Cathodal transcranial direct current stimulation over the Cz increases joint flexibility

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Abstract

Joint flexibility depends on both mechanical and neural factors. However, the contribution of neural factors is not fully understood. To test the hypothesis that the sensorimotor cortex is involved in joint flexibility, we investigated whether transcranial direct current stimulation (tDCS) over the Cz modifies ankle and wrist flexibility in healthy human participants. In eight male participants, range of motion of the left ankle and wrist were measured during a passive-dorsiflexion test. We also assessed passive torque, which represents involuntary resistance to dorsiflexion at the ankle. Participants performed passive-dorsiflexion tests before and after anodal, cathodal, and sham tDCS over the Cz. The current was applied for 10 min with an intensity of 2.0 mA during anodal and cathodal tDCS. Cathodal tDCS resulted in a 10.5% increase in range of motion of the ankle, but no significant increase in range of motion of the wrist. Neither anodal nor sham tDCS had a significant effect. Cathodal tDCS over the Cz may have affected neural factors, such as perception of joint angle or pain, because the passive torque at 0°, 5°, 10°, and 15°, which indicates mechanical effects, did not change. These results suggest that the sensorimotor cortex is involved in joint flexibility.

Keywords

joint flexibility; cerebral cortex; tDCS; neuromodulation; pain perception; range of motion; passive torque

1. Introduction

Flexibility is one of the components of fitness that is thought to be associated with exercise performance (Wilson et al., 1992) and incidence of muscular injury (Wilson et al., 1991). Flexibility is commonly evaluated by assessing joint range of motion (ROM), which depends on both mechanical and neural factors (Avela et al., 1999; Behm et al., 2013; Evetovich et al., 2003; Guissard and Duchateau, 2004, 2006; Guissard et al., 2001; Magnusson et al., 1996; Mizuno et al., 2013a, b; Morse et al., 2008; Wilson et al., 1992). While many studies of the mechanical factors have concluded that the muscle-tendon unit is important for joint ROM (Evetovich et al., 2003; Magnusson et al., 1996; Mizuno et al., 2013a, b; Morse et al., 2008; Wilson et al., 1992), the neural factors that affect joint ROM have not been fully investigated. For instance, it has been demonstrated that stretch tolerance, which means tolerance to stretching-induced pain, is one of the important limiting factors that affect the increase in joint ROM after static stretching (Magnusson et al., 1996, Mizuno et al., 2013a, Mizuno et al., 2013b). A previous study has reported that the increase in joint ROM immediately after and 5 min after static stretching for 5 min was due to both changes in mechanical factors related to the muscle-tendon unit and increased stretch tolerance, while the increase in joint ROM 10 min and 15 min after stretching was due to increased stretch tolerance alone (Mizuno et al., 2013a). It has also

been suggested that stretch tolerance might be related to the central nervous system (Magnusson, 1998), but the mechanism of stretch tolerance is still unclear. Furthermore, although a few studies have demonstrated the effect of spinal excitability on joint ROM, as measured using the H-reflex or tendon reflex (Avela et al., 1999; Behm et al., 2013; Guissard and Duchateau, 2004; Guissard et al., 2001), the contribution of the cerebral cortex to joint ROM remains unknown. However, because the cerebral cortex is involved in proprioception (Lephart et al., 1998) and some imaging studies have reported that the primary somatosensory cortex (S1) is involved in both pain perception and limb movement (Antal et al., 2008; Bingel et al., 2004; Bushnell et al., 1999; Dobkin et al., 2004; Francis et al., 2009; MacIntosh et al., 2004; Peyron et al., 2000; Porro et al., 2002), the excitability of the cerebral cortex associated with proprioception and cognitive function may also affect joint ROM.

Recently, transcranial direct current stimulation (tDCS), a non-invasive neuromodulation technique, has been used to modify cortical excitability (Nitsche and Paulus, 2000; Priori et al., 1998). tDCS modulates regional brain activity by altering the membrane potential of neurons (Liebetanz et al., 2002; Nitsche and Paulus, 2000). Furthermore, tDCS can increase or decrease cortical excitability in a polarity-dependent manner (Boggio et al., 2008; Fregni et al., 2006a; Fregni et al., 2006b; Jeffery et al., 2007;

Matsunaga et al., 2004; Nitsche and Paulus, 2000; Tanaka et al., 2009); that is, anodal tDCS can enhance cortical excitability, while cathodal tDCS can diminish it. Polarity-dependent changes in cortical excitability induced by tDCS are mediated by the activity of sodium and calcium ion channels in the neuronal membrane, and by the effectivity of receptors for N-methyl-D-aspartate-neurotransmitters (Liebetanz et al., 2002; Nitsche et al., 2003). Using this technique, Antal et al. (2008) demonstrated that cathodal tDCS over S1 decreased laser-stimulated pain perception. A recent meta-analysis also reported that anodal tDCS over the primary motor cortex (M1) increased sensory and pain thresholds, while anodal tDCS over S1 increased pain threshold (Vaseghi et al., 2014). These studies have suggested that modulation of S1 and/or M1 is related to pain perception (Antal et al., 2008; Vaseghi et al., 2014), although the effect of polarity remains controversial.

As mentioned above, cognitive function may affect joint ROM, especially considering that pain perception is a limiting factor for joint flexibility (Mizuno et al., 2013a, Mizuno et al., 2013b). Therefore, we predicted that tDCS stimulation to the S1 and/or M1 would modulate joint ROM as a result of modulating pain perception. Thus, in this study, to test the hypothesis that the sensorimotor cortex is involved in joint flexibility, we examined whether the application of tDCS over the sensorimotor foot area could modify ankle ROM in healthy participants. The position of the sensorimotor foot

area corresponds to the Cz, in reference to a previous study (Marshall et al., 2013). We positioned the electrode over the Cz to stimulate the foot sensorimotor cortex.

2. Materials and Methods

2.1. Participants

Ten healthy men volunteered for the study, and the final cohort consisted of eight men (mean ± SD; age, 25 ± 3 years; height, 170.8 ± 2.9 cm; weight, 65.3 ± 5.0 kg). Two participants were excluded based on the results of the Smirnov–Grubbs rejection test ($p < 0.01$) because the coefficient of variance (CV) and the value range for ankle ROM, wrist ROM, or passive torque at maximal dorsiflexion angle during pre-stimulation over a 3-trial period were too high [Subject 1: CV for ankle ROM, outlier (34.7), overall (10.4 ± 9.9); Subject 2: range for passive torque at maximal dorsiflexion angle, outlier (17.4 Nm), overall (4.9 ± 5.2 Nm)]. The results of tDCS for the outliers are summarized in the supplementary table. All participants had specifically studied sports science in graduate school. These men were right-leg-dominant, and none had a history of recent musculoskeletal injury or neuromuscular disease specific to the lower limb. They also had no history or current signs or symptoms of neurological or psychiatric disorders. The participants provided written informed consent for their participation in the experiments, which were conducted according to the principles of the Declaration of Helsinki. All participants were fully informed of the purposes, procedures, and possible risks of the study. The experimental protocol was approved by the Human Subjects Committee at

Chukyo University Graduate School of Health and Sports Sciences.

2.2. Experimental design

The present study was conducted as a single-center trial, as all participants were evaluated at a single study location. The experiment was designed as a double-blind trial. The order of tDCS stimulation was randomized, and the experimental design also contained sham stimulation as a control trial. All subjects repeatedly participated in anodal tDCS, cathodal tDCS, and sham tDCS experiments.

The participants visited the laboratory on four occasions, and the visits were separated by at least 24 h to prevent interference effects, based on a previous study that indicated that the effect of anodal tDCS at 2 mA for 10 min diminished 60 min after stimulation (Tanaka et al. 2009). The first visit involved a familiarization trial, and the subsequent three visits included the following experimental conditions: a) anodal tDCS, b) cathodal tDCS, and c) sham tDCS. During the familiarization trial, each participant practiced the passive-dorsiflexion test to minimize any potential learning effects and to adjust to the procedures. During the experimental trials, the participants underwent a pre-stimulation passive-dorsiflexion test, followed by one of the three types of stimulation, and a post-stimulation passive-dorsiflexion test (Fig. 1a). The post-stimulation passive-

dorsiflexion test was performed immediately after 10 min-tDCS. During the passive-dorsiflexion test, we measured passive torque (i.e., involuntary resistance to passive dorsiflexion) and ROM of the ankle and wrist. During the passive-dorsiflexion test, we measured passive torque (i.e., involuntary resistance to passive dorsiflexion) and ROM of the ankle and wrist. Before the pre-stimulation passive-dorsiflexion test, participants walked on a treadmill at 100 m/min for 5 min as a warm-up.

2.3. Passive-dorsiflexion test

To determine passive torque and joint ROM, each participant underwent a passive-dorsiflexion test. The passive-dorsiflexion test was performed using an approach similar to that described in previous studies (Mizuno et al., 2013a, Mizuno et al., 2013b, Morse et al., 2008). To assess ankle ROM, participants were secured to an isokinetic machine (Biodex System 3, Biodex, NY, USA) with their knee in full extension and the footplate fixed to their left foot. The lateral malleolus was aligned with the axis of the dynamometer. In this study, all reported ankle angles reflect the angle of the footplate, and the ankle angle was defined as 0° when the footplate was perpendicular to the floor. To assess wrist ROM, the participants were secured to an isokinetic machine (Biodex System 3, Biodex, NY, USA) with their left elbow flexed 90° and the grip held in their

left hand. The styloid process of the ulna was aligned with the axis of the dynamometer. All reported wrist angles reflected the angle of the wrist attachment, and the wrist angle was defined as 0° when the wrist attachment was parallel to the floor. Values were defined as positive for dorsiflexion of the ankle and the wrist. The foot and wrist of the participant were passively and isokinetically dorsiflexed at a speed of 1°/s from −30° for the foot and from 0° for the wrist, to the angle at which the participant felt discomfort and stopped the dynamometer by activating a safety trigger (Fig. 1b). To prevent reflex contraction due to pain, we defined the angle at which the participant subjectively felt discomfort as the maximal dorsiflexion angle. The maximal angle of the footplate or the wrist attachment was defined as the joint ROM. During this test, the passive torques generated on the footplate were measured when the ankle was submaximally dorsiflexed (i.e., 0°, 5°, 10°, and 15°) and at maximal dorsiflexion.

To correct for the effects of the weight of the attachment and the lower limb on torque, all measurements of passive torque were gravity corrected. Gravity correction was performed at −30° because passive torque around the ankle has been shown to be near zero at this angle (Kawakami et al., 1998). Therefore, to correct for gravity, we set the passive torque at zero at 30° of plantarflexion. Throughout the passive-dorsiflexion test, the participants were asked to completely relax, not offer any voluntary resistance, and to

wear an eye mask to eliminate any visual input from the immediate environment that might otherwise have provided them with a reference point for the joint ROM (Magnusson et al., 1998). Passive dorsiflexion of the ankle and the wrist was performed twice during the pre-stimulation passive-dorsiflexion test, but only once during the post-stimulation passive-dorsiflexion test. The trial in which the subject attained the greatest ankle ROM, during passive dorsiflexion for the pre-stimulation passive-dorsiflexion test, was used in subsequent analysis. Passive torque and ankle angle were converted from analog to digital at a sampling rate of 1.5 kHz (LX-10, TEAC, Tokyo, Japan). The order of ankle and wrist passive-dorsiflexion tests was counterbalanced among participants. When the first test was finished, the other assessment was started as soon as possible.

2.4. tDCS

tDCS was delivered by a DC Stimulator Plus (Neuroconn, Ilmenau, Germany), which is a battery-driven, constant current stimulator, using a pair of 5 × 7 cm sponge surface electrodes soaked in 0.9% NaCl. Prior to stimulation, the skin of each participant's scalp was cleaned carefully to reduce skin impedance and itching. One electrode was positioned over the Cz by using the international 10/20 system and the other electrode was placed on the center of the forehead. Current was applied for 10 min at an intensity

of 2.0 mA. According to previous studies, these parameters for tDCS sufficiently affect the leg area of the cerebral cortex (Jeffery et al., 2007; Tanaka et al., 2009). The type of stimulation (anodal or cathodal) refers to the polarity of the electrode over the Cz. For sham tDCS, the electrodes were placed in the same position as for active stimulation, but the current was turned off after 30 s. The order of the stimulation type was counterbalanced among participants.

2.5. Data reliability

To test measurement reliability, we performed an intraclass correlation coefficient (ICC) analysis using the simple replication reliability model (Fleiss, 1986) for the pre-tDCS measurement data. ICC analysis can be used to evaluate the agreement of repeated measures using between-subject variance (σ^2_{BS}) and within-subject variance(σ^2_{ws}). The ICC is calculated as, ICC = σ^2_{BS} / (σ^2_{BS} + σ^2_{ws}). This means that the smaller the within-subject variance(σ^2_{ws}) is, the more the ICC index approaches 1. Generally, an ICC value above 0.81 is regarded as almost perfect agreement between measurement values. To evaluate the day-to-day agreement for each measurement item, ICCs were calculated using the higher values from the two pre-tDCS measurements on three different days. The ICC values were 0.946, 0.924, and 0.935 for ankle ROM, wrist ROM,

and passive torque at the maximal dorsiflexion angle of the ankle, respectively.

2.6. Data analysis

To determine the interactions between time and the type of stimulation, the ROM of the ankle and wrist, and the passive torque at the maximal dorsiflexion angle of the ankle were assessed by using two-way repeated measures ANOVAs (time [pre- or post-stimulation] × type of stimulation [anodal, cathodal, or sham]). To determine the interactions between time, type of stimulation, and angle, the submaximal passive torque was assessed by using a three-way repeated measures ANOVA (time [pre- or post-stimulation] × type of stimulation [anodal, cathodal, or sham] × angle [0°, 5°, 10°, or 15°]). When appropriate, follow-up analyses were performed by using t-tests with Bonferroni corrections. Differences were considered statistically significant if $p \leq 0.05$. All data are reported as means ± SD; however, means ± SEM are used in the figures for the purpose of presentation.

3. Results

3.1. Ankle ROM

There was a significant two-way interaction between time and type of stimulation [$F_{(2, 14)} = 4.508$, $p = 0.031$, $\eta_p^2 = 0.392$]. *Post hoc* testing revealed that cathodal tDCS increased ankle ROM (Pre: 26.5 ± 7.7°, Post: 29.4 ± 8.1°, t = -3.48, df = 7, $p = 0.010$). However, no significant differences in ankle ROM were detected with anodal (Pre: 26.7 ± 7.4°, Post: 26.0 ± 8.3°, t = 0.86, df = 7, $p = 0.417$) or sham tDCS (Pre: 25.4 ± 7.6°, Post: 25.2 ± 7.2°, t = 0.15, df = 7, $p = 0.886$; Fig. 2a). Fig. 2b shows that all subjects demonstrated an increase in ankle ROM following cathodal tDCS (range: 1.0-26.2%).

3.2. Wrist ROM

There was no significant two-way interaction between time and type of stimulation [$F_{(2, 14)} = 3.136$, $p = 0.075$, $\eta_p^2 = 0.309$], and no significant main effects were detected for time [$F_{(1, 7)} = 3.315$, $p = 0.111$, $\eta_p^2 = 0.321$] or stimulation type [$F_{(2, 14)} = 1.395$, $p = 0.280$, $\eta_p^2 = 0.166$]. Pre- and post-stimulation wrist ROM did not significantly differ regardless of the stimulation type (anode: Pre 105.8 ± 16.2°, Post 100.5 ± 18.6°; cathode: Pre 103.6 ± 15.4°, Post 106.1 ± 17.8°; sham: Pre 103.4 ± 15.8°, Post 98.8 ±

17.8°; Fig. 3).

3.3. Passive torque at the maximal dorsiflexion angle of the ankle

There was no significant two-way interaction between time and type of stimulation [$F_{(2, 14)} = 2.983$, $p = 0.083$, $\eta_p^2 = 0.299$] and no significant main effects were detected for time [$F_{(1, 7)} = 0.984$, $p = 0.354$, $\eta_p^2 = 0.123$] or stimulation type [$F_{(2, 14)} = 0.991$, $p = 0.396$, $\eta_p^2 = 0.124$]. Passive torque at the maximal dorsiflexion angle did not change from pre- to post-stimulation, regardless of the stimulation type (anode: Pre 28.1 ± 9.5 Nm, Post 27.7 ± 11.0 Nm; cathode: Pre 27.3 ± 7.8 Nm, Post 31.3 ± 9.5 Nm; sham: Pre 27.5 ± 8.0 Nm, Post 27.8 ± 9.3 Nm; Fig. 4).

3.4. Submaximal passive torque of the ankle

For submaximal passive torque of the ankle, there were no significant three-way interactions among time, type of stimulation, and angle [$F_{(2.514, 17.599)} = 0.170$, $p = 0.887$, $\eta_p^2 = 0.024$] and there were no significant two-way interactions between time and type of stimulation [$F_{(2, 14)} = 0.389$, $p = 0.685$, $\eta_p^2 = 0.053$] or type of stimulation and angle [$F_{(1.526, 10.680)} = 2.200$, $p = 0.164$, $\eta_p^2 = 0.239$]. However, the interaction between time and

angle was significant [$F_{(3, 21)} = 4.132$, $p = 0.019$, $\eta_p^2 = 0.371$]. The statistical model was decomposed by using paired-samples *t*-tests with Bonferroni corrections (pre- vs. post-stimulation and 0° vs. 5° vs. 10° vs. 15° of ankle angle). *Post hoc* analyses revealed that submaximal passive torque increased with increasing ankle angle both pre- and post-stimulation (Pre: 0° vs. 5°, t = -6.76, df = 7, $p = 0.002$; 0° vs. 10°, t = -8.46, df = 7, $p < 0.001$; 0° vs. 15°, t = -7.50, df = 7, $p = 0.001$; 5° vs. 10°, t = -9.42, df = 7, $p < 0.001$; 5° vs. 15°, t = -7.62, df = 7, $p = 0.001$; and 10° vs. 15°, t = -6.55, df = 7, $p = 0.002$. Post: 0° vs. 5°, t = -7.44, df = 7, $p = 0.001$; 0° vs. 10°, t = -8.40, df = 7, $p < 0.001$; 0° vs. 15°, t = -7.44, df = 7, $p = 0.001$; 5° vs. 10°, t = -8.99, df = 7, $p < 0.001$; 5° vs. 15°, t = -7.40, df = 7, $p = 0.001$; and 10° vs. 15°, t = -6.42, df = 7, $p = 0.002$; Table 1).

4. Discussion

We examined whether the application of tDCS over the Cz can modify joint ROM in healthy participants to determine if the cerebral cortex plays a role in joint flexibility. The main findings of our investigation were that cathodal stimulation of the Cz significantly increased ankle ROM, whereas anodal and sham stimulation had no effect. Our results suggest that the sensorimotor cortex plays an important role in joint flexibility.

4.1. The mechanism of the increase in ankle ROM by cathodal tDCS

Although joint ROM depends on both mechanical and neural factors (Avela et al., 1999; Behm et al., 2013; Evetovich et al., 2003; Guissard and Duchateau, 2004, 2006; Guissard et al., 2001; Magnusson et al., 1996; Mizuno et al., 2013a, b; Morse et al., 2008; Wilson et al., 1992), the increase in ankle ROM by cathodal tDCS observed in our study (Fig. 2) was likely due to changes in neural factors, rather than mechanical factors. The submaximal passive torque of the ankle, which was used as an indicator of the mechanical effects of the muscle-tendon unit (Mizuno et al., 2013a; Mizuno et al., 2013b), did not change with tDCS (Table 1), suggesting that the mechanical properties of the ankle joint

in submaximal dorsiflexion did not change by cathodal tDCS.

Moreover, to show that the mechanical properties of the ankle joint did not change by tDCS in either the submaximal range or at the maximal dorsiflexion angle, we additionally investigated the change in passive torque by cathodal tDCS at a point close to the maximal dorsiflexion angle. Since ROM of the ankle was increased by cathodal tDCS in all subjects, we compared the passive torque at the maximal dorsiflexion angle in the pre-cathodal tDCS trial to the torque at the same angle in the post-cathodal tDCS trial. The result of the additional analysis revealed that cathodal tDCS created no change in the passive torque at a point close to the maximal dorsiflexion angle (from 27.3 ± 7.8 Nm to 26.9 ± 8.2 Nm, t = 0.48, df = 7, p = 0.65), suggesting that the cathodal tDCS did not change the mechanical properties of the ankle joint even at a point approaching the maximal dorsiflexion angle.

These results suggest that cathodal tDCS altered the excitability of the cerebral cortex, not the mechanical properties around the ankle joint, and then ankle joint flexibility increased.

4.2. The effect of cathodal tDCS on pain perception and proprioception

The increase in ankle ROM (Fig. 2) was likely due to decreased pain perception and/or sensory perception at the ankle, considering that the participants stopped passive dorsiflexion at the same level of perceived discomfort during the pre- and post-tDCS tests for passive-dorsiflexion.

One possibility is that the change in pain perception was caused by decreased sensorimotor cortex excitability after cathodal tDCS. Several imaging studies using functional magnetic resonance imaging (fMRI) have substantiated the involvement of S1 in pain perception (Bingel et al., 2004; Bushnell et al., 1999; Forss et al., 2005; Peyron et al., 2000; Porro et al., 2002). Peyron et al. (2000) reported that 15 out of 24 imaging studies showed significant S1 activation after painful stimulation, and specific nociceptive neurons are known to exist in S1 (Kenshalo and Isensee, 1983). Furthermore, Antal et al. (2008) showed that cathodal tDCS over S1 decreased laser-stimulated subjective pain perception of the hand, whereas anodal and sham tDCS had no effect. Their results indicated that cathodal tDCS over S1 decreased subjective pain perception. These prior results also suggest that the increase in ankle ROM observed in our study may be based on decreased pain perception secondary to decreased excitability of the cerebral cortex, which was caused by cathodal tDCS over the Cz.

Another possibility is that a change in proprioception was caused by decreased

sensorimotor cortex excitability after cathodal tDCS. It has been suggested that proprioception in the lower limb is mediated primarily by the central nervous system (Lephart et al., 1998). It has also been shown, by using fMRI, that active, passive, and electrically-stimulated ankle dorsiflexion activates several cortical regions, including the S1 region (Dobkin et al., 2004; Francis et al., 2009). Activation of the primary motor/primary somatosensory areas also appears to be significantly greater during large-amplitude ankle dorsiflexion compared to small-amplitude dorsiflexion (MacIntosh et al., 2004). This combination of evidence suggests that the excitability of the sensorimotor cortex is associated with a change in proprioception.

However, it is unclear whether the increase in ankle ROM after cathodal tDCS is based on altered pain perception and/or ankle proprioception because we did not directly and individually assess pain perception and sensory perception in relation to joint angle in this study. Additional studies are necessary to explore the effects of tDCS on pain and sensory perception in relation to joint angle.

4.3. Limitations

The present study has methodological limitations. First, it is impossible to stimulate a local brain region because the tDCS electrode (35 cm^2) is comparatively large.

Thus, we potentially stimulated part of the premotor cortex and the somatosensory association cortex, which is anterior and posterior to S1 and M1. Moreover, we may have induced effects in distant brain areas via a connectivity-driven network effect (Boros et al., 2008; Nitsche et al., 2005). Therefore, it is possible that we potentially changed pain perception and/or proprioception via the stimulation of other cortical areas in addition to the foot sensorimotor cortex. In this study, however, it is probable that we primarily stimulated the sensorimotor foot area, because the position of the sensorimotor foot area corresponds to the Cz according to a previous study (Marshall et al., 2013). It should also be noted that we observed a significant effect with tDCS only on the ankle and not on the wrist. Since we positioned the electrode on Cz to stimulate the foot area of the sensorimotor cortex, the stimulus would not reach the hand area by the tDCS parameters (2 mA, 10 min) used in this study. This result is consistent with the findings of a previous study in which anodal tDCS over the leg M1 area increased leg pinch force without altering the performance of a hand motor task (Tanaka et al., 2009).

Second, eight subjects would not be sufficient to cancel the effect of stimulation order. However, because all subjects demonstrated an increase in ankle ROM after cathodal tDCS, we suggest that the increase in ankle ROM after cathodal tDCS was not related to the order of stimulation.

5. Conclusions

In conclusion, we demonstrated that cathodal tDCS over the Cz for 10 min at 2 mA increased ankle joint ROM, whereas anodal and sham tDCS had no effect. As the Cz corresponds to the sensorimotor cortex (Marshall et al., 2013), this finding suggests that the sensorimotor cortex is involved in joint flexibility.

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Author contributions

Y. A. conceived and designed the experiments. T. M. designed and performed the experiments. Both authors discussed and interpreted results, and both authors wrote the manuscript. We have read and abided by the statement of ethical standards for manuscripts submitted to Neuroscience Research.

Competing financial interests

The authors declare no conflicts of interest.

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Figure Legends

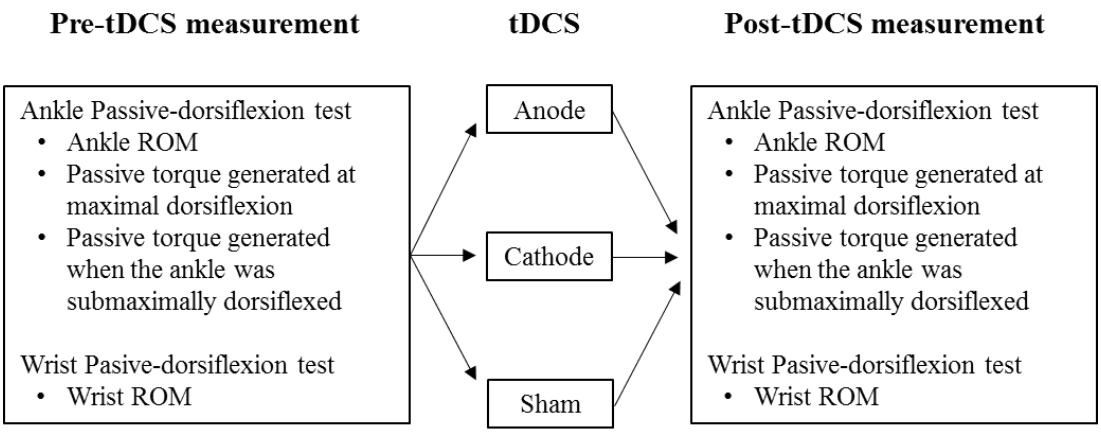
Figure 1: A schematic representation a) The experimental design. Range of motion (ROM) of the left ankle and wrist, and passive torque of the left ankle are measured during a passive-dorsiflexion test. All subjects participate repeatedly in anodal transcranial direct current stimulation (tDCS), cathodal tDCS, and sham tDCS experiments. b) Picture of the passive-dorsiflexion test of the ankle joint. The foot of the participant is passively and isokinetically dorsiflexed at a speed of 1°/s from 30° of plantarflexion to the angle at which the participant feels discomfort and stops the dynamometer by activating a safety trigger.

Figure 2: Ankle joint range of motion (ROM) before and after each type of transcranial direct current stimulation (tDCS) a) Absolute values for pre- and post-stimulation ankle ROM b) Individual change rate in post-stimulation ankle ROM using the pre-stimulation period as a baseline. *Significantly different from pre-stimulation ($p < 0.05$). Data are expressed as mean ± SEM.

Figure 3: Wrist joint range of motion (ROM) before and after each type of transcranial direct current stimulation (tDCS). Data are expressed as mean ± SEM.

Figure 4: Passive torque at the maximal dorsiflexion angle of the ankle before and after each type of transcranial direct current stimulation (tDCS). Data are expressed as mean ± SEM.

a


Pre-tDCS measurement
tDCS
Post-tDCS measurement
Ankle Passive-dorsiflexion test
• Ankle ROM
• Passive torque generated at maximal dorsiflexion
• Passive torque generated when the ankle was submaximally dorsiflexed
Wrist Pasive-dorsiflexion test
• Wrist ROM
Anode
Cathode
Sham
Ankle Passive-dorsiflexion test
• Ankle ROM
• Passive torque generated at maximal dorsiflexion
• Passive torque generated when the ankle was submaximally dorsiflexed
Wrist Pasive-dorsiflexion test
• Wrist ROM

b

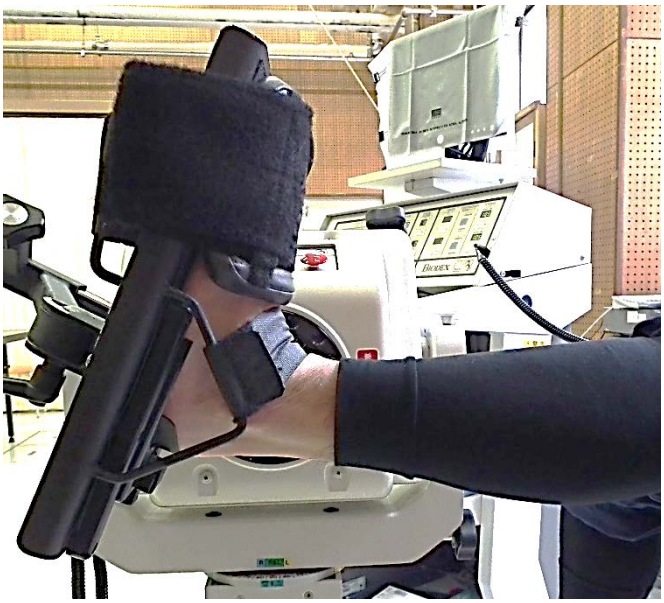


Fig. 1

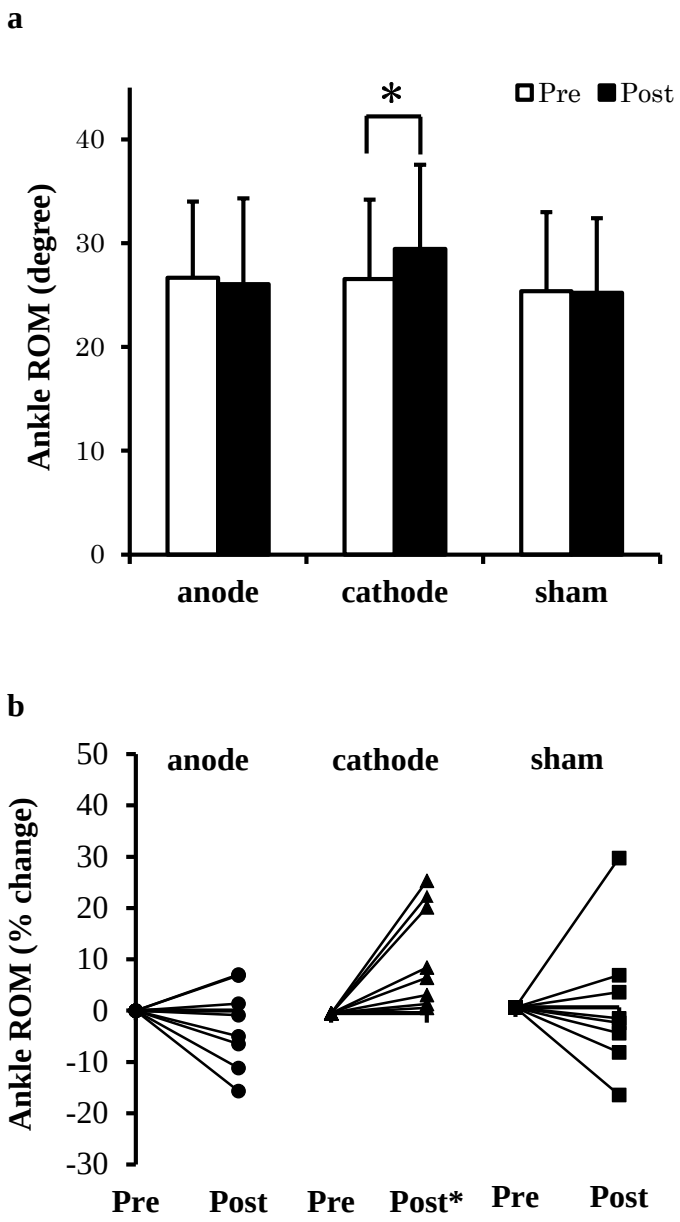

a
*
Pre
Post
Ankle ROM (degree)
40
30
20
10
0
anode
cathode
sham
b
anode
cathode
sham
Ankle ROM (% change)
50
40
30
20
10
0
-10
-20
-30
Pre
Post
Pre
Post*
Pre
Post

Fig. 2

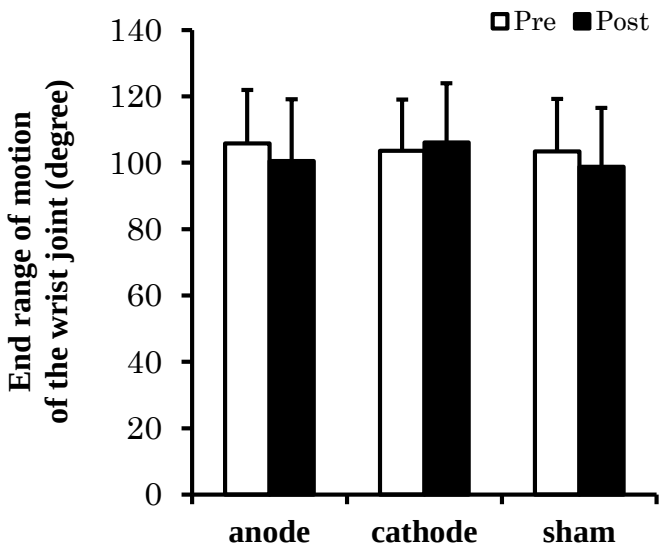

Pre
Post
End range of motion
of the wrist joint (degree)
140
120
100
80
60
40
20
0
anode
cathode
sham

Fig. 3

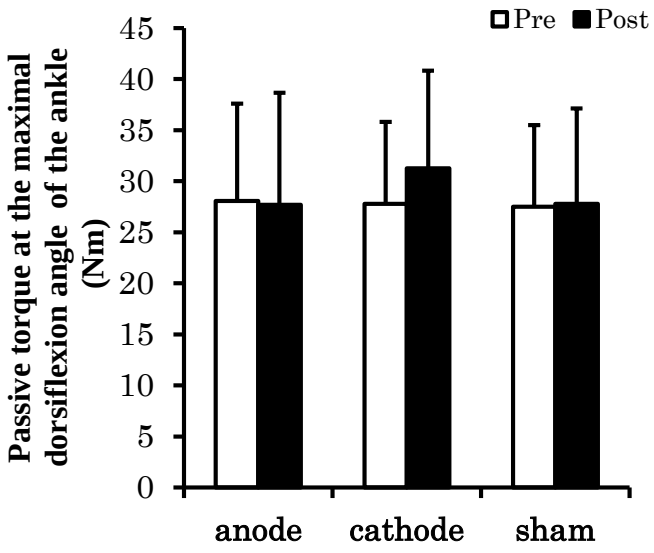

Pre
Post
Passive torque at the maximal dorsiflexion angle of the ankle (Nm)
45
40
35
30
25
20
15
10
5
0
anode
cathode
sham

Fig. 4

Table 1 Passive torque (Nm) at 0°, 5°, 10°, and 15° pre- and post-stimulation

		Dorsiflexion angle (degrees)			
		0	**5**	**10**	**15**
Pre	**Anode**	6.4 ± 1.2	8.2 ± 1.9	10.7 ± 2.6	14.3 ± 4.2
	Cathode	6.4 ± 1.5	8.1 ± 2.0	10.8 ± 2.8	14.4 ± 4.3
	Sham	6.7 ± 1.5	8.5 ± 2.1	11.4 ± 2.9	15.3 ± 4.6
Post	**Anode**	6.5 ± 1.4	8.6 ± 2.1	11.0 ± 2.9	14.9 ± 4.5
	Cathode	6.2 ± 1.5	8.2 ± 2.2	11.0 ± 2.9	14.5 ± 4.7
	Sham	6.8 ± 1.5	8.7 ± 2.2	11.8 ± 3.3	15.7 ± 4.9

Values are means ± SD.

Supplementary Table 1. The results of transcranial direct current stimulation for the outliers

	Anode		Cathode		Sham		CV	Range
	Pre	Post	Pre	Post	Pre	Post		
Ankle ROM (Degree)								
Subject 1	23.9	17.6	14.1	15.0	13.3	17.7	34.7†	10.6
Subject 2	25.9	26.2	19.2	17.9	26.6	23.2	17.3	7.5
Wrist ROM (Degree)								
Subject 1	86.2	75.8	66.1	73.1	84.2	67.4	14.1	20.2
Subject 2	108.9	17.7	97.1	96.8	115.1	111.0	8.6	18.0
Passive torque at maximal dorsiflexion angle (Nm)								
Subject 1	39.7	31.3	37.5	44.4	29.5	33.2	14.6	9.6
Subject 2	56.8	59.8	46.6	40.5	63.9	53.6	15.7	17.4†

The coefficient of variance (CV) and the value range were calculated by using pre-stimulation values over a 3-trial period. CV = S.D. / Mean × 100, Range = Max – Min

†These two participants were excluded based on the results of the Smirnov–Grubbs rejection test ($p < 0.01$) because the CV or the value range were too high.