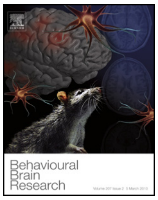
Behavioural Brain Research 253 (2013) 78–89

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr



Research report

Beyond the silence: Bilateral somatosensory stimulation enhances skilled movement quality and neural density in intact behaving rats

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HIGHLIGHTS

- tDCS over SI promoted quantitative and quantitative aspects of skilled movements.
- Bilateral tDCS selectively enhanced the quality of movement components.
- Bilateral tDCS enhanced neural density in the SI area.
- Cortical thickness was not affected by the anodal stimulation.

ARTICLE INFO

Article history:
Received 17 May 2013
Accepted 11 July 2013
Available online xxx

Keywords:
tDCS
Skilled reaching behavior
Somatosensory cortex
Motor cortex
Skilled movement

ABSTRACT

It is thought that a close dialogue between the primary motor (M1) and somatosensory (S1) cortices is necessary for skilled motor learning. The extent of the relative S1 contribution in producing skilled reaching movements, however, is still unclear. Here we used anodal transcranial direct current stimulation (tDCS), which is able to alter polarity-specific excitability in the S1, to facilitate skilled movement in intact behaving rats. We hypothesized that the critical role of S1 in reaching performance can be enhanced by bilateral tDCS. Pretrained rats were assigned to control or stimulation conditions: (1) UnAno: the *unilateral* application of an anodal current to the side contralateral to the paw preferred for reaching; (2) BiAno1: *bilateral* anodal current; (3) BiAno2: a *bilateral* anodal current with additional 30 ms of 65 μA pulses every 5 s. Rats received tDCS (65 μA; 10 min/rat) to the S1 during skilled reach training for 20 days (online-effect phase). After-effect assessment occurred for the next ten days in the absence of electrical stimulation. Quantitative and qualitative analyses of online-effects of tDCS showed that UnAno and BiAno1 somatosensory stimulation significantly improve skilled reaching performance. Bilateral BiAno1 stimulation was associated with greater qualitative functional improvement than unilateral UnAno stimulation. tDCS-induced improvements were not observed in the after-effects phase. Quantitative cytoarchitectonic analysis revealed that somatosensory tDCS bilaterally increases cortical neural density. The findings emphasize the central role of bilateral somatosensory feedback in skill acquisition through modulation of cortico-motor excitability.

1. Introduction

The somatosensory (S1) cortex is a central structure involved in motor learning and the acquisition of skilled movement. It closely cooperates with the primary motor cortex (M1), which has been widely studied in the integration of afferent input from subcortical processing systems such as brain stem and basal ganglia to cortex [1,2]. Specifically, several lines of evidence derived from lesion studies confirm modular control of skilled reaching performance by M1 [3–6]. However, a close interaction with sensory systems such as the S1 is essential for movement execution and motor learning [7,8].

Skilled reaching movements represent a well-characterized outcome of M1 and S1 cooperation in that they encompass a fixed sequence of characteristic movement components [9]. As opposed to the well-characterized role of the M1 in producing and adjusting the sequence of components involved in skilled reaching movements, less is known about the relative contribution of the S1 *vs.* M1. In the somatosensory domain, it is still unclear to what extent and

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http://dx.doi.org/10.1016/j.bbr.2013.07.022

at which movement component the sensory cortex, particularly S1, is involved in skilled reaching movement. The S1 integrates inputs from different parts of the body into somatotopic maps, which represent complete topological sensory profiles of body organs [10,11]. Because of its wide array of dynamic interactions [12], the study of S1 provides insight into general mechanisms of cortical dynamics related to both motor and sensory information processing function. Unlike M1, evidence of a significant causal link between injury-induced motor impairment and lesion in S1 is provided in few studies dealing with post-lesion functional deficits [13–15]. Such studies typically portrait a general picture of the S1 role in motor learning [16].

The purpose of the present study was to investigate the role of S1 in skilled motor learning processes. Using a complementary approach to inducing focal cortical lesions, we applied anodal transcranial direct current stimulation (tDCS) over S1 in intact behaving rats. The procedure that delivered subthreshold electrical current to the S1 region was used because tDCS can focally affect the level of hemispheric excitability and modulate spontaneous neuronal activity in a polarity-dependent manner [17,18]. Cortical excitability, therefore, can be either increased by anodal stimulation or decreased by cathodal stimulation [19]. We proposed that anodal tDCS enhances the local excitability within S1 thus selectively supporting the acquisition and performance of skilled reaching movements.

2. Materials and methods

2.1. Subjects

Adult male Long-Evans rats ($n = 24$), weighing 450–630 g at the beginning of the experiment were initially subjected to bilateral skull trephination for electrode placement and transcranial direct current stimulation (tDCS). All testing was performed during the light phase of the cycle at the same time of day. Animals were food restricted prior to testing in the skilled forelimb reaching task, and maintained at about 90% of their initial body weight throughout the experiment. To maintain body weight, rats were given an additional amount of food in their home cage at least 3–4 h after completion of the behavioural training and testing. Because animals were housed in pairs, they were weighed daily throughout the experiment in order to monitor their food consumption. All procedures and experimental manipulation including animal handling, food restriction, electrostimulation and behavioural testing were performed under protocols approved by the Animal Care Committee of the University of Lethbridge in compliance with the guidelines of the Canadian Council on Animal Care.

2.2. Experimental design

Animals were randomly assigned to the following groups: Sham, UnAno, BiAno1, and BiAno2; number of rats for each group: $n = 6$. The *online-effect* of tDCS was assessed by testing animals in the reaching task for 20 consecutive days concurrently with the application of tDCS. Rats also were tested for 10 additional days in the skilled reaching task without the application of tDCS for any potential *after-effect* of the stimulation. For qualitative movement analysis, reaching performance was video recorded every 5 days of testing. Following the behavioural assessments rats were euthanized for histological analyses.

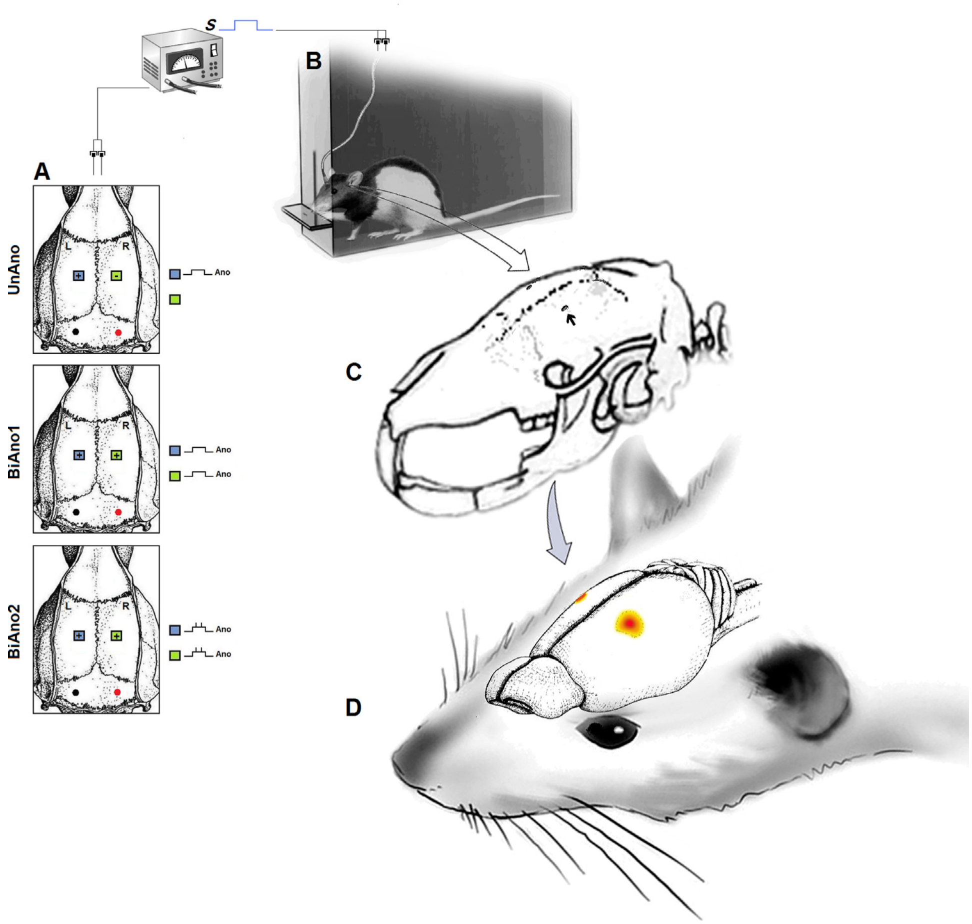

Fig. 1. (A) Approximate locations where the anodal direct currents stimulated somatosensory cortex. Blue and green boxes show the dominant and non-dominant hemispheres, respectively. Black dots reflect reference and red dots represent ground electrodes. Signs + and − indicate the polarity of the currents applied to the different groups. (B) Schematic representation of an intact behaving rat in skilled forelimb reaching task connected to a stimulation source. Each test session required the rats to reach for 20 food pellets. (C and D) Diagrams of a rat skull showing approximate bilateral somatosensory points of anodal stimulation (grey arrow) and also underlying somatosensory cortical areas (red-colour spot). *S*, stimulation; Ano, anode; UnAno, unilateral anode; BiAno, bilateral anode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

2.3. *Skilled forelimb reaching task*

Analyses of skilled forelimb reaching were based on earlier descriptions [9,20]. Each training and test session required the rats to reach for 20 food pellets. Baseline training was considered complete once the success rates reached asymptotic levels. At particular time points, the rats' reaching performance was video recorded from a frontal view using a digital camcorder (Canon ZR70 MC) at 30 frames/s with a shutter speed of 1/500. The tapes were analysed frame-by-frame on a Sony DV player. Quantitative analysis included percentage of successes and reaching attempts. A successful reach was defined as obtaining the pellet on the first attempt, withdrawing the paw with the pellet through the slit and releasing the pellet into the mouth. Percent reaching success was calculated by counting the number of successful reaches divided by the number of pellets (20) given in each session multiplied by 100. Moreover, an attempt was defined as a repeated forelimb movement towards the pellet and obtaining the pellet after more than one reach (Fig. 1B; [21]).

Qualitative movement analysis of limb movements was performed for the first three successful reaches of each limb. Eleven reaching movement components [(1) *Orient*, (2) *Limb lift*, (3) *Digits close*, (4) *Aim*, (5) *Advance*, (6) *Digits open*, (7) *Pronation*, (8) *Grasp*, (9) *Supination 1*, (10) *Supination 2*, and (11) *Release*] and 35 subcomponents were scored according to earlier descriptions [9]. Each movement component was rated on a three-point scale: 0 point, movement absent; 0.5 point, the movement was present but abnormal; 1 point, the movement was normal.

2.4. *Transcranial direct current stimulation (tDCS)*

Using stereotaxic coordinates (AP: ±1.60 mm; ML: ±2.80 mm), one small hole was drilled in the skull over the somatosensory cortex of each hemisphere. Two stimulation electrodes (screws) were fixed to the skull on top of the dura, without entering the brain. A screw implanted at the back of the skull served as a reference electrode.

Rats from the stimulation groups received 10 min of tDCS concurrently with the skilled reaching testing. Three different protocols of stimulation were applied to compare tDCS online- and after-effects on motor learning: (1) the UnAno or *unilateral anode* protocol consisted of the application of 65 μA anodal direct constant current for 10 min to a single hemisphere in relation to the animal's preferred limb; (2) in the BiAno1 or *bilateral anode1* protocol animals received a bilateral 65 μA anodal direct constant current for 10 min; and (3) in the BiAno2 or *bilateral anode2* protocol animals received 65 μA anodal direct constant current in the left and right hemispheres simultaneously. Additionally, group BiAno2 received a train of 30 ms pulses of 65 μA applied every 5 s to both hemispheres. Rats in all groups were connected to the stimulator (Grass Medical Stimulator, QUINCY. MASS. USA; Fig. 1A, C and D) during all reaching trials, but the stimulator was switched on only for the stimulation groups and it was off for all groups during after-effects (no-stimulation) sessions.

2.5. *Histology and analysis*

Rats were euthanized with an overdose of sodium pentobarbital (300 mg/kg i.p.) and intracardially perfused with saline (0.9%; 200 mL/rat) followed by 4% paraformaldehyde (PFA; 200 mL/rat). Brains were removed, post-fixed for 24 h in 4% PFA and cryoprotected in 30% sucrose and 4% paraformaldehyde at 4 °C for coronal sectioning (40 μm) and cresyl-violet staining. The stained sections were examined under a microscope (Zeiss, Germany) and images were captured using an AxioCam camera (Zeiss, Germany) for histological analysis and presentation. From the coronal sections, measures were made of cortical thickness and neural density in both hemispheres of all groups. The experimenter was blind to the experimental groups.

Cortical thickness. A summary of the measurement procedure for cortical thickness (adapted from [22,23]), using 10 representative histology sections, is shown in Fig. 2A and *a*. Briefly, four points (medial, central, lateral, and ventrolateral) on ten coronal sections (planes 10, 13, 17, 21, 24, 27, 32, 36, 41, 45) from each brain were selected based on Paxinos and Watson [24]. The most rostral section measured was located at ∼2.20 mm anterior to bregma and the most caudal section at ∼−6.30 mm posterior to bregma. For each point, a vector was considered from the tangent of the outer edge to the inner edge of the cortex. ImageJ software 1.47b (http://imagej.nih.gov/ij; NIH, USA) was used to record up to eight measurements of cortical thickness from each coronal section, four from each hemisphere [23].

Neural density and cytoarchitectonics. Neural density analysis (quantitative cytoarchitectonics) was performed using ImageJ 1.47b (http://imagej.nih.gov/ij) based on earlier descriptions [25]. A calibration was made by step tablet (http://rsb.info.nih.gov/ij/download.html) for optical density prior to the analysis of density. Two approximate planes (planes 27 and 32, ∼−1.88 mm and

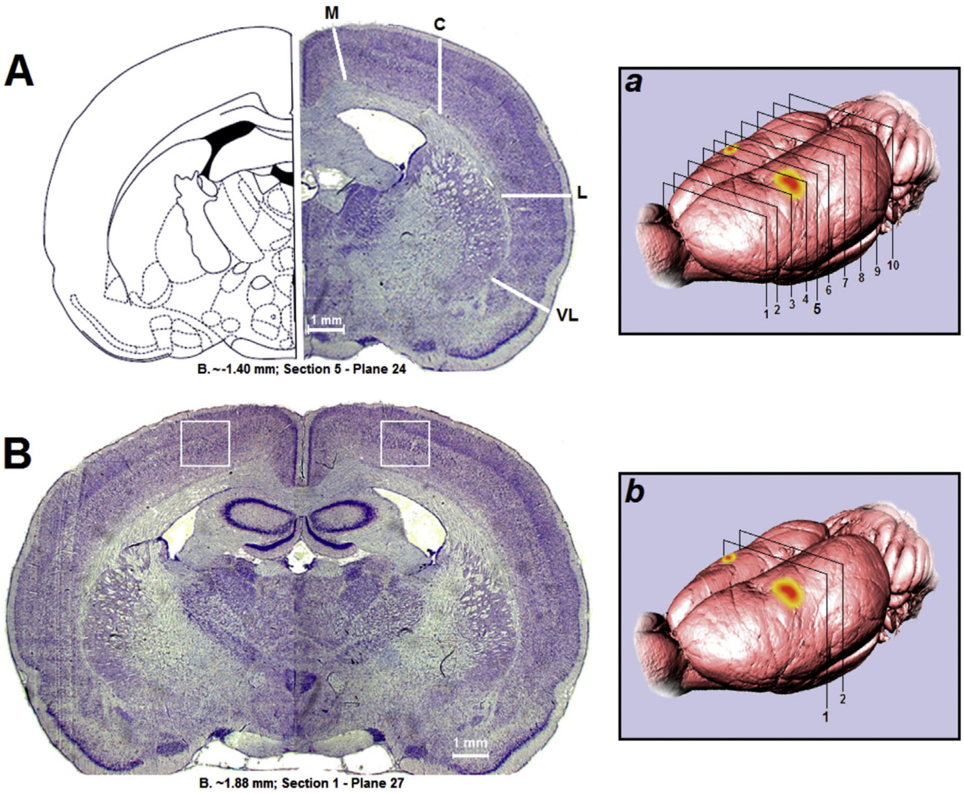

Fig. 2. (A) Cortical thickness. Four points (Medial, Central, Lateral, and Ventrolateral) on ten coronal sections (planes 10, 13, 17, 21, 24, 27, 32, 36, 41, 45) from each brain were selected. Panel *a* shows ten approximate points of the sections, from the most rostral section located at ∼2.20 mm anterior to bregma to the most caudal section at ∼−6.30 mm posterior to bregma. (B) Quantitative cytoarchitectonics. Densitometry measures based on absolute grey value index shown by approximate planes (planes 27 and 32, ∼−1.88 mm and ∼−3.14 mm posterior to bregma, respectively) of stained sections in each brain. White squares on left and write hemispheres represent two regions of interest (S1HL, S1FL) that have been determined within the cortical regions adjacent to the stimulation area. All plates adopted from Paxinos and Watson [24].

∼−3.14 mm posterior to bregma respectively; [24]) of stained sections in each brain were selected. Two regions of interest (ROI) were determined within the somatosensory cortical regions, adjacent to the stimulation area of each hemisphere (Fig. 2B and *b*). Both left and right ROIs included the same cortical regions and nearly all six cortical layers. An absolute grey value index or the average grey value within the ROIs was separately measured for each region [25,26].

2.6. Statistical analysis

All analyses were performed using SPSS 16.0. (SPSS Inc., USA, 1989–2007). Dependent variables in skilled reaching including quantitative (success percent, number of attempts) and qualitative (11 movement components of reaching performance) measures were averaged and analysed for both online-effect (20 days) and after-effect sessions (10 days) using repeated measure of analysis of variance (ANOVA). *Post hoc* analysis was used for motor measures to adjust for multiple comparisons between different groups. Furthermore, repeated measure ANOVA was conducted with Groups, Components and Days effects for all independent variables. To test for correlations between behavioural measures and neural density (grey index value) Pearson's correlation coefficients were determined. Differences in between-group and within-group comparisons were also assessed with independent and dependent samples *t*-tests for both behavioural and histological data, with $P < 0.05$ set as the significance level. All data are presented as mean ± standard error of the mean.

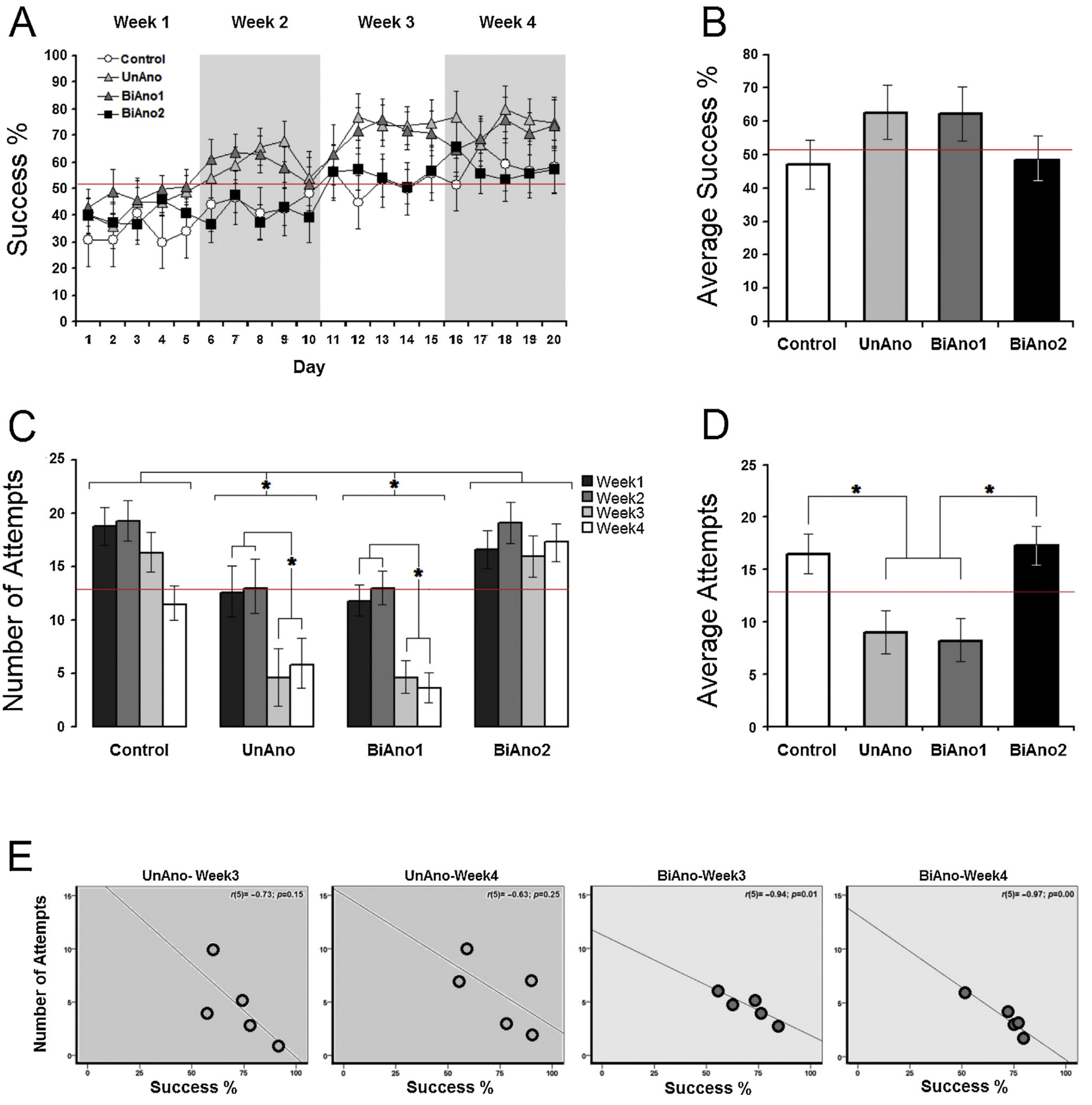

Fig. 3. (A) Time course of daily reaching success in all experimental groups during stimulation sessions. Every five-day period in this experiment counts for a one-week stimulation trial. (B) Average reaching success percent in each group during 20 days stimulation. Despite a slight increase in success percent in UnAno and BiAno1 groups, no significant between-groups difference was observed. (C) The number of reaching attempts on the last day of each five-day period (Weeks 1–4), and (D) average attempts for each group. As noted, an attempt was defined as a repeated forelimb movement towards the pellet and obtaining the pellet after more than one reach. Rats in UnAno and BiAno1 groups indicated significantly decreased attempts on Weeks 3 and 4 when compared with controls and group BiAno2. (E) Correlation between the number of attempts and success percent on Weeks 3 and 4 in groups UnAno (top, dark grey; $n = 5$) and BiAno1 (down, light grey; $n = 5$). Reaching success was negatively correlated to the number of attempts only in the group BiAno1. Asterisks indicate significances: $^*P < 0.05$; ANOVA. *UnAno*, unilateral anode; *BiAno*, bilateral anode.

3. Results

3.1. tDCS promotes greater reaching success: online effects

Success percent. Skilled motor control was assessed in a reaching task in which animals were required to reach for food pellets with their preferred forelimb. During the first 20 days of tDCS application (online-effect), success rates in all groups increased beyond baseline levels (Fig. 3A). Animals in groups UnAno and BiAno1 indicated better reaching performance than controls and BiAno2 animals. No significant main effect of Group, however, was observed ($P = 0.321$). Within-subject effects indicated a significant main effect of Day ($F^{19,36} = 6.81$, $P < 0.001$) but no significant interaction between Group and Day ($P = 0.992$). Furthermore, panel B in Fig. 3 shows the average success rate of all groups. Average reaching success in both UnAno and BiAno1 groups was considerably higher than in other groups (UnAno: 62.7 ± 8.06 and BiAno1: 62.3 ± 8.06 *vs.* BiAno2: 48.43 ± 7.36 and Control: 47.12 ± 7.36) indicating that both unilateral and bilateral application of tDCS, although not significantly, improved reaching performance in the online-effect period.

Number of reaching attempts: Fig. 3C shows the changes in the number of reaching attempts across four weeks of assessment of tDCS online effects on reaching performance. Although reaching attempts slowly dropped in the last two weeks in controls, UnAno and BiAno1 groups were able to show fast improvement of attempts in Weeks 3 and 4 of testing. Group BiAno2, on the other hand, showed no noticeable improvement in their attempts across the four weeks of reaching trials. There was a significant main effect of Group ($F^{3,18} = 12.53$, $P < 0.001$; $\eta^2 = 0.67$) and Week ($F^{3,18} = 14.60$, $P < 0.001$; $\eta^2 = 0.44$), but no significant interaction between Groups and Weeks ($P = 0.103$). Average attempts across four weeks for all groups shown in Fig. 3D indicates that average attempts in groups UnAno (9.00 ± 1.41) and BiAno1 (8.25 ± 1.41) were significantly lower than controls (16.50 ± 1.29) and BiAno2 (17.29 ± 1.29; $P = 0.005$; $P < 0.01$). No significant difference was found in the average number of attempts between the controls and BiAno2 animals ($P = 0.972$) and UnAno and BiAno1 animals ($P = 0.981$). Unlike reaching success, therefore, reaching attempts significantly decreased by concurrent tDCS applied either unilaterally or bilaterally to the left and right somatosensory cortices of both hemispheres during the online-effect assessment phase. Furthermore, as shown in subpanels *a* and *b* rats with high total reaching success score (groups UnAno and BiAno1) had a lower total number of attempts. However, the correlation between total reaching success and number of attempts in Weeks 3 and 4 was significant only in group BiAno1 (Week 3: $r^5 = -0.95$, $P < 0.01$; Week 4: $r^5 = -0.97$, $P < 0.001$) (Fig. 3E).

Movement trajectories: Individual movement scores served as qualitative measures of skilled forelimb reaching capacity during the first 20 days of tDCS treatment (online-effect assessment). No

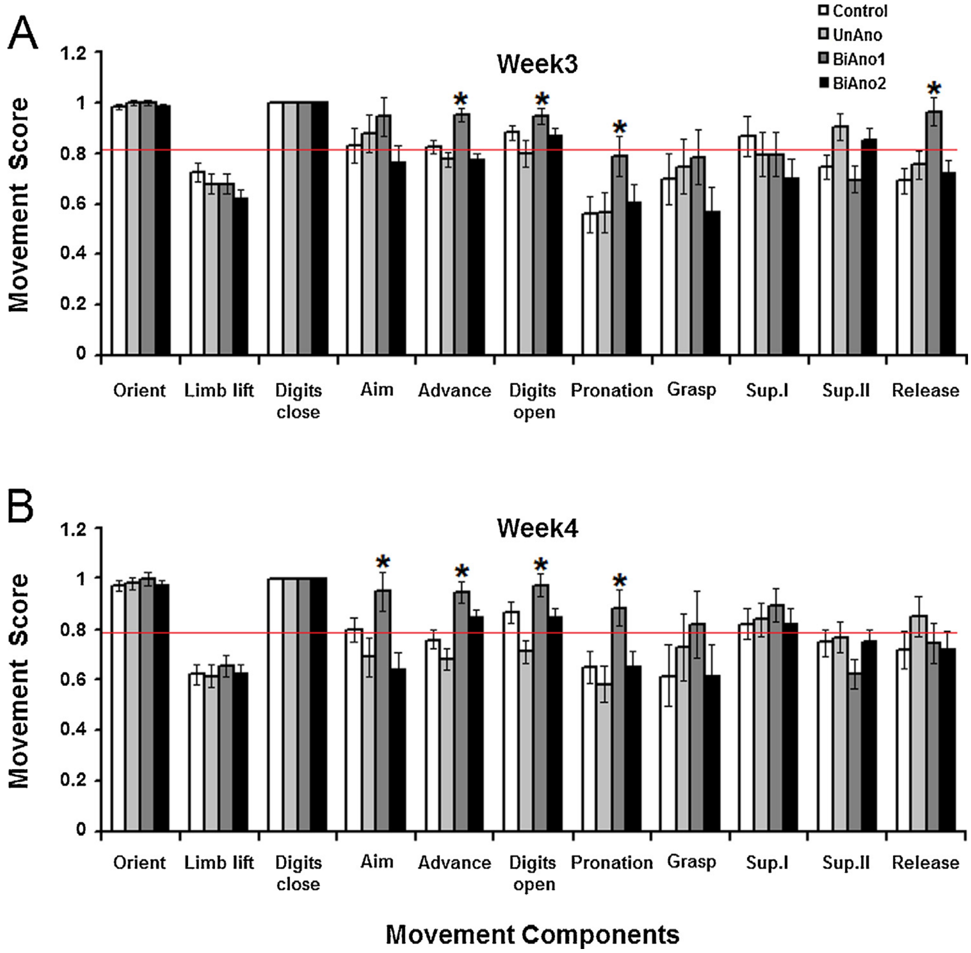

Fig. 4. (A and B) Qualitative analysis of individual reaching movement components for Weeks 3 and 4 during online-effect phase. No significant difference was observed between groups on the first and second weeks. Note, however, that only rats in the group BiAno1 improved reaching performance by higher quality in Advance, Digits Open, Pronation, and Release on Week 3. Moreover, they could also functionally benefit from the tDCS on week four when they significantly showed more enhanced quality of Aim, Advance, Digits Open, and Pronation. Red horizontal lines represent grand mean. Asterisks indicate significant difference: $^*P = 0.05$, ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

significant difference was observed between groups during Weeks 1 and 2 of treatment. However, performance during Weeks 3 and 4 revealed significant qualitative changes in reaching performance induced by tDCS (Fig. 4A and B). In Week 3, there was a significant main effect of Group ($F^{3,18} = 3.24$, $P < 0.05$; $\eta^2 = 0.35$, ANOVA), Components ($F^{10,18} = 19.52$, $P < 0.001$; $\eta^2 = 0.52$, ANOVA) and significant interactions between Group and Components ($F^{30,180} = 2.36$, $P < 0.001$; ANOVA). Further analysis revealed that only rats with BiAno1 treatment improved reaching performance in Advance, Digits Open, Pronation, and Release (Fig. 5A–C) in the third week when compared to other experimental groups (all Ps < 0.05). These findings indicate that, unlike other groups, BiAno1 rats showed enhanced ability to adjust body position when approaching the slit. Higher scores in components Digits Open and Pronation enabled them to move digits and limb more freely towards pellets. Improved Release, finally, provided rats an opportunity to accomplish the task by more accurate movements of digits and efficient position in head and body to extract the pellet from the paw.

The enhanced quality of reaching performance induced by tDCS was also observed in the same group in Week 4 when all groups were ultimately assessed for the online effects of stimulation. A significant main effect of Group ($F^{3,18} = 4.81$, $P < 0.05$; $\eta^2 = 0.44$, ANOVA), Component ($F^{10,18} = 14.73$, $P < 0.001$; $\eta^2 = 0.45$, ANOVA) and a significant interaction of Group by Component ($F^{30,180} = 1.52$, $P < 0.05$; ANOVA) was observed in Week 4. Similar to Week 3, only rats in the BiAno1 group functionally benefited from the tDCS in Week 4 and displayed enhanced quality of Aim, Advance, Digits Open, and Pronation compared to the other experimental groups (all Ps < 0.05). During Week 4, therefore, the beneficial effect of tDCS induced more efficient elbow abduction to the body midline accompanied by better position of digits towards the target. Moreover, qualitative measures of reaching performance in group BiAno1 in Weeks 3 and 4 of the online-effect phase and overall changes in neural density (left and right) did not reveal a significant correlation.

3.2. *Limited after effects in the absence of tDCS*

Rats were tested for ten additional days in the skilled reaching task without the application of tDCS to evaluate potential after-effects of the stimulation [18,27].

Success percent. As illustrated in Fig. 6A, the improvement in success rate in the after-effect trials from days 1 to 10 (Control: 36.66 *vs.* 61.66; UnAno: 39.00 *vs.* 54.00; BiAno1: 36.00 *vs.* 57.00; BiAno2: 36.66 *vs.* 50.00) indicated that all experimental groups were able to improve their reaching skills as the testing proceeded in the absence of tDCS. No significant difference between the groups in percent success was found during after-effect sessions ($P = 0.837$). However, a main effect of Day ($F^{9,18} = 5.07$, $P < 0.001$; ANOVA) was observed suggesting that all animals improved their reaching performance across ten testing days regardless of their experimental treatment. No interaction effect of Group and Day was observed ($P = 0.853$). Panel B in Fig. 6 also indicates that the improved reaching performance observed in groups UnAno and BiAno1 in online-effect assessment disappeared during ten days of after-effect sessions.

Number of reaching attempts. All animals were assessed for the number of reaching attempts. There were no significant differences in the number of attempts between the four groups (Control: 8.83 ± 2.05; UnAno: 11.3 ± 2.25; BiAno1: 10.5 ± 2.25; BiAno2: 13.66 ± 2.05; $P = 0.337$; Fig. 6C and D) at the end of after-effect testing sessions. No significant effects of Weeks ($P = 0.188$) or interaction between Groups and Weeks ($P = 0.664$) were observed.

Movement components. Analysis of eleven reaching components at the after-effect time points showed no overall Group difference in the reaching components (Week 1: $P = 0.843$; Week 2: $P = 0.384$) indicating that all animals revealed similar qualities in reaching components in the absence of tDCS. As illustrated in Fig. 7A and B, movement components in all groups showed similar profiles of changes, and the previously observed online-effects in group BiAno1 disappeared when tested in after-effect assessment sessions.

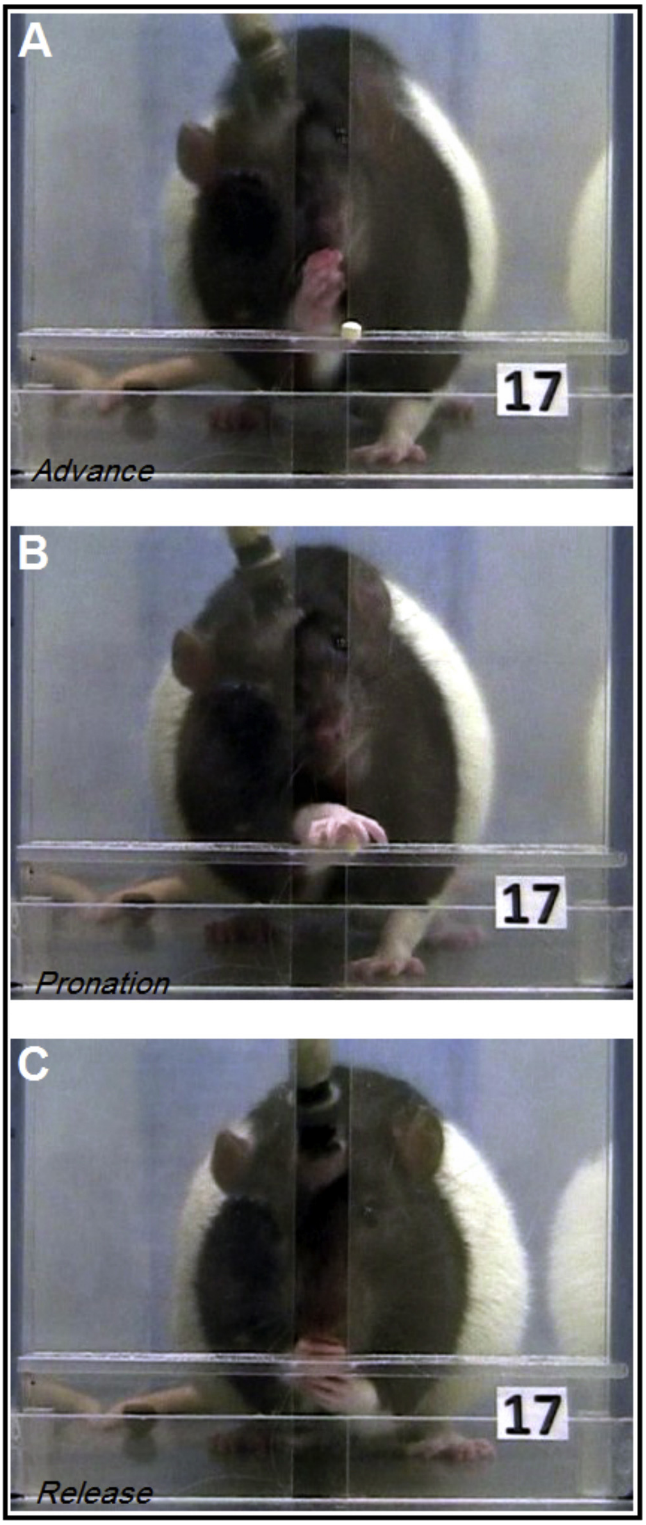

Fig. 5. Photographs illustrating (A) Advance, (B) Pronation, and (C) Release, three qualitative components of reaching performance in a rat with skull cap construct within skilled forelimb reaching task.

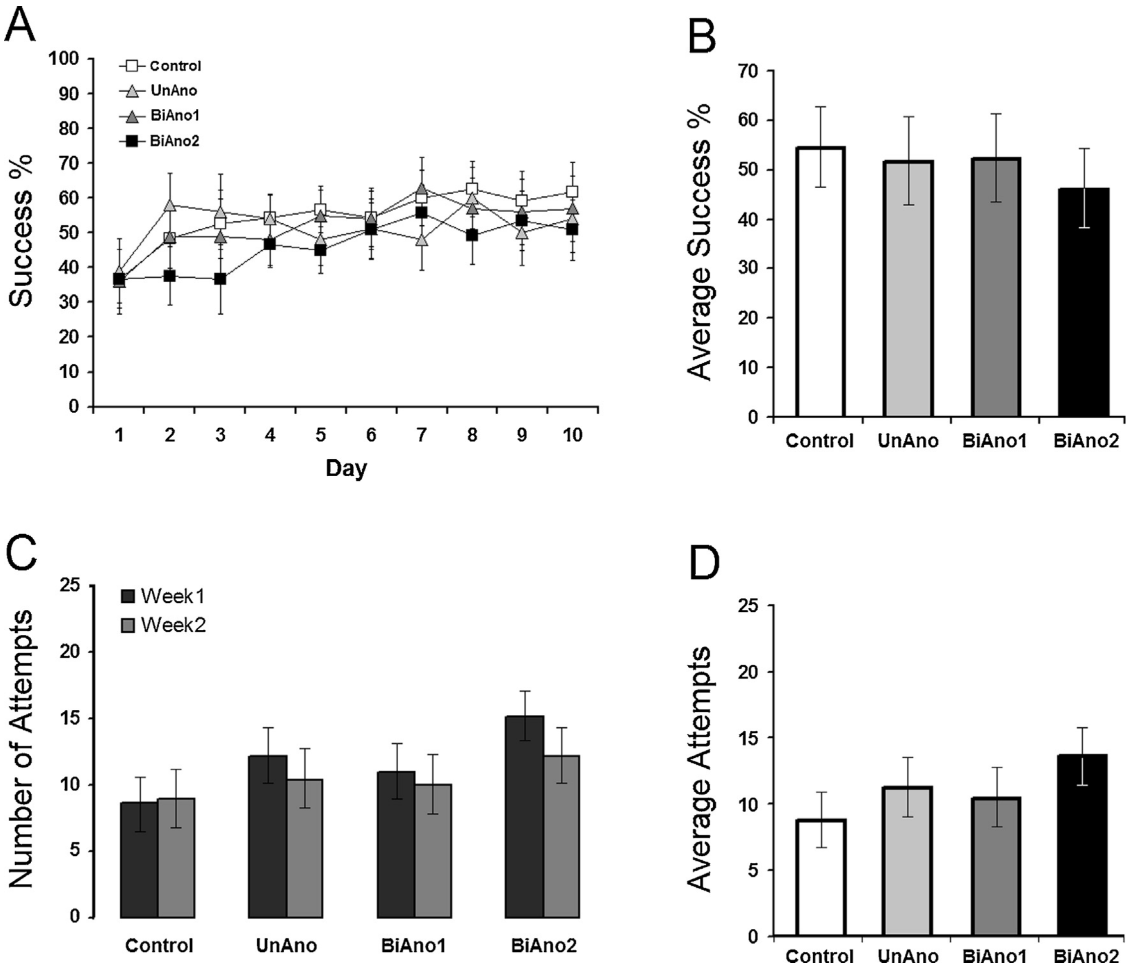

Fig. 6. (A) Time course of daily reaching success in all experimental groups and (B) the average reaching success percent in each group during after-effect sessions (Weeks 1 and 2) indicated that all experimental groups were able to improve their reaching skills as the testing was proceeded without the tDCS application in two weeks. (C and D) Number of attempts during the after-effect assessment. There was no significant difference in the number of attempts between four groups. *UnAno*, unilateral anode; *BiAno*, bilateral anode.

3.3. Bilateral tDCS permanently increases cortical neural density

Cortical thickness. Fig. 8A summarizes cortical thickness measurements following online- and after-effect assessments of the tDCS application. Cortical thickness was measured in the medial, central, lateral, and ventrolateral portions in both hemispheres. No main effect of Group ($P = 0.086$) was found, although rats in the group BiAno2 showed a slightly thinner cortex in both hemispheres compared to other groups. Furthermore, *post hoc* analysis for multiple between-group comparisons showed no significant differences between experimental groups.

Neural density. Summary of tDCS-related changes in cortical neural density is shown in Fig. 8B. The density of neurons in both (left and right) regions of interest (S1HL and S1FL; [24]) in BiAno1 animals was higher compared to other groups. The most noticeable changes in neural density were observed in the UnAno and BiAno2 groups where the left and right hemispheres were compared. Neural density in the right hemisphere in both groups increased. These main findings were confirmed by the statistical analysis. There was a significant effect of Group ($F^{3,19} = 3.191$, $P < 0.05$; $\eta^2 = 0.33$, ANOVA), but no main effect of Side ($P = 0.078$) or interaction between Group and Side ($P = 0.509$). *Post hoc* analysis confirmed that neural density in the BiAno1 group increased when compared to Controls ($P < 0.05$), the UnAno ($P = 0.016$) and BiAno2 ($P = 0.014$) groups. Dependent samples *t*-test also confirmed significant differences in left and right neural density observed in UnAno and BiAno2 groups.

Overall, histological analysis indicated that tDCS applied over the intact somatosensory cortex was capable of increasing neural density in corresponding cortical regions. Cortical thickness, however, was unaffected by tDCS.

4. Discussion

The primary aim of the present study was to analyse the impact of unilateral and bilateral delivery of anodal tDCS to the somatosensory cortex (SI) on fine motor control in rats. The results amount to four main functional and structural findings: (1) tDCS over SI promoted quantitative aspects of skilled reaching movements measured by the number of attempts. Somatosensory stimulation also decisively, although not significantly, enhanced reaching success, an alternate indicator for the improved skilled movement. (2) Chronic bilateral anodal tDCS was associated with greater qualitative alterations than unilateral stimulation, leading to enhanced quality of movement components in Aim, Advance, Digits open, Pronation, and Release in the online-effect assessments. (3) All motor improvements induced by somatosensory stimulation disappeared during the after-effect assessment phase. (4) Finally, bilateral tDCS (BiAno1) enhanced neural density in the SI area. Cortical thickness, however, was not affected by the anodal

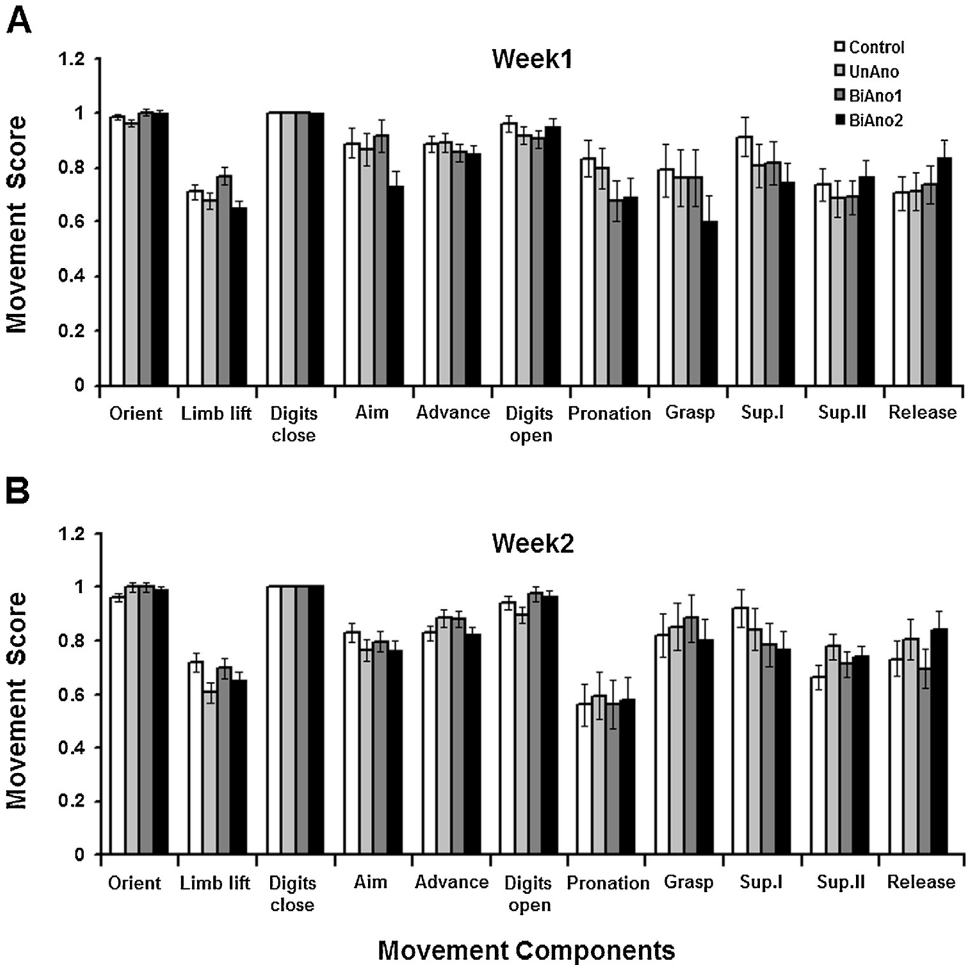

Fig. 7. Qualitative analysis of individual reaching movement components for Weeks 1 and 2 of after-effect phase. No significant difference was observed between groups.

stimulation. Taken together, our findings provide evidence that bilateral contribution of S1 to skilled movement elicited by anodal focal stimulation is selective and limited to selected movement components, and may be associated with structural changes in cortical neural density.

4.1. Somatosensory tDCS results in specific improvements in skilled reaching movement performance

Skilled reaching movements evolved in relation to food handling and refer to movements of the limbs, paws and digits for catching, holding, and manipulating objects. A line of empirical efforts to examine and analyse skilled movement in animals are focused on the study of rat skilled reaching [28–31] with a task in which an animal reaches through a slit to attain a single food pellet located in a small notch on a shelf [9,32].

To investigate the level of bilateral somatosensory involvement in skilled reaching, we used a pellet reaching task as an object-location task. Learning this task involves a relatively fixed and organized sequence of sensory-modulated actions [9]. Thus, skilled limb movements represent a movement pattern which is clearly recognizable from instant to instant and can be easily disrupted or enhanced by any immediate intervention. As an action pattern, skilled limb movement, therefore, can have some elements that supposedly are produced, supported and monitored by alternative subunits mapped to adjacent structures, such as interactions by motor and somatosensory cortices.

Skilled reaching performance consists of eleven movement components [9]. Our analysis in the present study showed that bilateral somatosensory activation, however, had considerable impact on only five movement components. While improved quality of Release in the online-effect phase was replaced with the high quality of component Aim in Week 4, other components (Advance, Digits open, Pronation) still consistently improved in both Weeks 3 and 4. Although each of these three components belong to the same phase of reaching movement (*Reaching & Pronation*; in [33]), each form different motor elements for the entire movement sequence to accomplish the task.

Furthermore, two characteristic features of reaching movement in rodents, targeting and particularly hand shaping, in contrast to primates are controlled by haptic information provided by the perioral area [1]. Thus, reaching in rats requires an integrated somatosensory feedback and its complementary role in hand and digit shaping. The extent to which neurons in S1 make connections with motor neurons to a precise handling and manipulation has not been extensively studied in rats. Our data, therefore, provides behavioural findings to suggest that the involvement of the somatosensory information in the rat reaching movement and its corresponding neural substrate is more complex than has been thought.

The non-significant correlation between behavioural and morphological changes in this study indicate that bilateral somatosensory stimulation enhanced reaching performance in Weeks 3 and 4. This change is revealed by elevated success rates, reduced number of attempts and improved quality of

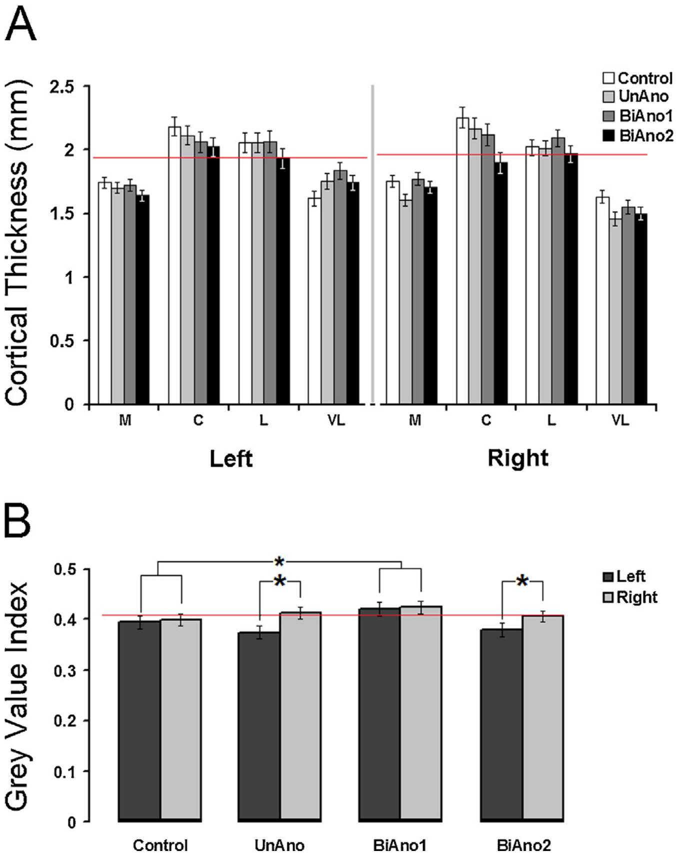

Fig. 8. (A) Cortical thickness. The thickness of the cortex measured in the medial, central, lateral, and ventrolateral points of both hemispheres showed no main effect of Group. Also, group BiAno2, although not significantly showed slightly thinner cortex in both sides compared to other groups. (B) Quantitative cytoarchitectonics-Neural density. Note that the density of neurons reflected by grey value index (GVI) in both left and right regions of interests in group BiAno1 significantly increased compared to the other groups. Red horizontal lines represent grand mean. Asterisks indicate significant difference: $^{*}P = 0.05$, ANOVA. *UnAno*, unilateral anode; *BiAno*, bilateral anode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

specific movement components in the BiAno1 group. However, this behavioural improvement was not correlated with increased cortical neural density. Therefore, functional augmentation on the last weeks of online-effect assessment may be a late reflection of enhanced bilateral somatosensory cortex excitability induced by tDCS, possibly in absence of tDCS-induced cortical changes. This functional plasticity can be attributed to a flexible association between sensation and action [34] in S1 and its pre-existing neuronal circuits [35] that become re-activated by the immediate bilateral somatosensory anodal stimulation. This finding supports the observation of return to baseline movement performance in the after-effect phase. These tDCS-induced online behavioural changes may bear implications for the neuroanatomical substrate of skilled reaching movements.

4.2. tDCS modulates specific somatosensory correlates of reaching performance

Different central structures, usually through lesion studies, were shown to be involved in skilled reaching movements. It is now widely accepted that motor cortex lesions in M1 disrupt reaching movements [29,36–38]. Moreover, connected regions such as striatum [30,39,40], red nucleus [41], cerebellum and the inferior olive [75] can affect skilled reaching movement. Damage to the descending pathways, including the corticospinal tract (CST; [42,43]), rubrospinal tract (RST; [76]), tectospinal and reticulospinal tracts [44], also impairs the execution of skilled forelimb movements. Hence, the organization of the motor system in rats allows different motor elements to be controlled by different anatomical subsystems.

The contribution of the S1 in skilled movements, however, has received less attention than M1, perhaps because the primary motor and somatosensory cortices of rodents such as rats overlap considerably [1]. Therefore, in the context of lesion studies, it can be associated with heterogeneous functional consequences. Evidence indicates that somatosensory input plays a crucial role in the precision of hand control [77] and the acquisition of new motor skills [45]. It is also hypothesized that the somatosensory feedback during object manipulation provides complementary information to the motor cortex, thus allowing for fine motor adjustments, regulation of prehension strength, and precision grasping [7]. More importantly, M1 receives bilateral somatosensory input which is somatotopically organized [46]. Because M1 neurons receive tactile and proprioceptive input [47] during hand and paw use, skilled reaching performance may closely reflect the quality of cortical motor and sensory integration.

The present findings also indicate that the S1 involvement in reaching movement components is relatively specific to the movement pattern. This is reflected by both the two time courses of interventions (online- *vs.* after-effect phases) and the enhanced quality of skilled movement which was restricted to only a few components. The present study, therefore, provides complementary data indicating that somatosensory projections to the M1 is necessary to the success and quality of digital dexterity. For instance, although precision has been previously attributed to M1 [48,49], our findings suggest that bilateral somatosensory input is particularly important in the advance-to-pronation portion of a reaching movement. The anatomical basis and physiological properties of this input to the motor cortex still await further clarification, but it is possible that the activity originates from the S1 cortex through associative connections to M1 [50,51] or corticocortical pathways mediated by pyramidal tract neurons (PTNs).

The improving effect of bilateral *vs.* unilateral stimulation over S1 on movement components also highlights bi-hemispheric collaboration of somatosensory signals in reaching movements of intact rats. An increased activation of ipsilateral S1 during hand movement in stroke patients [52] and a bilateral participation of S1 in monkey [53] and rat [54] reaching movements have been reported. The finding in the present study, however, is the first to suggest that neurons in contra- and ipsilateral S1 in intact rats contribute to the proprioceptive and cutaneous guidance of ongoing movements in reaching task. This aspect of our findings provides new insights to the discussion of complementary somatosensory feedback particularly from the ipsilateral cortex to accomplish a motor task [53]. If anodal tDCS can potentiate bilateral somatosensory contribution to skilled reaching movements in intact behaving animals, which aspect of cortical activity may benefit from focal cortical stimulation?

4.3. tDCS affects skilled reaching performance through altered cortical excitability

tDCS affects brain functions through modulation of cortical excitability, such as an increase by anodal (surface-positive) or a decrease by cathodal (surface-negative) stimulation. The main assumption behind the application of anodal tDCS is that it increases the spontaneous firing rate and the excitability of cortical neurons by depolarizing the membrane ([18,55]; see [56], for review). Depending on the research hypotheses and questions, different stimulation protocols can be employed, including one anode and one cathode [57], one anode and two cathodes [58], one anode [59,60] and one cathode only [61], as well as two anodes and two cathodes [62]. The anode-cathode tDCS protocol [57] used in the present study was chosen based on its promise to maximize synchrony between cortical regions [56].

The procedure used in this experiment was induced online functional effects only in animals with bilateral somatosensory excitability (BiAno1). These behavioural changes failed to persist in the after-effect phase in the absence of stimulation. It is widely accepted that tDCS effects are multifactorial and are capable of generating changes in different neurobiological systems [74] such as neurotransmitter functions [63] and plasticity linked to neutrophic actions, including those exerted by brain-derived neurotrophic factor (BDNF; [64]). Furthermore, it has been previously suggested that polarization effects of the neuronal membrane are responsible for the short-term effects of tDCS, whereas the long-lasting effects are caused by the modulation of *N-Methyl-D-aspartate* (NMDA) receptor strength [78] or changes in protein synthesis [65]. Although, the effects underlying tDCS cannot be simplified to only one mechanism, the short and online behavioural effect of anodal tDCS in group BiAno1 seems to be caused by changes in the neuronal polarization and an increased neuronal firing rate in the stimulated regions. tDCS-induced changes in sodium and calcium channels [66] and in glial cells [67] seem to be potential modulators of the excitability-enhancing online-effect of anodal tDCS. Moreover, because it is possible that the increased firing rate of S1 neurons during bilateral stimulation involves transcallosal connections and callosal glutametergic input [68], some features of the functional plasticity in the present study can be attributed to the increased interneuron activity, probably mediated by callosal intermispheric projections to both M1 and S1 to improve somatosensory capacities and consequently to enhance the M1 activity. Somatosensory glutomatergic pathways to M1 neurons, therefore, may play a key role to potentiate the ipsilateral and contralateral excitatory feedback during the stimulation.

tDCS-induced functional plasticity might originate from transient modifications of synaptic efficacy similar to mechanisms underlying long-term potentiation (LTP) of synaptic activity [57]. Some features of this plasticity mediated by enhanced tDCS-dependent synaptic potentiation have been previously reported in which anodal tDCS elicited a short lasting synaptic potentiation [64]. An alternative possible mechanism for functional online effects of tDCS includes enhanced psychomotor activity by alleviating the effects of stress and depression [69,70]. While stress is known to reduce skilled reaching performance, administration of antidepressants was shown to enhance skilled reaching success [71,72].

On the other hand, the enhanced focal cortical excitability is not only polarity-dependent, but also determined by the stimulation intensity [55]. Group BiAno2 in the present experiment received higher intensity of anodal direct current to both hemispheres. Both functional and structural data in this group failed to show improvement in reaching movement and neural density indicating that the 64 μA protocol for 10 min accompanied by an increased intensity of the current every 5 s, although not disrupting, could not improve skilled movement as the group BiAno1 did. The paradoxical face of the finding, when compared to BiAno1, refers to the neutral (not improving and not disrupting) effect of higher intensity of the stimulation. Did the protocol of the stimulation really create a new challenge to adjacent, even subcortical regions? If this is the case sometimes, as reported by an early study [73], can the neutral effect of the BiAno2 protocol be attributed to, for instance, simultaneous inhibitory inputs from deeper cerebral layers re-activated by the increased current intensity? The robust effectiveness of the protocol used for the BiAno1 group, on the other hand, showed that the applied current intensity in the protocol remained within safe limits along with improving effects, although all findings in both groups still need to be confirmed by further animal research.

A cytoarchitectonic measure of the cerebral cortex [23,25,26] was also used to reveal any potential morphological changes in different stimulation groups. Although the assessment of the cortical thickness did not show any difference between stimulation groups, further histological examination suggested that only rats in the group BiAno1 increased neural density measured by grey value index (GVI, [25]). We also failed to find a significant correlation between the enhanced GVI and functional improvements during online-effect phase in BiAno1 rats indicating that the observed behavioural improvement probably did occur independently or in absence of the tDCS-induced morphological alteration in the BiAno1 group. Several neurobiological mechanisms have been reported in relation to the long-lasting structural effects of focal tDCS ([18,59,79]; see [74] for review). The discrepancy between the online functional improvement and aftereffect-related structural plasticity, however, refers to an important aspect of tDCS-induced changes in this study: transient behavioural improvement does not necessarily represent a particular gross anatomical reconstruction, and conversely these anatomical changes seem difficult to be translated into some definite behavioural enhancement.

5. Conclusion

The present observations confirm that alteration of bilateral cortical excitability of S1 not only improves skilled reaching accuracy, but also selectively enhances the quality of movement components. The functional changes confirm motor control of contralateral limbs, but also suggest a vital role of the ipsilateral hemisphere in the control of skilled reaching. Our study confirms the existence of associative connections between S1 and M1 [35], although it is still unclear how the bilateral somatosensory interaction modifies the individual movement components of skilled reaching.

The present results suggest that tDCS serves as a valid approach to study regional and interhemispheric control of behaviour in the intact brain. However, it remains to be determined how the enhanced interhemispheric interaction selectively influences skilled reaching performance. The additive value of tDCS-induced morphological plasticity provides further support for the idea that tDCS may induce local and distant plastic changes [74]. The main task for future studies, therefore, becomes to identify how modulation of facilitatory and/or inhibitory pathway activity can improve motor and somatosensory capacities, particularly in clinical conditions.

Disclosure/conflict of interest

There are no conflicts of interest.

Acknowledgements

This research was supported by Alberta Innovates – Health Solutions (AI-HS) to GM and AL, the Canadian Institutes of Health Research (CIHR) to GM and Natural Sciences and Engineering Research Council of Canada (NSERC) to AL. GM is a Senior Scholar of Alberta Innovates-Health Solutions.

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