

## Safety of Anodal Transcranial Direct Current Stimulation with Respect to Blood-Brain Barrier Permeability in the Rat

NAR Nik-Mohd-Afizan<sup>1,2,#</sup>, Yumiko Watanabe<sup>1,#</sup>, Ichiro Takashima<sup>1</sup>

<sup>1</sup>Human Informatics Research Institute, National Institute of Advanced Industrial Science and Technology, 1-1-1 Umezono, Tsukuba, Japan

<sup>2</sup>Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

<sup>#</sup>These authors contributed equally to this work.

### Abstract

Transcranial direct current stimulation (tDCS) is currently used based on its potential to treat a wide range of neurological diseases. Although previous studies have demonstrated an intensity-dependent effect of anodal tDCS, few studies have evaluated the safety of this procedure regarding the permeability of the blood-brain barrier (BBB). Therefore, the present study aimed to determine the safety of anodal tDCS in terms of BBB permeability in rats because maintenance of BBB function during tDCS is particularly important to protect the brain from foreign substances, and to maintain a stable environment. For the present study, an electrode was directly fixed onto the cranium and anodal tDCS was applied using a constant current stimulator that delivered a 500  $\mu$ A current for 30 min. Possible BBB dysfunction was assessed by intravenously administering Evans blue dye and performing immunohistochemical analyses of the tight junction protein Claudin-5. Anodal tDCS did not affect BBB permeability or Claudin-5 expression levels, even under relatively high current stimulus conditions; specifically, 144.9 A/m<sup>2</sup> for 30 min (i.e., 260820 C/m<sup>2</sup>). Although further studies will be necessary, the present results indicate that anodal tDCS is relatively safe compared to cathodal tDCS.

**Keywords:** Electric stimulation, tDCS, permeability, Blood brain barrier, Claudin-5.

*Accepted June 13, 2017*

### Introduction

Transcranial direct current stimulation (tDCS) is a noninvasive procedure that is currently used to treat a wide range of neurological and psychiatric diseases, including post-stroke recovery, depression, and Parkinson's disease [1-3]. Essentially, tDCS utilizes a direct current with low intensity that is passed into the brain via scalp electrodes. Anodal (positive) stimulation is thought to facilitate the depolarization of neurons, while cathodal (negative) stimulation hyperpolarizes the resting membrane potential and reduces neuronal firing [4]. Therefore, previous studies have proposed anodal stimulation to the lesional hemisphere and/or cathodal stimulation to the contralateral non-lesional hemisphere for stroke rehabilitation, which is expected to normalize the post-stroke bihemispheric imbalance in transcallosal inhibition [5,6].

A polarity-specific effect of tDCS is a particularly important factor for clinical therapy. For example, it has been well documented that cathodal tDCS suppresses seizures in patients with epilepsy [7,8]. In addition, a study of patients with aphasia showed that cathodal tDCS over the

left frontotemporal areas improves picture naming accuracy, whereas anodal tDCS fails to induce any changes [9]. In contrast, one study found that anodal tDCS improves word recognition memory in patients with Alzheimer's disease, whereas cathodal tDCS worsens the performance [10]. Moreover, the efficacy of anodal tDCS delivered to primary motor areas has been demonstrated for improving motor function in patients with Parkinson's disease [3]. As recent studies have shown that anodal stimulation boosts synaptic plasticity [11,12], anodal tDCS is considered to be the more effective stimulation technique, compared to cathodal tDCS, when attempting to improve behavioral and cognitive function in patients with neurodegenerative disorders [3,13].

Notably, anodal tDCS has been shown to have intensity-dependent effects during cognitive tasks in both human and animal studies [13,14]. For example, Iyer et al. examined the effects on cognitive function of tDCS in healthy subjects [13] and found that verbal fluency significantly improved following anodal stimulation at 2 mA, while there were no significant effects after stimulation with 1 mA. Similarly, the effects of repetitive anodal tDCS were tested in a rat model of

Alzheimer's disease; significant changes in spatial learning and memory were seen under the strong stimulus condition [14]. Therefore, it appears that higher stimulus intensities result in greater improvements in human tDCS studies.

However, there are major concerns regarding the safety of tDCS, particularly under conditions of increased current intensity or prolonged stimulus duration. A previous study investigating the safety limits of cathodal tDCS in rats indicated that brain lesions develop at a current density of 142.9 A/m<sup>2</sup> with durations longer than 10 min [i.e., 85,740 C/m<sup>2</sup>]; [15]. However, to the best of our knowledge, few studies have investigated the safety of anodal tDCS. The application of anodal tDCS in a mouse ischemic stroke model induced augmented derangement of the blood-brain barrier (BBB) during the acute phase of stroke [16]. The BBB is a dynamic interface that separates the brain from circulating blood and usually protects the brain from potentially harmful foreign substances, including viruses, bacteria, and toxins, which are circulating in the blood by preventing their leakage from blood vessels. Therefore, it is important that BBB function remain as normal as possible during tDCS procedures, to protect the brain from these substances and to maintain a stable environment.

The present study investigated the safety of anodal tDCS from the perspective of BBB permeability. To achieve the maximum clinical effects of anodal tDCS stimulation in a safe manner, it will be important to understand the relationship between stimulation intensity and BBB permeability. Therefore, the present study was conducted to investigate whether relatively strong anodal tDCS applied to the normal brain would have an influence on BBB permeability.

## **Materials and Methods**

### ***Animals***

The present study included adult male Wistar rats (10-15 weeks) weighing 250-350 g (n = 30). We excluded female rats because cyclical reproductive hormones may regulate selective permeability of the BBB [17]. All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the National Institute of Advanced Industrial Science and Technology (AIST; Tsukuba, Japan). The rats were housed with ad libitum food and water and maintained on a 12-h light/dark cycle.

### ***Surgical Procedures and Electrode Implantation***

To apply the DCS, a screw with a defined contact area (3.45 mm<sup>2</sup>) was embedded into the intact skull of each rat during a surgical procedure that was modified from a previous study [15]. Prior to screw implantation, the rats were anesthetized with urethane (1.2 g/kg, intraperitoneal). Then, the scalp and underlying tissues were removed and the screw was implanted 1.5 mm to the right and 2 mm anterior of bregma. The screw was stopped halfway through the skull and the stimulus current was applied to the brain through the thinned skull and dura via the screw.

### ***DCS Application***

During the application of the direct current (anodal, cathodal, and sham), the rats were kept under urethane anesthesia; an adequate level of anesthesia was verified by checking the tail-pinch reflex. The counter electrode, which was composed of a large rubber plate electrode with a wet sponge pad, was placed onto the ventral thorax of the anesthetized rats and the DCS was continuously applied, at a current intensity of 500  $\mu$ A for 30 min, with a constant current stimulator (DC Stimulator plus, NeuroConn; Ilmenau, Germany). This current intensity corresponds to a charge density of 260,820 C/m<sup>2</sup>, which is strong enough to produce scars on the cortical surface during cathodal stimulation [15]. A higher current intensity was selected because the present study aimed to examine the effects of anodal stimulation on BBB permeability during relatively strong stimulation intensities. The current intensity was ramped up for 15s, rather than switching it on and off directly, to avoid the stimulation break effect [18]. For the sham stimulation, the electrodes were placed on the skull in the same manner but the stimulator was switched off [19].

### ***Evans Blue Dye Injection***

BBB permeability to macromolecules was assessed and quantified using Evans Blue dye [EBD, 961 Da, Sigma-Aldrich; St. Louis, MO, USA]; [20] that was prepared in a 2% solution using 0.9% saline. This solution (4 mL/kg) was slowly injected into the femoral vein immediately after the tDCS procedure; the femoral vein was cannulated prior to the tDCS to facilitate dye injection. The dye was allowed to circulate in the vasculature for 90 min prior to euthanization.

### ***Determination of BBB Permeability***

Following the experimental procedures, the rats were deeply anesthetized and transcardially perfused with a 0.9% saline solution. Next, the brains were removed and EBD extravasation into the brains was quantified to assess BBB permeability. Following decapitation, the brain tissues were weighed and homogenized in 500  $\mu$ L of 0.1 M phosphate-buffered saline (PBS) and 500  $\mu$ L of trichloroacetic acid (TCA, Sigma-Aldrich). The samples were incubated at 4°C for 1 h, centrifuged at 10,000 g for 30 min, and then the supernatants were diluted with 1:3 volume of 95% ethanol. Next, absorbance was measured by a 96-well plate reader with a wavelength range of 620-680 nm (Spectra MAX Gemini; Molecular Devices, Sunnyvale, CA, USA).

The total Evans blue content ( $\mu$ g) was determined according to an external standard curve using serial dilutions of the stock dye solution (1:2 dilution factor in a concentration range of 1.23-4.56  $\mu$ g/mL). EBD extravasation was analyzed with the following linear regression:  $Y = aX + b$ , where Y is the measured absorbance intensity and X is the concentration of the dye; the outputs are expressed as micrograms of EBD ( $\mu$ g)/milligram of tissue weight (mg). The differences in dye content between the anodal, cathodal, and sham samples reflected the EBD extravasation levels.

## Immunohistochemistry

Immunohistochemical staining was performed to evaluate the spatial distribution of the Claudin-5 protein after electrical stimulation. Briefly, the rats were transcardially perfused with ice cold 0.9% saline (pH 7.4) followed by ice cold 4% paraformaldehyde in PBS (pH 7.4). Next, the rat brains were immersed in the same solution for 30 min and then incubated overnight in a 30% sucrose solution. Subsequently, the brains were embedded in OCT compound (Tissue-Tek; Sakura Finetek USA, Inc., Torrance, CA, USA) on dry ice and stored at  $-80^{\circ}\text{C}$  until further use.

Frozen coronal sections (14  $\mu\text{m}$  thick) were prepared using a microtome. Next, the sections were pre-heated at  $37^{\circ}\text{C}$  for 30 min and then rinsed three times with PBS. The non-specific binding sites were blocked with 5% bovine serum albumin (BSA) in PBS at room temperature for 1 h and then the sections were incubated with mouse anti-Claudin-5 conjugated with Alexa Fluor 488 (1:100; Molecular Probes, Portland, OR, USA) in PBS containing 5% BSA at  $4^{\circ}\text{C}$  overnight. Subsequently, the samples were washed three times in PBS and mounted using mounting medium. Finally, the sections were photographed and observed under a fluorescence microscope (Biozero BZ-8000, Keyence; Osaka, Japan) and the captured images were analyzed with image densitometry using Image J software (National Institutes of Health, Bethesda, MD, USA).

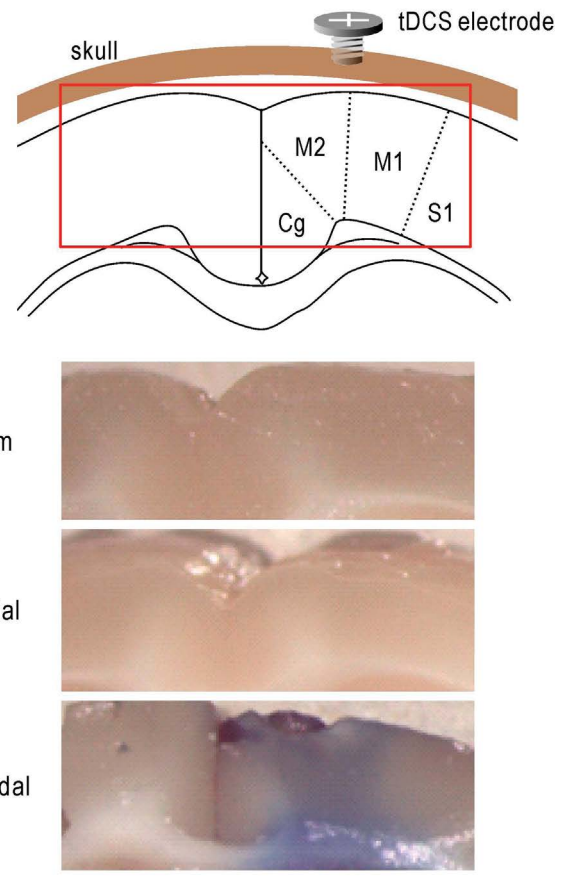
## Statistical Analysis

All quantitative data are expressed as means  $\pm$  standard error of the mean (SEM). Statistical comparisons were done with one-way analysis of variance (ANOVA) followed by a Tukey–Kramer post-hoc analysis for intergroup comparisons. P values  $< 0.05$  were considered to indicate statistical significance. All statistical analyses were performed with SPSS for Windows (ver. 11.5; SPSS Inc., Chicago, IL, USA).

## Results

Figure 1 illustrates EBD extravasation after the sham, anodal, and cathodal stimulations. Clear EBD leakage was detected in the cathodal condition. A visual inspection revealed that cathodal stimulation resulted in a focal brain lesion directly beneath the stimulation electrode. On the other hand, visible signs of lesions were not detected after the anodal or sham stimulations. Therefore, the extensive dye leakage under the cathodal condition seems to have resulted from the neuronal and vascular damage associated with the cathodic current.

Next, the absorbance signals corresponding to the amount of EBD in the brain were quantified. Figure 2A depicts the standard curve that was used to determine the concentration of albumin-EBD in the brain samples. The cathodal condition resulted in a significant increase in EBD concentration ( $12.51 \pm 1.004 \mu\text{g/g}$ ; Figure 2B), while the anodal condition resulted in very little EBD leakage ( $1.588 \pm 0.451 \mu\text{g/g}$ ) and did not significantly differ from the sham-stimulated condition ( $1.765 \pm 0.523 \mu\text{g/g}$ ). These results indicate that anodal stimulation



**Figure 1.** Coronal brain section showing the blue color of Evans Blue dye (EBD) in the cathodal stimulation condition. Sections were obtained from the cortex under the stimulating electrode. M1, primary motor cortex; M2, secondary motor cortex; S1, primary somatosensory cortex; Cg, cingulate cortex. Scale bar: 1.0 mm.

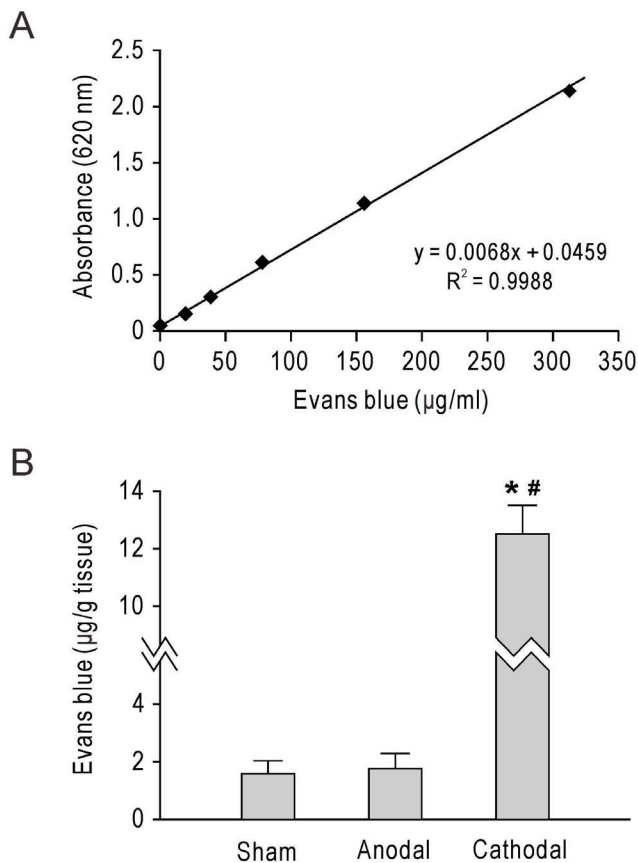
did not increase BBB permeability, even at current intensity levels that produce scars during cathodal stimulation.

Finally, the expression levels of Claudin-5 were examined in cryosections of brain tissue obtained after the tDCS stimulation. Immunofluorescent images of the cerebral vessels did not reveal significant differences in the expression of Claudin-5 between the sham- and anodal-stimulated groups (Figure 3). On the other hand, the percentage of Claudin-5-positive vessels exhibited a significant decrease in the cathodal-stimulated group.

## Discussion

The present study evaluated the safety of anodal tDCS in terms of BBB permeability in rats, and found that anodal tDCS did not affect BBB permeability, even at relatively high current stimulations; specifically,  $144.9 \text{ A/m}^2$  for 30 min (i.e.,  $260,820 \text{ C/m}^2$ ).

Several studies have examined the safety of tDCS in humans [13,21]. For example, Iyer et al. assessed the safety of tDCS in healthy subjects by applying stimulation at  $2 \text{ mA}$  ( $0.8 \text{ A/m}^2$ ) for 20 min [i.e.,  $960 \text{ C/m}^2$ ; 13]. These authors concluded that the limited exposure of the prefrontal cortex to tDCS is safe because there were no visually discernible

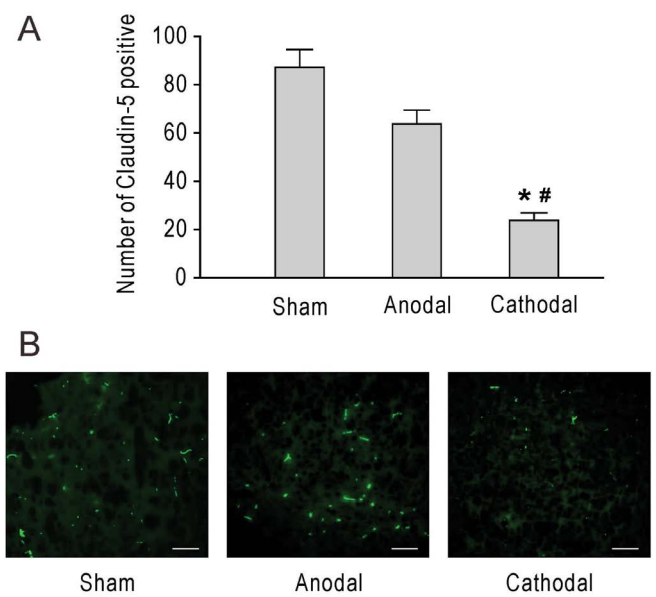


**Figure 2 (A&B):** A: Standard curve used to estimate the concentration of albumin-EBD in the brain samples. B: Quantification of EBD. Data are expressed as means  $\pm$  SEM ( $n = 5$ ). Group data were compared by one-way ANOVA and the Tukey–Kramer multiple comparison test. The cathodal condition resulted in a significant increase in EBD concentration compared with the control (sham) ( $*P < 0.05$ ); and compared with the anodal condition ( $\#P < 0.05$ ). EBD, Evans Blue dye; SEM, standard error of the mean; ANOVA, analysis of variance.

changes in simultaneous electroencephalography (EEG) recordings. According to a recent review [22], conventional tDCS protocols employ current intensities that range from 0.1 mA (as a sham) to 4.0 mA and the majority of studies apply intensities from 1.0-2.0 mA. Additionally, the conventional duration of stimulation is from 4 s to 40 min. Therefore, the conventional charge is typically limited to 7.2 C [e.g., 40 min and 3 mA]; [22]; that is, a stimulation corresponding to 2,057 C/m<sup>2</sup> when conventional tDCS electrodes (35 cm<sup>2</sup>) are used.

Much higher stimulation intensities have been applied in animal studies. For example, Liebetanz et al. estimated that the charge density for a lesion size that is theoretically zero would correspond to 52,400 C/m<sup>2</sup> for cathodal stimulation [15]. In the present study, anodal stimulation was applied at a charge density of 260,820 C/m<sup>2</sup>, which is approximately five times larger than the safety limit for cathodal stimulation, and there were no visible signs of cortical lesions or leakage of EBD extravasations.

On the other hand, Peruzzotti-Jametti et al. reported that the application of anodal tDCS in a mouse ischemic stroke model induced augmented derangement of the BBB during the acute phase of stroke [16]. Therefore, the present study



**Figure 3 (A&B):** Quantitative analysis of Claudin-5-positive cells per section (mean  $\pm$  SEM of seven different sections per animal,  $n = 4$  animals per group). The immunoreactivity of Claudin-5 decreased in the cathodal condition compared with the control (sham) ( $*P < 0.05$ ); and compared with the anodal condition ( $\#P < 0.05$ ). B: Representative images of Claudin-5 expression under each condition. SEM, standard error of the mean. Scale bar: 100  $\mu\text{m}$ .

attempted to elucidate the mechanisms underlying the effects of electrical stimulation, with a focus on BBB permeability. Tight junction, occludin, and claudin proteins represent the major components that comprise intercellular junctions between cells. Of these proteins, the expression levels of Claudin-5 were examined in the present study because this protein is prominent at several junctions between apical epithelial cells and goblet cells in the BBB macroenvironment, and the up- and downregulation of Claudin-5 are the main determinants of tight junction properties in the BBB [23]. The immunohistochemical analyses in the present study revealed that Claudin-5 levels were not affected by anodal stimulation (Figure 3), which is in contrast to the findings of Peruzzotti-Jametti et al. [16]. This discrepancy may be due to the fact that anodal tDCS was applied to normal healthy brains in the present study, rather than to injured brains as in the previous study. When anodal tDCS is applied to damaged brains, the safety limit would likely be lower than that observed in the present study.

The present study implies that when anodal tDCS is applied at high stimulus intensities there will be more prominent effects. Anodal tDCS has already shown benefits for patients with neurodegenerative and neuropsychiatric disorders [1-3,10]; however, the efficacy and effect size of tDCS is occasionally less clear [24-27]. In addition, current tDCS treatment protocols can only induce acute and short-duration beneficial effects on cognitive function [28]. Therefore, an increase in current dose may produce reliable and long-term stable outcomes from tDCS treatments. The results of the present study show that the integrity of BBB function under a relatively large anodal tDCS current dose is guaranteed, highlighting the

importance of systematic dose-finding research for anodal tDCS therapy. We believe that a higher stimulus current could be delivered to targeted brain regions in humans to widen the range of applicable diseases for anodal tDCS. For example, use of a ring electrode may be promising to effectively produce focal electrical fields in the brain, with a minimum increase in stimulus current [29].

Nevertheless, we should be very careful when increasing anodal tDCS current intensity in clinical settings. Some studies have reported that tDCS produces adverse outcomes, such as pain, headache, itching, and a burning sensation [30-32]. Furthermore, high currents from anodal tDCS can increase the risk of mood changes, even if the BBB integrity is maintained, inducing epilepsy and influencing brain stem activity [33]. Therefore, further studies employing healthy and diseased animal models, as well as human clinical trials, will be necessary to determine how to attain the maximum benefits of this treatment, while still ensuring the safety of its recipients.

## Conclusion

The present study investigated the safety of anodal tDCS in terms of BBB permeability using rats. The results showed that anodal tDCS did not affect BBB permeability or the expression levels of Claudin-5, even at relatively high current stimulations that should not be used for cathodal stimulation. Although further studies are needed, the present findings suggest that anodal tDCS can be used at higher stimulation intensities than cathodal tDCS to obtain the maximum benefits of treatment.

## Acknowledgements

We thank Drs. Hidetoshi Inagaki, Motomichi Doi for their helpful advice on absorbance measurement. We also thank Dr. Noriyuki Higo and Ms. Yoko Sugiyama for histology staining. This work was supported by a Grant-in-Aid for Scientific Research to I.T. (15K12780, 17H01810) and Y.W. (24730641, 16K04443) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the New Energy and Industrial Technology Development Organization (NEDO). Part of this work was also supported by a continuous professional development (CPD) of the Malaysian Ministry of Higher Education (MOHE).

## References

1. Feng WW, Bowden MG, Kautz S. Review of transcranial direct current stimulation in poststroke recovery. *Top Stroke Rehabil* 2013; 20: 68-77.
2. Kuo MF, Paulus W, Nitsche MA. Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *Neuroimage* 2014; 85: 948-960.
3. Broeder S, Nackaerts E, Heremans E, et al. Transcranial direct current stimulation in Parkinson's disease: Neurophysiological mechanisms and behavioral effects. *Neurosci Biobehav Rev* 2015; 57: 105-117.

4. Medeiros LF, de Souza IC, Vidor LP, et al. Neurobiological effects of transcranial direct current stimulation: A review. *Front Psychiatry* 2012; 3: 110.
5. Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol* 2006; 5: 708-712.
6. Nair DG, Renga V, Lindenberg R, et al. Optimizing recovery potential through simultaneous occupational therapy and non-invasive brain-stimulation using tDCS. *Restor Neurol Neurosci* 2011; 29: 411-420.
7. Yook SW, Park SH, Seo JH, et al. Suppression of seizure by cathodal transcranial direct current stimulation in an epileptic patient - a case report -. *Ann Rehabil Med* 2011; 35: 579-582.
8. Varga ET, Terney D, Atkins MD et al. Transcranial direct current stimulation in refractory continuous spikes and waves during slow sleep: a controlled study. *Epilepsy Res* 2011; 97: 142-145.
9. Monti A, Cogiamanian F, Marceglia S, et al. Improved naming after transcranial direct current stimulation in aphasia. *Neurol Neurosurg Psychiatry* 2008; 79: 451-453.
10. Ferrucci R, Mameli F, Guidi I, et al. Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology* 2008; 71: 493-498.
11. Rohan JG, Carhuatanta KA, McInturf SM, et al. Modulating Hippocampal Plasticity with In Vivo Brain Stimulation. *J Neurosci* 2015; 35: 12824-12832.
12. Podda MV, Cocco S, Mastrodonato A, et al. Anodal transcranial direct current stimulation boosts synaptic plasticity and memory in mice via epigenetic regulation of Bdnf expression. *Sci Rep* 2016; 6: 22180.
13. Iyer MB, Mattu U, Grafman J, et al. Safety and cognitive effect of frontal DC brain polarization in healthy individuals. *Neurology* 2005; 64: 872-875.
14. Yu X, Li Y, Wen H, et al. Intensity-dependent effects of repetitive anodal transcranial direct current stimulation on learning and memory in a rat model of Alzheimer's disease. *Neurobiol Learn Mem* 2015; 123: 168-178.
15. Liebetanz D, Koch R, Mayenfels S, et al. Safety limits of cathodal transcranial direct current stimulation in rats. *Clin Neurophysiol* 2009; 120: 1161-1167.
16. Peruzzotti-Jametti L, Cambiaghi M, Bacigaluppi M, et al. Safety and efficacy of transcranial direct current stimulation in acute experimental ischemic stroke. *Stroke* 2013; 44: 3166-3174.
17. Wilson AC, Clemente L, Liu T, et al. Reproductive hormones regulate the selective permeability of the blood-brain barrier. *Biochim Biophys Acta* 2008; 1782: 401-407.
18. Wachter D, Wrede A, Schulz-Schaeffer W, et al. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. *Exp Neurol* 2011; 227: 322-327.

19. Fregni F, Liebetanz D, Monte-Silva KK, et al. Effects of transcranial direct current stimulation coupled with repetitive electrical stimulation on cortical spreading depression. *Exp Neurol* 2007; 204: 462-466.
20. Yen LF, Wei VC, Kuo EY, et al. Distinct patterns of cerebral extravasation by Evans blue and sodium fluorescein in rats. *PLoS One* 2013; 8: e68595.
21. Nitsche MA, Niehaus L, Hoffmann KT, et al. MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. *Clin Neurophysiol* 2004; 115: 2419-2423.
22. Bikson M, Grossman P, Thomas C, et al. Safety of transcranial direct current stimulation: Evidence based update 2016. *Brain Stimul* 2016; 9: 641-661.
23. Wallez Y, Huber P. Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis. *Biochim Biophys Acta* 2008; 1778: 794-809.
24. Tremblay S, Lepage JF, Latulipe-Loiselle A, et al. The uncertain outcome of prefrontal tDCS. *Brain Stimul* 2014; 7: 773-783.
25. Kuo MF, Paulus W, Nitsche MA. Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *Neuroimage* 2014; 85: 948-960.
26. Gonsalvez I, Baror R, Fried P, et al. Therapeutic noninvasive brain stimulation in Alzheimer's disease. *Curr Alzheimer Res* 2017; 14: 362-376.
27. Bystad M, Grønli O, Rasmussen ID, et al. Transcranial direct current stimulation as a memory enhancer in patients with Alzheimer's disease: a randomized, placebo-controlled trial. *Alzheimers Res Ther* 2016; 8: 13.
28. Hsu WY, Ku Y, Zanto TP, et al. Effects of noninvasive brain stimulation on cognitive function in healthy aging and Alzheimer's disease: a systematic review and meta-analysis. *Neurobiol Aging* 2015; 36: 2348-2359.
29. Datta A, Bansal V, Diaz J, et al. Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimul* 2009; 2: 201-207.
30. Furubayashi T, Terao Y, Arai N, et al. Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. *Exp Brain Res* 2008; 185: 279-286.
31. Brunoni AR, Amadera J, Berbel B, et al. A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int J Neuropsychopharmacol* 2011; 14: 1133-1145.
32. Matsumoto H, Ugawa Y. Adverse events of tDCS and tACS: A review. *Clin Neurophysiol Pract* 2017; 2: 19-25.
33. Redfearn JW, Lippold OC, Costain R. A preliminary account of the clinical effects of polarizing the brain in certain psychiatric disorders. *Br J Psychiatry* 1964; 110: 773-785.

**Correspondence to:**

Ichiro Takashima,  
Human Informatics Research Institute,  
National Institute of Advanced Industrial Science and  
Technology (AIST)  
1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan  
E-mail: i.takashima@aist.go.jp