of the measurement. Group II showed changes in cognitive function from day 7. From the 21st day, experimental Group III showed the same level of decline in cognitive function after previously showing evident decline($p<0.05$) (Table 1).

Differences among groups in times to start movement in the open-field test showed significant decreases from day 7 onward. Specifically, in the results of the post hoc test on day 7, experimental Group II showed a more significant decline than Group I ($p<0.05$). Conversely, on day 14, Group II showed the most significant decrease in the post hoc test, and Group III also showed a significant decrease ($p<0.01$). On day 21, Group II ($p<0.001$) showed a more significant decrease than Group I ($p<0.01$). On day 28, every group showed a significant decrease compared to experimental Group I ($p<0.001$). The paired-t test was conducted to check variations according to the time intervals between measurements. Experimental Group I didn't show any significant differences up to the 21st day, but then showed a significant difference on day 28 ($p<0.05$). Experimental Group II showed a significant difference on day 7 ($p<0.05$), and the results were the same for each subsequent measurement. Group III showed a significant decrease from day 14 onward ($p<0.05$) (Table 2).

With respect to differences among groups according to time in acetylcholine content, significant differences were found on both day 14 and day 28, and experimental groups II and III showed more significant differences ($p<0.001$) than Group I. In particular, experimental Group II showed the most significant increase in acetylcholine content ($p<0.001$). In the post hoc test, significant differences were found on day 14 for experimental groups II and III, which both showed more significant increases in acetylcholine content than did group I ($p<0.01$). Experimental Group II ($p<0.001$) and Group III ($p<0.05$) both showed significant increases. There were also significant differences on day 28 with groups II and III showing more significant increases than group I ($p<0.01$). Experimental groups II and III showed the most significant increases ($p<0.001$ and $p<0.05$, respectively) (Table 3).

DISCUSSION

A treatment for Alzheimer's disease has not yet been found, but research is continuing into potential treatments. Transcranial direct current stimulation, or tDCS, can control the activity of the motor cortex in a kind of cortical stimulation. It has been reported that this method is comfortable and safe to apply in the clinical setting[10]. While the effect of tDCS comes from control of synaptic activity within the cerebral cortex, excitement of the corticospinal pathway depends on the level of the membrane potential[11, 12]. In this study, after induction of Alzheimer-like dementia with intraperitoneally injected scopolamine, the time it took Sprague-Dawley rats to start movement and reach a food pallet in the open-field test was measured on days 7, 14, 21, and 28 of tDCS. The results show significant differences

Table 1. Open Field Test pass times (sec)

Group	Day				
	pre	7 days	14 days	21 days	28 days
I	94.13±11.55	92.94±15.11	90.19±14.82	88.38±17.04	85.56±15.82
II	93.25±11.69	79.75±10.66††**	72.38±9.76††**	66.38±8.82††**	58.75±10.20††**
III	93.81±13.26	89.50±9.14	82.63±8.53†	75.38±7.93†*	68.31±11.69††*
p	0.996	0.010	0.001	0.000	0.000

All values mean±SD. Group I: Scopolamine injection only: Group II: Scopolamine injection + Tacrine: Group III: Scopolamine injection + tDCS

Table 2. Open Field Test start times (sec)

Group	Day				
	pre	7 days	14 days	21 days	28 days
I	298.13±5.44	295.63±8.92	293.75±10.88	291.56±13.95	288.94±15.57*
II	297.19±6.57	280±15.16†*	263.13±13.28††*	243.13±18.52††*	223.75±13.42††*
III	297.81±5.44	292.19±18.53***	283±20.32††*	266.88±15.48††*	252.5±8.69††*
p	0.974	0.027	0.000	0.000	0.000

All values mean±SD. Group I: Scopolamine injection only: Group II: Scopolamine injection + Tacrine: Group III: Scopolamine injection + tDCS

Table 3. Acetylcholine activity in each group (ng/mg protein)

Group	Acetylcholine activity	
	14 days	28 days
I	60.95±6.35	23.96±4.89
II	76.25±6.67†††***	34.28±3.73†††***
III	73.54±6.34†††***	30.24±2.88†††***
p	0.000	0.000

All values mean±SD. Group I: Scopolamine injection only: Group II: Scopolamine injection + Tacrine: Group III: Scopolamine injection + tDCS

from the control group in the groups given tDCS. Experimental group II showed the most significant difference. The post hoc test revealed that experimental group II showed the most significant decrease in time compared to experimental Group I. Group III showed a significant decrease on day 21. In the open-field and "gluttony" test, experimental group I showed impairment with no change in time or in frequency of errors over the 28 days, whereas the other groups, times to reach food and the number of errors decreased. This result is considered a result of improved cognition based on the conclusion of a previous study[13].

In conclusion, we confirmed that the application of tDCS after induction of cognitive impairment with scopolamine has positive effects on behavioral reactions, restoration of cognitive ability, and biochemical change. The effect of tDCS was maintained over a period of time (at least 28 days). This research provides basic data that suggest tDCS is a possible new treatment method for patients with cognitive impairment, as it changes the excitatory biochemistry of brain cells in a noninvasive way.

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