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Full-length original article

Anodal tDCS of contralateral hemisphere modulates ipsilateral control of spinal motor networks targeting the paretic arm post-stroke

running title: Ipsilateral tracts in stroke motor recovery

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Highlights

Anodal transcranial direct current stimulation was applied over the undamaged hemisphere in hemiparetic stroke subjects.

The aim was to explore effects induced by stimulation of the undamaged motor cortex in spinal motor networks.

Activation of the undamaged hemisphere reveals an ipsilateral control onto spinal motor networks of the hemiparetic side.

Abstract

Objective: The role of ipsilateral motor cortex efferent pathways in the transmission of voluntary command to spinal motor nuclei remains controversial in humans. In healthy subjects, their implication in cortical control is hidden by predominant role of crossed corticospinal tract. However, evidence from electrophysiological and imaging studies suggest that ipsilateral tracts may contribute to functional recovery after unilateral brain damage. This randomized-sham control study aims to explore to what extent ipsilateral tracts from the undamaged hemisphere may strengthen corticospinal control onto spinal motor networks following stroke.

Method: Anodal transcranial direct current stimulation (tDCS) was combined with monosynaptic H-reflex method to evaluate the variations of reciprocal inhibition (RI) in wrist flexors in 21 stroke participants.

Results: Anodal tDCS decreased RI in wrist flexors in stroke participants in both arms. tDCS unmasks an ipsilateral control from the undamaged hemisphere onto spinal motor networks controlling affected arm muscles in stroke participants. In the unaffected (contralateral) arm, effects in stroke participants were opposite to those induced in healthy subjects.

Conclusion: Stimulation of the ipsilateral undamaged cortex in stroke participants induces modulation of motor networks controlling the hemiparetic side.

Significance: Rehabilitation could leverage stimulation of the undamaged hemisphere to enhance motor recovery post stroke.

Introduction

Post stroke spontaneous recovery is possible, but often remains incomplete. Most survivors still exhibit some motor impairment that further affect their daily life activities. Cortico-subcortical lesions induced by stroke affect motor cortex efferent pathways that mainly project onto spinal networks and onto the contralateral hemisphere.

Among the alterations following stroke, disruption in the interhemispheric connections has been identified as a mechanism contributing to motor impairment. It has been well described that both hemispheres are interconnected through transcallosal fibres that relay mutual interhemispheric inhibition (Cracco et al., 1989, Ferbert et al., 1992, Boroojerdi et al., 1996). In humans, transcranial magnetic stimulation (TMS) studies have reported a high abnormal interhemispheric inhibition from the unaffected hemisphere to the affected hemisphere following hemiparetic stroke (Daskalakis et al., 2002, Ward et al., 2003, Murase et al., 2004, Fregni et al., 2005, Hummel et al., 2005) which likely interferes with functional recovery (Boroojerdi et al., 1996, Nowak et al., 2009). These findings laid on the basis of the interhemispheric post stroke imbalance model from which stems the rationale that non-invasive brain stimulation methods such as transcranial direct current stimulation (tDCS), repetitive TMS or theta burst stimulation may help to restore mutual transcallosal inhibition following stroke. To this end, neuromodulation methods have been extensively investigated either to upregulate neuron excitability in the lesional motor cortex or inversely to downregulate excitability in the undamaged motor cortex and even to modulate simultaneously both hemispheres (Simonetta–Moreau 2014, Kubis 2016, Lefaucheur et al., 2014, 2017, 2020). Lately, some evidence from electrophysiological and imaging studies have come at odds with the abnormal interhemispheric inhibition from the undamaged hemisphere and support that plastic changes occurring in spared motor areas located not only in the ipsilesional but also in the contralesional hemisphere may contribute to recovery (Chollet et al., 1991, Gerloff et al., 2006, Lotze et al., 2006, 2012, Di Pino et al., 2014, Allman et al., 2016, Mohapatra et al., 2016). Indeed, it was shown that the down regulation of the undamaged hemisphere excitability may get worse performance in the paretic upper limb in stroke subjects with more severe impairment (Ward et al. 2006, 2007, Bradnam et al. 2012, McDonnell and Stinear, 2017). All together, these findings gave rise to the vicariation model which assumes that activity in residual networks may surrogate lost functions in the damaged area.

Besides causing cerebral lesion, stroke induces disruption in corticospinal pathways that arise primarily from the motor cortex and project to the spinal motoneurons. Accordingly, the brain stimulation approach mentioned above could strengthen residual cortical control onto spinal neurons by recruiting the corticospinal projections that remain functional after stroke. Ipsilateral corticospinal projections have been identified in both mammals and humans thanks to physiological and anatomical studies (Kuypers, 1964, Nathan et al., 1990, Lemon, 2008). However, TMS studies reported that ipsilateral motor evoked potentials are scarce in healthy subjects and occur uneasily in forearm and hand muscles even with strong voluntary contraction and high TMS intensity (Wasserman et al. 1991, 1994, Ziemann et al., 1999, Tazoe and Perez, 2014). Based on these findings, it was assumed that ipsilateral corticospinal projections poorly contribute in the transmission of voluntary motor commands to spinal neurons in healthy subjects. Nevertheless, ipsilateral motor evoked potentials have been observed in several pathological conditions including congenital brain lesions and acquired brain lesions, such as stroke (Benecke et al., 1991, Mazevet et al., 2003, Alagona et al., 2001). Consistent with these findings, ipsilateral motor pathways from the undamaged hemisphere are relevant candidates to substitute the crossed corticospinal tract in transmission of the motor command to the spinal cord after stroke. There is some literature addressing the effects of excitatory stimulation of the undamaged hemisphere on motor recovery in paretic side (Rushmore et al., 2013, Cunningham et al., 2015, Buetefisch, 2015, McDonnell and Stinear, 2017). Non-invasive brain stimulation methods were used in healthy subjects and in neurological patients to explore corticospinal control onto spinal motor neurons (Pierrot-Deseilligny and Burke, 2012). However, the effects of excitatory stimulation of the undamaged hemisphere on excitability of spinal motor networks controlling the hemiparetic side have been rarely investigated (Mazevet et al., 2003, McCambridge et al., 2018).

Reciprocal inhibition (RI) is an essential spinal neuron network that orchestrates the contraction in antagonistic muscles. It is composed of Ia afferent from neuromuscular spindle and Ia inhibitory interneurone that projects on the motoneurone of the antagonists. It was shown that motoneurone and Ia interneurone are controlled in parallel from the brain to produce coordinated contraction of agonists and relaxation of antagonists (Lundberg, 1970). Given its supraspinal controls, transmission in RI pathways was found decreased at rest in spastic patients with multiple sclerosis (Crone et al., 1994, Morita et al., 2001) and in hemiplegic patients (Nakashima et al., 1989, Crone et al., 2003).

Electrophysiological experiments in humans provides evidence that RI between flexor and extensor in the forearm differs from the "true Ia reciprocal inhibition" described in the cat and in humans at ankle or at elbow levels. Thereby, it has been suggested that interneurons mediating RI between wrist flexor and extensor probably share more characteristics with Ib interneurons than with Ia inhibitory interneurons (Aymard et al., 1995, Wargon et al., 2006). Regarding their descending controls, Ib interneurons receive a cortical excitation and a powerful controlling input from the reticulospinal tract and thus are influenced by contra and ipsilateral descending controls (Crosby et al., 1962).

The present study aims to stimulate undamaged motor cortex using excitatory anodal tDCS in order to investigate the possible role of ipsilateral corticospinal pathways in transmission of voluntary motor commands to spinal neuron networks. Previously, in healthy subjects, we showed that excitatory (anodal) tDCS applied over the motor cortex was able to modulate excitability in various contralateral motor neuron networks in the spinal cord including RI (Roche et al., 2009, 2011, 2012). Twenty-one stroke patients were investigated by using the same method developed in healthy subjects (Roche et al., 2009). The results obtained in the present experiments provide evidence that activation of the undamaged hemisphere recruits ipsilateral descending pathways that project on spinal networks involved in the motor control of the paretic side and may contribute to functional recovery following stroke.

Materials and Methods

Ethical Approval

The experiments described here were performed in accordance with the Declaration of Helsinki guidelines (World Medical Association). The study was approved by the local ethical committee (CPP Île-de-France VI-Pitié-Salpêtrière). All subjects gave written informed consent before participating in the experiments.

Participants

A randomized sham-controlled study was performed in twenty-one stroke participants with unilateral stroke. Hemiparetic participants from two Physical Medicine and Rehabilitation departments (Pitié-Salpêtrière Hospital and Sainte Marie Foundation) were included during their regular post-stroke rehabilitation training. Stroke participants aged 28 to 78 years (mean 54.8 ± 2.8 years, 4 women) were recruited (Table 1). They were enrolled if they fulfil the following inclusion criteria: hemiparetic subjects following unilateral stroke de novo, age over 18 years and able to understand the consent form.

Exclusion criteria were suffering from other neurological disorders, psychiatric disorders, having contraindications to tDCS such having of epileptic seizures, using serotonergic or dopaminergic drugs, having a metallic implant (i.e. pacemaker, cochlear implant...), having drug or alcohol addiction. Sixteen out of 21 participants were recorded twice to test both active and sham tDCS with a washout period of one week. Four subjects participated in only one session as they were discharged from the rehabilitation departments before undergoing the second experiment. One participant did not wish to continue the study.

Data obtained in stroke participants were compared to data from 38 healthy subjects (age ranged from 21 to 57 years, mean age: 32.88 ± 11.8 years; 13 men) that were collected in our previous studies (Roche *et al.*, 2009; Lackmy-Vallée *et al.*, 2014).

Transcranial direct current stimulation of the motor cortex

tDCS was delivered using a NeuroConn DC-stimulator (NeuroConn GmbH Company, Ilmenau, Germany) *via* two conductive rubber electrodes placed in saline-soaked sponges (5x7 cm). The anode (active electrode) was positioned 2cm lateral from Cz along the line connecting C3-Cz-C4, to stimulate the primary motor cortex (M1) of the upper limb area (Figure 1A) (Brasil-Neto

et al., 1992, Yousry et al., 1997). The anode was applied over the unaffected hemisphere and the cathode was placed over the contralateral supra-orbital region (see Figure 1A). Current was delivered in anodal polarity to increase excitability in the unaffected motor cortex. Two conditions were tested: 1) active tDCS, the current was delivered for 20 minutes, 2) sham tDCS using the same set-up but current was applied only for 120 seconds at the beginning and for 30 seconds at the end of stimulation to mimic the ramp up and ramp down sensations as commonly perceived in the active condition. In both conditions, current intensity was set at 1.75 mA. The current was ramped up and down over the first and last 8 seconds of stimulation. Constant current flow was controlled and monitored by a volt-meter during stimulation. At the end of each session, the participants were asked about their perception of tDCS. Some of them reported a tickling sensation when the current ramped up but this sensation faded after a few minutes. None of the 16 participants recording twice reported differences between sham and anodal conditions which confirmed that they were blinded to the condition tested.

Measurement of spinal network excitability

Electromyogram (EMG) recordings

Stroke participants with mild or moderate motor impairments were transferred to a comfortable reclining armchair. For those who were not able to walk, they were kept seated in their wheelchair. Armrests were set with a slight 60° shoulder abduction, elbows were semi-flexed and wrists were extended. EMG activity was recorded at rest from flexor carpi radialis (FCR) and extensor carpi radialis (ECR) using bipolar surface electrodes placed over the muscle belly. Note that both upper limbs (affected and unaffected sides) were recorded in the same run (Figure 1A). EMG activity was amplified ($\times 5000 - 10,000$) and filtered with a band-pass filter (100 Hz–3 kHz) through a Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK). EMG activity was sampled and digitized at 2 kHz (Power 1401 A/D board, Cambridge Electronic Design). EMG data were stored on a computer for offline analyses.

Electrical stimuli

Test and conditioning stimulations consisted of percutaneous electrical stimuli (rectangular shocks of 1 ms duration) delivered at 0.33 Hz through bipolar electrodes (brass half sphere of 3 cm diameter) applied to the peripheral nerve. Electrical stimuli were delivered with a constant-current stimulator (D7SA Digitimer Ltd, Welwyn Garden City, UK). Test stimulation

evoking monosynaptic H-reflexes in FCR was delivered to the median nerve using electrodes placed 2 cm below the elbow in the medial side into the cubital fossa. Peak to peak amplitude of the maximal H-reflexes (Hmax) in FCR was firstly estimated to adjust the unconditioned H-reflex at 50 % of Hmax amplitude. Attention was paid to keep constant amplitude of unconditioned H-reflex over time since the sensitivity of H-reflex to facilitation or inhibition can vary with its unconditioned size (Crone et al., 1990). EMG activity from FCR and ECR were recorded and displayed on an oscilloscope screen online and then offline analyses were performed. The amplitude of the maximal motor response (Mmax) was measured at the end of the experiment to normalize the data. We had to ensure that the unconditioned H-reflex was large enough to evaluate the effects of the conditioning stimulation and to follow its variations for the 40 minutes of the experiment. In a few participants, it was not possible to evoke a complied H-reflex in FCR on both sides. Twenty out of 21 stroke participants exhibited an H-reflex in FCR in the affected arm and 16 out of 21 participants on the unaffected side (Table 2). Conditioning stimuli were delivered through electrodes placed 2 cm above the elbow on the external part of the arm in the spiral groove to stimulate the radial nerve. The motor threshold was determined by tendon palpation and by EMG displayed on the oscilloscope. The intensity of the conditioning stimuli was set between 0.9 to $0.95 \times MT$. The mean intensity of the conditioning stimulation was $0.92 \pm 0.02 MT$ on the affected side and $0.94 \pm 0.02 MT$ on the unaffected side. The interstimulus interval (ISI) was the time interval between test and conditioning stimuli at which the degree RI was maximum. The ISI was determined using 0.5 ms steps in the range $-1 ms / + 3 ms$ (Day et al., 1984).

Experimental procedure

To avoid carry-over effects, the two conditions (sham and anodal tDCS) were separated by one week. The degree of RI from ECR to FCR in both arms was recorded in the same run before tDCS. First, experimental parameters such as unconditioned H-reflex amplitude, conditioning stimulation intensity and ISI were determined in each limb (Figure 1A). One run lasted 3 minutes and consisted of 60 H-reflexes in FCR: 30 H-reflexes evoked in affected arm (15 unconditioned and 15 conditioned-reflexes) and 30 H-reflexes elicited in the unaffected arm (15 unconditioned and 15 conditioned-reflexes) (Figure 1B). Stimulations were driven by Signal software and randomly alternated every 3 sec by side (affected *vs.* unaffected arm) and by H-reflexes (unconditioned *vs.* conditioned). Once all parameters were set, they remained

unchanged throughout the experiment in stroke participants as well as in healthy subjects. Then, the mean degree of RI in each upper limb was evaluated for 10 minutes (3 successive runs) corresponding to baseline period. Then, tDCS electrodes were attached on the skull with adhesive tape. The current was delivered either for 2 minutes (sham intervention) or for 20 minutes (anodal tDCS) (Nitsche and Paulus, 2000). Variations of degrees of RI were monitored by repetitive measurements for 20 minutes split in two periods of 10 minutes (Per1 tDCS and Per2 tDCS). The tDCS electrodes were removed at the end of the 20 minutes. To evaluate the post-effects, the degree of RI in each side was assessed for 10 minutes after the end of tDCS application. Each period of 10 minutes was composed of 3 runs of 60 H-reflexes (Figure 1C).

Data analysis

Data expression

The H-reflex size was determined by averaging peak-to-peak amplitudes expressed as a percentage of the maximal motor response (M_{max}). The ratio H_{max}/M_{max} was expressed as a percentage. The degree of RI was defined as $((\text{unconditioned H-value} - \text{conditioned H-value}) / \text{unconditioned H-value}) \times 100$. Table 2 sums up the individual values of H_{max}/M_{max} ratio and mean RI estimated at baseline and in Per2. Data were shown as mean \pm Standard Error of the mean (SEM).

Statistics

Statistical analyses were performed using Sigma plot 12.5 software and JMP software for the modelling part. The alpha significance level was fixed at 0.05 and the results were considered statistically significant only if $p < 0.05$. Homoscedasticity and normality (Shapiro-Wilk test) were firstly verified to allow parametric analyses. Alternatively, non-parametric methods were used.

The present series of experiments was performed in hemiparetic stroke participants. Two conditions were tested anodal (active) and sham tDCS. H-reflexes in the FCR were evoked in the unaffected and in the affected arms. From the 21 hemiparetic stroke participants, 5 subjects attended to only one session and in 5 subjects an H-reflex in FCR could not be evoked in both sides.

To deal with those missing data and to study sources of variability, statistical analysis was organized in two stages: a first descriptive step and a “modelling” step (mixed model). This allowed to whole process the data without excluding subjects with missing data.

The first "descriptive step" was performed to draw on an overview of effects induced by anodal tDCS directly on the degree of RI in stroke participants. The main factors of influence on the degree of RI were investigated separately (side: contralateral or ipsilateral, condition: sham or anodal tDCS, time period: baseline, Per1 tDCS, Per2 tDCS, Post tDCS). When comparing two means (side or condition), *t*-test or non-parametric Wilcoxon test were performed depending on verification of conditions (normality and equal variance). For comparison of time period, One-way repeated measures (rm) ANOVA or Friedmann test (rm ANOVA on ranks) were performed depending of verification of conditions. One-way rm ANOVA were followed (if significant) by a “post-hoc analysis” using Newman-Keuls method in order to specify origin of differences.

The second “modelling” step, was performed using mixed models in order to estimate the possible influence of clinical characteristics of stroke subjects on effects induced by anodal tDCS. To avoid inter-subject variability of RI at baseline, we calculated the mean value of RI_t (Per1, Per2, Post tDCS) – RI_{t0} (baseline). The model was based on mean $RI_t - RI_{t0}$ as a function of time period (fixed effect), participants (random effect) and successively gender, age or covariables such as delay elapsed since the stroke, brain lesion location and two clinical outcome measures: Medical Research Council (MRC) scale to estimate muscle strength and the modified Ashworth scale (MAS) to evaluate spasticity. This was done for each side (contralateral or ipsilateral). Because of the small number of subjects, only models with one cofactor have been kept.

Lastly, we previously studied in healthy subjects the effects of anodal tDCS onto RI evaluated in both arms using the same experimental design. Despite the mean age between two populations were not equal (see Table 1), we have applied a mixed model in order to explore differences between two populations considering parameters of experience and covariables.

Results

Unconditioned H-reflex in FCR and RI at baseline estimated in both arms

Before reporting the effects of tDCS onto RI in stroke participants, the following paragraph presents the main features of unconditioned H-reflex and RI at baseline (so without tDCS application)

Twenty out of 21 stroke participants exhibited an H reflex in FCR on the affected arm. Sixteen out of 21 participants exhibited an H reflex in FCR on the unaffected arm (Table 2). The mean latency of FCR H-reflex estimated in the affected arm was 18.07 ± 0.5 ms and 18.28 ± 0.7 ms in the unaffected arm. In the 16 subjects who exhibited H-reflex in both arms, the mean latency in the affected arm was 18.14 ± 0.6 ms and 18.28 ± 0.7 ms in the unaffected arm (paired-*t*- test, $P = 0.881$, see Table 2). In those subjects, the mean Hmax/Mmax ratio was larger on the affected arm ($46.76 \% \pm 6.0$) with regards to the unaffected arm ($30.42 \% \pm 4.7$) (paired-*t*- test, $P = 0.006$, see example in Figure 1B). The H_{max}/M_{max} ratios in both arms were within the range previously observed in spastic stroke subjects (Aymard et al., 2000; Lamy et al., 2009).

To ensure that experimental conditions were stable, offline analyses were computed to verify that the unconditioned H-reflexes evoked in FCR on both arms did not vary throughout the experiment: the affected arm (rm-ANOVA on ranks, $ddl = 3$, $Q = 2.820$, $P = 0.420$); the unaffected arm (rm-ANOVA on ranks, $ddl = 3$, $Q = 1.923$, $P = 0.589$).

The distribution of RI estimated at baseline (without tDCS) on affected and unaffected sides stressed an important inter-individual variability in stroke participants especially in the affected side ($P < 0.001$) (data not shown).

Effects of sham stimulation in both arms

Figure 2A shows the variations of RI estimated in FCR on the unaffected arm, i.e. contralateral to anode position in the sham condition. Twelve out of 16 stroke participants exhibited an H reflex in FCR on the unaffected side took part in experiments with sham tDCS. Sham tDCS was inefficient to modulate RI (rm-ANOVA, $ddl = 47$, $F = 1.164$, $P = 0.194$, see Table 3).

Figure 2C represents the variations of RI estimated in FCR of the affected arm, i.e. ipsilateral to anode position. Sixteen out of 20 stroke participants exhibited an H reflex in FCR on the affected side were recorded in sham. Sham tDCS did not modulate RI considering differences between time period ($ddl = 63$, $F = 2.366$, $P = 0.084$, see Table 3).

This is consistent with our previous studies since sham tDCS was inefficient to induce effects in stroke subjects as in healthy subjects irrespective to the side.

Modulations of RI induced by anodal tDCS

Results obtained in the unaffected arm (contralateral)

Figure 2B shows the variations of RI estimated in FCR of the unaffected arm, i.e. contralateral to anode position. Sixteen participants that exhibited H reflex in FCR on the unaffected side were recorded with anodal tDCS. Anodal tDCS decreased RI (rm-ANOVA, $ddl = 63$, $F = 3.001$, $P = 0.04$) during the 20 minutes of stimulation. *Post hoc* analyses showed differences between baseline *vs.* Per1 tDCS and between baseline *vs.* Per2 tDCS (see Table 3).

Results obtained in the affected arm (ipsilateral)

The main result of this series of experiments is depicted in Figures 2D which represents the variations of RI estimated in FCR of the affected arm, i.e ipsilateral to anode position. All participants that exhibited H-reflex in FCR on the affected side were recorded with anodal tDCS. Anodal tDCS decreased RI ($ddl = 79$, $F = 3.633$, $P = 0.018$) that persisted beyond the stimulation duration, since *post hoc* analyses showed a difference between baseline *vs.* Post tDCS (see Table 3).

Factors that could influence the effects induced by tDCS in hemiparetic stroke subjects

In a second step using mixed model, we investigated the possible influence of covariable i.e. age, gender or clinical characteristics of stroke participants on the effects induced by tDCS on $RI_t - RI_{t0}$. The clinical factors investigated were post-stroke onset, brain lesion location (cortico-subcortical lesion or subcortical lesion) and two clinical outcome measures (MRC and MAS). To avoid the inter-individual variability of RI estimated at baseline, the mean RI_t (Per1, Per2, Post tDCS) $- RI_{t0}$ (baseline) were calculated.

To focus on the effects induced by tDCS, only the data collected during active tDCS condition were considered ($n = 20$) and each arm (contralateral, ipsilateral) were considered independently. Then, $RI_t - RI_{t0}$ was considered as a linear function of time period (fixed effect), participants (random effect) and successively one cofactor previously described. From those 18 models (9 cofactors x 2 sides), only MRC score of the elbow flexor evaluated in the affected

arm (ipsilateral side) showed a significant influence ($P < 0.05$). This suggests that the more severe impairment in forearm, the less RI was depressed by anodal tDCS. However, because of multiplication of tests, we should be careful with such conclusion.

Comparison of changes in RI induced by anodal tDCS in stroke subjects vs. healthy subjects

We previously studied in healthy subjects, using the same experimental design, the effects of anodal tDCS onto RI evaluated in both arms (contralateral and ipsilateral to the anodal tDCS). Figure 3 shows the modulations of RI induced by anodal tDCS observed in stroke participants and in healthy subjects. Obviously, descriptive comparison of RI modulations obtained in both groups showed qualitative differences.

The baseline value was lower in stroke participants with respect to healthy subjects irrespective of side (contralateral $P_{\text{baseline}} < 0.001$; ipsilateral: $P_{\text{baseline}} < 0.001$). To ensure that these differences were not influenced by the population characteristics (age and gender), variations of RI ($RI_t - RI_{t0}$) were studied in a mixed model with time period, status, and conditions as fixed effects and subjects as random effect. Given the sample size and the difference of mean ages between groups, results from linear models need to be considered cautiously. However, statistical analyses confirmed that age or gender have no significant effect.

The model supports the results obtained from the descriptive step: 1) in the contralateral upper limb, effects induced by anodal tDCS were different ($P_{\text{status}} < 0.001$), anodal tDCS led to a decreased RI in stroke participants (Figure 3A), while in healthy subjects RI was increased during tDCS application (Figure 3B), and the degree of RI returned to its baseline value at the end of stimulation in both populations. 2) In the ipsilateral arm, anodal tDCS did not modulate RI in healthy subjects (Figure 3D), while in stroke participants it induced a decrease of RI which outlasted tDCS application ($P_{\text{status}} = 0.02$) (Figure 3C).

Discussion

In this series of experiments, we investigated changes induced by anodal tDCS on spinal neuron networks relaying RI in wrist flexors in stroke participants. The degree of RI was estimated in the affected and in the unaffected arm and the modulations of RI were followed during and after tDCS application. To the best of our knowledge, very few studies have investigated the changes in spinal networks excitability in the unaffected side. Likewise, RI in the unaffected arm has

never been studied in unilateral stroke participants. The first paragraph of the discussion is focused on the comparison between the degrees of RI estimated at baseline in both arms in stroke participants regarding to in healthy subjects (data from our previous studies). Unexpectedly, modulations of RI induced by tDCS applied over the undamaged hemisphere in the contralateral arm (unaffected arm) are reversed regarding that observed in the contralateral arm in healthy subjects. The second paragraph of the discussion sets out the hypotheses likely to explain this reversal. Finally, the main finding of the present study i.e. the effects of the tDCS stimulation on the ipsilateral side in stroke participants and its potential functional significance are presented in the last paragraphs of the discussion.

Stroke participants exhibited a reduced RI in flexor wrist muscles in both upper limbs at baseline

Before tDCS application, RI at baseline in the affected and unaffected arms in stroke participants was lower than that evaluated in healthy subjects. In healthy subjects, there is no significant asymmetry between the mean values of RI obtained in dominant and non-dominant sides (Elodie Garrec, PhD thesis, 2019). The reduction of RI that we observed here in the affected arm is consistent with results of previous studies that reported modifications of the excitability in various spinal networks following stroke such as i) a clear reduction of RI and presynaptic inhibition assessed with D1 inhibition of the FCR H-reflex, homosynaptic depression in the affected arm of hemiparetic subjects (Nakashima et al., 1989, Aymard et al., 2000, Lamy et al., 2009) ii) a hyperexcitability of the propriospinal neurons controlling wrist motoneurons (Mazevet et al., 2003). Abnormalities of neural network transmission following stroke are generally interpreted as a defect in supraspinal controls acting on spinal neuron networks. Indeed, it was shown in animal experiments that spinal neuron networks are influenced by supraspinal inputs mainly relayed by corticospinal tract and reticulospinal tract (Lundberg and Voorhoeve, 1962). In the cat, it was well described that the motoneuron and its corresponding interneuron mediating RI receive similar segmental and descending controls (Lundberg, 1970). Likewise, experiments in humans provided evidence that pathways mediating RI from extensors to flexors are influenced by excitatory corticospinal inputs (Day et al., 1984, Rothwell et al., 1984, Cavallari et al., 1984, Cowan et al., 1986, Kudina et al., 1993). Therefore, it is not surprising that the cortical lesions induced by stroke lead to a decrease

of the excitatory cortical inputs and thus to a subsequent decrease in neuron excitability in the spinal cord.

Regarding the unaffected arm, a reduced RI was found in stroke participants compared to healthy subjects. This result aligns with few studies performed in the unaffected side. For example, in the upper limb, pathological stretch reflexes have been observed on both sides in hemiparetic subjects (Thilmann et al., 1990) and slight muscle weakness was measured in unaffected arm in subject with unilateral stroke (Colebatch and Gandevia, 1989). In the lower limb, reciprocal Ia inhibition from ankle flexor to extensors is almost inexistent in unaffected leg in hemiparetic subjects (Crone et al., 2003) while results addressing presynaptic inhibition remain controversial. Some studies reported that presynaptic inhibition of the unaffected limb was close to the one observed in healthy subjects (Aymard et al., 2000, Crone et al., 2003) whereas Lamy et al. (2009) found a depressed presynaptic inhibition in both limbs in stroke subjects. Even though, the unaffected side in hemiparetic subjects is usually referred as the intact side, abnormalities may occur and would deserve to be more consistently investigated.

Anodal tDCS oppositely modulates RI in stroke participants compared to healthy subjects

Previously in healthy subjects, we studied RI directed to FCR motoneurons but also RI directed to ECR motoneurons. We have shown that contralateral anodal tDCS increases RI directed to FCR (Roche et al., 2009) and decreases RI directed to ECR (Lackmy-Vallée et al., 2014). We hypothesized that opposite modulations induced by anodal tDCS on pathways relaying RI onto flexor and extensor motoneurons at wrist level reflect an asymmetric control onto flexor and extensor motor nuclei. This asymmetric control may take place either at supraspinal level or at segmental level.

Unexpectedly, in stroke participants in conditions similar as to healthy subjects, tDCS decreases RI directed to FCR in the unaffected arm (contralateral side to anodal tDCS). A first hypothesis is that this reversal is linked to differential imbalance of interhemispheric inhibition following stroke. As stressed in the introduction, the mutual inhibition between ECR and FCR cortical motor areas has been disrupted leading to an over activation of the corresponding area in the undamaged hemisphere (Daskalakis et al., 2002; Ward et al., 2003, Murase et al., 2004, Fregni et al., 2005, Hummel et al., 2005). In healthy subjects, deNoordhout et al. (1999) suggested that the cortico-motoneuronal synaptic connections are stronger on wrist and finger extensor

motoneurons than on flexor motoneurons. It thus may be assumed that the imbalance in interhemispheric inhibition following stroke differentially affects the motor area in charge of wrist extensors and flexors, and so it would be more detrimental for wrist extensor motor areas. The reversal of the asymmetry in favor of wrist flexor motor area would result in a predominant control acting on flexor motoneurons and likely on the RI interneurons-leading to a reduced RI from extensors to flexors.

A second hypothesis relies on the possible asymmetry in organization and pattern of connections of inhibitory interneurons mediating reciprocal inhibition in flexor and extensor motor nuclei. Animal experiments showed that i) maximal Ia IPSPs are much larger in flexor than in extensor motoneurons (Eccles and Lundberg, 1958) ii) Ia interneurons activated from flexor Ia afferents inhibit Ia interneurons activated from extensor Ia afferents, and vice versa as Hultborn et al. (1976) showed that there is a mutual inhibition between interneurons mediating RI. In humans, Baldissera et al. (1987) using monosynaptic H-reflex method in *extensor to flexor* wrist muscles provided evidence supporting the existence of mutual inhibition between opposite interneurons mediating RI at wrist level. In healthy subjects, RI relayed to wrist extensor motoneurons was found stronger than that transmitted to wrist flexor motoneurons (Lackmy-Vallée et al., 2014). Stroke causes a defect in corticospinal control onto reciprocal inhibition pathways, which probably leads to strengthen this asymmetry in transmission of RI in flexor and extensor motor nuclei, and so depresses more the RI directed from *extensor to flexor* wrist muscles. From this perspective, application of anodal tDCS over the undamaged motor cortex would increase the mutual inhibition in favor of flexor inhibitory interneurons and so would lead to a decreased RI from *extensor to flexor* wrist muscles.

Anodal tDCS over the undamaged hemisphere modulates the RI in the affected upper limb in stroke participants

Previously in healthy subjects, we have shown that anodal tDCS has no effects on RI between wrist motor nuclei in the ipsilateral upper limb. By contrast, in stroke participants, anodal tDCS modulates RI directed to flexor motoneurons to the ipsilateral (affected) upper limb. This finding supports the hypothesis that anodal tDCS may unmask an ipsilateral control from the undamaged hemisphere onto spinal neurons of the affected arm in hemiparetic stroke subjects. Anatomical and behavioral experiments in animals (Kuypers, 1964, Armand and Kuypers, 1980) and post-mortem studies in humans have reported the existence of ipsilateral

corticospinal tract projecting onto neuron networks in the cervical spinal cord (Nathan et al., 1990). However, in healthy subjects, TMS studies reported that ipsilateral motor evoked potentials (MEP) are inexistent in relaxed muscles even though it could be recorded in some upper limb muscles during strong contraction of the target muscles with high TMS intensity (Wasserman et al., 1991, 1994, Ziemann et al., 1999, Alagona et al., 2001, Chen et al., 1997, 2003). In hemiparetic subjects, ipsilateral MEP in the affected arm are more frequent than in healthy subjects and latency of ipsilateral MEP is consistently longer than contralateral MEP (Chollet et al., 1991, Benecke et al. 1991, Turton et al., 1996, Netz et al., 1997, Ziemann et al., 1999). Imaging studies using positron emission tomography and functional MRI revealed that ipsilesional premotor and supplementary motor area are more likely to be activated during the affected upper limb movement in stroke subjects (Ward et al., 2007). Together these findings suggest that ipsilateral projections from the undamaged hemisphere may be unmasked and play a role to compensate damage in crossed corticospinal pathways following stroke. The existence of ipsilateral corticospinal tract may help to supply cortical inputs onto spinal neuron networks controlling the affected upper limb after stroke. Please note that, such electrophysiological investigations conducted in humans do not allow to conclude if ipsilateral control from the undamaged hemisphere onto RI pathways is mediated by uncrossed corticospinal tract or if it goes through indirect pathways such as cortico-reticulospinal tracts that fed inhibitory spinal interneurons.

Potential functional significance

To date, the contribution of the undamaged hemisphere in functional motor recovery is under debate. Some studies reported that, in stroke subjects with poor clinical recovery, cortical activity was bilateral and, in some cases, weighted towards the undamaged hemisphere (Loubinoux et al., 2003, Ward et al., 2003, 2007, Mazevet et al., 2003). In severely affected subjects, the undamaged hemisphere excitability gradually increases as motor function recovers, indicating reorganization toward the undamaged hemisphere (Stinear et al., 2008). This increase in the undamaged motor area excitability is also marked by facilitation of ipsilateral descending projections to the affected upper limb muscles (Turton et al., 1996, Netz et al., 1997). Other studies pointed out activity in the motor area of the undamaged hemisphere during complex task performance with the affected hand in subjects with near-complete motor recovery (Gerloff et al., 2006, Lotze et al., 2006). However, more recent studies using diffusion

weighted imaging have suggested that contribution of the undamaged hemisphere in functional adaptation following stroke depends on the residual integrity of white matter tracts from the lesioned hemisphere, thus the undamaged hemisphere activity is enhanced in subjects with more disruption in corticospinal tract from the lesioned hemisphere and with a greater upper limb impairment (Johansen-Berg et al., 2002, Ward et al., 2003, 2006, 2007, Lotze et al., 2006, Stinear et al., 2007). In subjects experiencing good recovery, the undamaged hemisphere excitability decreases over time (Stinear et al., 2008). This discrepancy raises the question as to what extent facilitation of ipsilateral projections from the undamaged hemisphere may be of benefit for recovery of motor function following stroke.

Altogether, these findings suggest that decreasing activity of the undamaged motor area by the non-invasive brain stimulation in order to restore the interhemispheric inhibition between both hemispheres may be not suitable for all stroke subjects. Instead, neuromodulation protocols should be tailored regarding subject's hallmarks to optimize rehabilitation strategy. In this view, approaches combining clinical measures with diffusion weighted imaging have emerged in attempt to predict the outcome of stroke subjects that undergo rehabilitation training. (Stinear et al., 2010, 2012, Jang et al., 2010, Bradnam et al., 2013). Regarding the clinical characteristics of stroke participants, we observed that participants with moderate impairments in the forearm were more responsive to our tDCS protocol: the higher MRC score at the elbow flexor, the deeper was the RI decrease in the unaffected arm. As mentioned in the introduction, interneurons relaying RI in the wrist muscles differ from inhibitory interneurons that mediated "true Ia RI" described at the ankle and elbow levels in humans. At the wrist level, onto inhibitory interneurons converge group I afferents from the antagonistic muscle and group I afferents originating from the biceps and triceps muscles (Aymard et al., 1995). This pattern of connection may explain results stressed by our mixed model test. This raises the question why the mixed model test did not show an influence of the MRC scores of the wrist flexor and extensor? A hypothesis is that in our group of stroke participants, the MRC scores at the wrist level were more diffuse than those estimated at the elbow level. The marked decrease of RI observed in stroke participants with moderate impairment in the elbow flexor muscles are in accordance with the hypothesis mentioned above suggesting an asymmetric control at supraspinal level or at segmental level in favor on flexor motor nuclei. It seems that group I afferents from the elbow flexor muscle facilitate more FCR inhibitory interneurons leading to a reduced RI from ECR to FCR due to the mutual inhibition. Given the sample size and multiple

variables tested by the mixed model test, further experiments are needed. However, this model did not show influence of other characteristics tested. Nevertheless, this study provides the first clues in attempt screening participants who may be more responsive to this kind of tDCS protocol. We were unable to determine that well-responded patients to tDCS protocol were those having mild impairment post-stroke or having a good recovery.

Nevertheless, standard methods used in electrophysiological investigations provide evidence that ipsilateral projections from the undamaged hemisphere may help to strengthen the residual corticospinal control onto spinal neuron networks involved in the upper limb motor function in stroke people. This opens a way to new approaches combining electrophysiological investigations, imaging studies and clinical evaluations to tailor the use of neuromodulation methods and optimize the effects in unilateral stroke.

Conflict of interest: The authors have no conflicts of interest to declare and have no competing financial interests.

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Author contribution:

Dr Wanalee Klomjai, Dr Mounir El Mendili: data collection, data interpretation, drafting the manuscript for intellectual content. Dr Alain Giron: statistics, data interpretation, drafting of the manuscript for intellectual content. Dr Nicolas Roche, Dr Eleonore Bayen, Dr Claire Aymard, Pr Pascale Pradat-Diehl: stroke participant inclusions, clinical evaluations, drafting of the manuscript for intellectual content. Dr Rose Katz, Dr Alexandra Lackmy-Vallée: Study design, experimental design, study supervision, data interpretation, drafting of the manuscript for intellectual content, generation of figures and tables.

References

- Alagona G, Delvaux V, Gérard P, De Pasqua V, Pennisi G, Delwaide PJ, et al. Ipsilateral motor responses to focal transcranial magnetic stimulation in healthy subjects and acute-stroke patients. *Stroke* 2001;32(6):1304–1309.
- Allman C, Amadi U, Winkler AM, Wilkins L, Filippini N, et al. Ipsilesional anodal tDCS enhances the functional benefits of rehabilitation in patients after stroke. *Sci Transl Med* 2016;8(330):330re1..
- Armand J, Kuypers HG. Cells of origin of crossed and uncrossed corticospinal fibers in the cat: a quantitative horseradish peroxidase study. *Exp Brain Res* 1980;40(1):23–34.
- Aymard C, Chia L, Katz R, Lafitte C, Pénicaud A. Reciprocal inhibition between wrist flexors and extensors in man: a new set of interneurons? *J Physiol* 1995;487(1):221–235.
- Aymard C, Katz R, Lafitte C, Lo E, Pénicaud A, Pradat-Diehl P, et al. Presynaptic inhibition and homosynaptic depression: a comparison between lower and upper limbs in normal human subjects and patients with hemiplegia. *Brain* 2000;123:1688-702.
- Baldissera F, Cavallari P, Fournier E, Pierrot-Deseilligny E, Shindo M. Evidence of mutual inhibition of opposite Ia interneurons in the human upper limb. *Exp Brain Res* 1987;66(1):106–114.
- Benecke R, Meyer BU, Freund HJ. Reorganisation of descending motor pathways in patients after hemispherectomy and severe hemispheric lesions demonstrated by magnetic brain stimulation. *Exp Brain Res* 1991;83(2):419–426.
- Bradnam LV, Stinear CM, Barber PA, Byblow WD. Contralesional hemisphere control of the proximal paretic upper limb following stroke. *Cereb Cortex* 2012;22(11):2662–2671.
- Bradnam LV, Stinear CM, Byblow WD. Ipsilateral motor pathways after stroke: implications for non-invasive brain stimulation. *Front Hum Neurosci* 2013 8;7:184.
- Brasil-Neto JP, McShane LM, Fuhr P, Hallett M, Cohen LG. Topographic mapping of the human motor cortex with magnetic stimulation: factors affecting accuracy and reproducibility. *Electroencephalogr Clin Neurophysiol* 1992;85(1):9–16.
- Borojerd B, Diefenbach K, Ferbert A. Transcallosal Inhibition in cortical and subcortical cerebral vascular lesions. *J Neurol Sci* 1996;144:160-70.
- Buetefisch CM. Role of the Contralesional Hemisphere in Post-Stroke Recovery of Upper Extremity Motor Function. *Front Neurol* 2015 16;6:214.
- Cavallari P, Fournier E, Katz R, Pierrot-Deseilligny E, Shindo M. Changes in reciprocal Ia inhibition from wrist extensors to wrist flexors during voluntary movement in man. *Exp Brain Res* 1984;56:574-6
- Chen R, Cohen LG, Hallett M. Role of the ipsilateral motor cortex in voluntary movement. *Can J Neurol Sci* 1997;24(4):284–291.
- Chen R, Yung D, Li JY. Organization of ipsilateral excitatory and inhibitory pathways in the human motor cortex. *J Neurophysiol* 2003;89:1256-64.

- Chollet F, DiPiero V, Wise RJ, Brooks DJ, Dolan RJ, Frackowiak RS. The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography. *Ann Neurol* 1991;29:63-71.
- Colebatch JG, Gandevia SC. The distribution of muscular weakness in upper motor neuron lesions affecting the arm. *Brain* 1989;112 :749-63.
- Cowan JM, Day BL, Marsden C, Rothwell JC. The effect of percutaneous motor cortex stimulation on H reflexes in muscles of the arm and leg in intact man. *J Physiol*. 1986;377:333-47.
- Cracco RQ, Amassian VE, Maccabee PJ, Cracco JB. Comparison of human transcallosal responses evoked by magnetic coil and electrical stimulation *Electroencephalogr Clin Neurophysiol* 1989;74:417-24.
- Crone C, Hultborn H, Mazières L, Morin C, Nielsen J, Pierrot-Deseilligny E. Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat. *Exp Brain Res* 1990;81:35-45.
- Crone C, Nielsen J, Petersen N, Ballegaard M, Hultborn H. Disynaptic reciprocal inhibition of ankle extensors in spastic patients. *Brain* 1994;117 (Pt 5):1161-8.
- Crone C, Johnsen LL, Biering-Sørensen F, Nielsen JB. Appearance of reciprocal facilitation of ankle extensors from ankle flexors in patients with stroke or spinal cord injury. *Brain* 2003;126:495-507.
- Crosby E.C., Humphrey T. et Lauer E. W. Correlative anatomy of the nervous system. The Macmillan Company, N.Y.,1962.
- Cunningham DA, Varnerin N, Machado A, Bonnett C, Janini D, Roelle S, et al. Stimulation targeting higher motor areas in stroke rehabilitation: A proof-of-concept, randomized, double-blinded placebo-controlled study of effectiveness and underlying mechanisms. *Restor Neurol Neurosci* 2015;33:911-26.
- Daskalakis ZJ, Christensen BK, Fitzgeralds PB, Roshan L, Chen R. The mechanisms of the interhemispheric inhibition in the human motor cortex. *J Physiol* 2002;543(Pt 1):317–326.
- Day BL, Marsden CD, Obeso JA, Rothwell JC. Reciprocal inhibition between the muscles of the human forearm. *J Physiol*.1984;349:519-34.
- deNoordhout AM, Rapisarda G, Bogacz D, Gérard P, De Pasqua V, Pennisi G, Delwaide PJ. corticomotoneuronal synaptic connections in normal man: an electrophysiological study. *Brain* 1999;122:1327-40.
- Di Pino G, Pellegrino G, Assenza G, Capone F, Ferreri F, Formica D, Ranieri F, Tombini M, Ziemann U, Rothwell JC, Di Lazzaro V. Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat Rev Neurol* 2014 ;10(10):597-608.
- Eccles RM, Lundberg A. The synaptic linkage of direct inhibition. *Acta Physiol Scand* 1958;43(3–4):204–215.
- Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. *J Physiol* 1992;453:525-46.

Fregni F, Boggio PS, Mansur CG, Wagner T, Ferreira MJ, Lima MC, et al. Transcranial direct current stimulation of the unaffected hemisphere in stroke patients. *Neuroreport* 2005 ;16:1551-5.

Gerloff C, Bushara K, Sailer A, Wassermann EM, Chen R, Matsuoka T, et al. Multimodal imaging of brain reorganization in motor areas of the contralesional hemisphere of well recovered patients after capsular stroke. *Brain* 2006;129:791-808.

Hultborn H, Illert M, Santini M. Convergence on interneurons mediating the reciprocal Ia inhibition of motoneurons. I. Disynaptic Ia inhibition of Ia inhibitory interneurons. *Acta Physiol Scand* 1976;96:193-201.

Hummel F, Celnik P, Giraux P, Floel A, Wu WH, Gerloff C, et al. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain* 2005;128:490-9.

Jang SH. Prediction of motor outcome for hemiparetic stroke patients using diffusion tensor imaging: A review. *NeuroRehabilitation* 2010;27:367-72.

Johansen-Berg H, Dawes H, Guy C, Smith SM, Wade DT, Matthews PM. Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. *Brain* 2002;125:2731-42.

Kubis N. Non-Invasive Brain Stimulation to Enhance Post-Stroke Recovery. *Front Neural Circuits* 2016. 27;10:56.

Kudina L, Ashby P, Downes L. Effects of cortical stimulation on reciprocal inhibition in humans. *Exp Brain Res* 1993;94:533-8

Kuypers HG. The descending pathways to the spinal cord, their anatomy and function. *Prog Brain Res* 1964;11:178-202.

Lackmy-Vallée A, Klomjai W, Bussel B, Katz R, Roche N. Anodal transcranial direct current stimulation of the motor cortex induces opposite modulation of reciprocal inhibition in wrist extensor and flexor. *J Neurophysiol* 2014;112:1505-15.

Lamy JC, Wargon I, Mazevet D, Ghanim Z, Pradat-Diehl P, Katz R. Impaired efficacy of spinal presynaptic mechanisms in spastic stroke patients. *Brain* 2009;132(Pt 3):734-48.

Lefaucheur JP, André-Obadia N, Antal A, Ayache SS, Baeken C, Benninger DH, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin Neurophysiol* 2014 ;125(11):2150-2206.

Lefaucheur JP, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clin Neurophysiol* 2017;128:56-92.

Lefaucheur JP, Aleman A, Baeken C, Benninger DH, Brunelin J, Di Lazzaro V, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014-2018). *Clin Neurophysiol* 2020;131(2):474-528

Lemon RN. Descending pathways in motor control. *Annu Rev Neurosci* 2008;31:195-218.

Lotze M, Markert J, Sauseng P, Hoppe J, Plewnia C, Gerloff C. The role of multiple contralesional motor areas for complex hand movements after internal capsular lesion. *J Neurosci* 2006;26:6096-102.

- Lotze M, Beutling W, Loibl M, Domin M, Platz T, Schminke U, et al. Contralesional motor cortex activation depends on ipsilesional corticospinal tract integrity in well-recovered subcortical stroke patients. *Neurorehabil Neural Repair* 2012;26:594-603.
- Loubinoux I, Carel C, Pariente J, Dechaumont S, Albucher JF, Marque P, et al. Correlation between cerebral reorganization and motor recovery after subcortical infarcts. *Neuroimage* 2003;20:2166-80.
- Lundberg A, Voorhoeve P. effects from the pyramidal tract on spinal reflex arcs. *Acta Physiol Scand* 1962;56:201-19.
- Lundberg A.(1970) The excitatory control of the Ia inhibitory pathway. In *Excitatory synaptic mechanisms*, ed Andersen, P. & Jansen, J.K.S., pp. 333-340. Oslo: Universitetsforlaget
- Mazevet D, Meunier S, Pradat-Diehl P, Marchand-Pauvert V, Pierrot-Deseilligny E. Changes in propriospinally mediated excitation of upper limb motoneurons in stroke patients. *Brain* 2003;126:988-1000
- McCambridge AB, Stinear JW, Byblow WD. Revisiting interhemispheric imbalance in chronic stroke: A tDCS study. *Clin Neurophysiol* 2018;129:42-50.
- McDonnell MN, Stinear CM. TMS measures of motor cortex function after stroke: A meta-analysis. *Brain Stimul* 2017;10(4):721-734.
- Mohapatra S, Harrington R, Chan E, Dromerick AW, Brededa EY, Harris-Love M. Role of contralesional hemisphere in paretic arm reaching in patients with severe arm paresis due to stroke: A preliminary report. *Neurosci Lett* 2016 23;617:52-8
- Morita H, Crone C, Christenhuis D, Petersen NT, Nielsen JB. Modulation of presynaptic inhibition and disynaptic reciprocal Ia inhibition during voluntary movement in spasticity. *Brain* 2001;124(Pt 4):826-37.
- Murase N, Duque J, Mazzocchio R, Cohen LG. Influence of interhemispheric interactions on motor function in chronic stroke. *Ann Neurol* 2004;55:400-9.
- Nakashima K, Rothwell JC, Day BL, Thompson PD, Shannon K, Marsden CD. Reciprocal inhibition between forearm muscles in patients with writer's cramp and other occupational cramps, symptomatic hemidystonia and hemiparesis due to stroke. *Brain* 1989;112:681-97.
- Nathan PW, Smith MC, Deacon P. The corticospinal tracts in Man. Course and location of fibres at different segmental levels. *Brain* 1990;113:303-24.
- Netz J, Lammers T, Hömberg V. Reorganization of motor output in the non-affected hemisphere after stroke. *Brain* 1997;120:1579-86.
- Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol* 2000;527 :633-9.
- Nowak DA, Grefkes C, Ameli M, Fink GR. Interhemispheric competition after stroke: brain stimulation to enhance recovery of function of the affected hand. *Neurorehabil Neural Repair* 2009;23:641-56.
- Pierrot-Deseilligny E, Burke D. General methodology. In: *The Circuitry of the Human Spinal Cord*. New York: Cambridge Univ. Press, 2012. p. 28 –36.

Roche N, Lackmy A, Achache V, Bussel B, Katz R. Impact of transcranial direct current stimulation on spinal network excitability in humans. *J Physiol* 2009;587:5653-64.

Roche N, Lackmy A, Achache V, Bussel B, Katz R. Effects of anodal transcranial direct current stimulation over the leg motor area on lumbar spinal network excitability in healthy subjects. *J Physiol* 2011;589:2813-26.

Roche N, Lackmy A, Achache V, Bussel B, Katz R. Effects of anodal tDCS on lumbar propriospinal system in healthy subjects. *Clin Neurophysiol* 2012;123:1027-34.

Rothwell JC, Day BL, Berardelli A, Marsden CD. Effects of motor cortex stimulation on spinal interneurons in intact man. *Exp Brain Res* 1984;54(2):382-384.

Rushmore RJ, DeSimone C, Valero-Cabré A. Multiple sessions of transcranial direct current stimulation to the intact hemisphere improves visual function after unilateral ablation of visual cortex. *Eur J Neurosci* 2013;38:3799-807.

Simonetta-Moreau M. Non-invasive brain stimulation (NIBS) and motor recovery after stroke. *Ann Phys Rehabil Med* 2014;57(8):530-542.

Stinear CM, Barber PA, Smale PR, Coxon JP, Fleming MK, Byblow WD. Functional potential in chronic stroke patients depends on corticospinal tract integrity. *Brain* 2007; 130: 170-80

Stinear CM, Barber PA, Coxon JP, Fleming MK, Byblow WD. Priming the motor system enhances the effects of upper limb therapy in chronic stroke. *Brain* 2008 ;131(:1381-90.

Stinear C. Prediction of recovery of motor function after stroke. *Lancet Neurol* 2010; 9: 1228-32.

Stinear CM, Barber PA, Petoe M, Anwar S, Byblow WD. The PREP algorithm predicts potential for upper limb recovery after stroke. *Brain* 2012;135:2527-35.

Tazoe T, Perez MA. Selective activation of ipsilateral motor pathways in intact humans. *J Neurosci* 2014 15;34:13924-34.

Thilmann AF, Fellows SJ, Garms E. Pathological stretch reflexes on the "good" side of hemiparetic patients. *J Neurol Neurosurg Psychiatry* 1990;53:208-14.

Turton A ,Wroe S, Trepte N, Fraser C, Lemon RN. Contralateral and ipsilateral EMG response to transcranial direct current stimulation during recovery of arm and hand function after stroke. *Electroencephalogr Clin Neurophysiol* 1996;101:316-28.

Ward NS, Brown MM, Thompson AJ, Frackowiak RS. Neural correlates of outcome after stroke: a cross-sectional fMRI study. *Brain* 2003;126:1430-48.

Ward NS, Newton JM, Swayne OB, Lee L, Thompson AJ, Greenwood RJ, et al. Motor system activation after subcortical stroke depends on corticospinal system integrity. *Brain* 2006;129:809-19.

Ward NS, Newton JM, Swayne OBC, Lee L, Frackowiak RS, Thompson AJ, Greenwood RJ et al. The relationship between brain activity and peak grip force is modulated by corticospinal system integrity after subcortical stroke. *Eur J Neurosci* 2007;25(6):1865-1873.

Wargon I, Lamy JC, Baret M, Ghanim Z, Aymard C, Pénicaud A, et al. The disynaptic group I inhibition between wrist flexor and extensor muscles revisited in humans. *Exp Brain Res* 2006 ;168(1-2):203-17.

Wassermann EM, Pascual-Leone A, Hallett M. Cortical motor representation of the ipsilateral hand and arm. *Exp Brain Res* 1994;100:121-32.

Wassermann EM, Fuhr P, Cohen LG, Hallett M. Effects of transcranial magnetic stimulation on ipsilateral muscles. *Neurology*. 1991;41:1795-9. Erratum in: *Neurology* 1992;42:1115.

Yousry TA, Schmid UD, Alkadhi H, Schmidt D, Peraud A, Buettner A, Winkler P. Localization of the motor hand area to a knob on precentral gyrus. A new landmark. *Brain* 1997;120:141-57.

Ziemann U, Ishii K, Borgheresi A, Yaseen Z, Battaglia F, Hallett M, Cincotta M, Wassermann EM. Dissociation of the pathways mediating ipsilateral and contralateral motor-evoked potentials in human hand and arm muscles. *J Physio* 1999;518:895-906.

Legends

Table 1. Clinical features of stroke participants.

Stroke participants: rank, sex (male (M), female(F)), age of the participants at the time of the investigation (years), lesion, time = time elapsed between stroke and the first electrophysiological investigation, type = origin of the lesion ischemia or hemorrhage, site = cortical or subcortical, N/A= not available

Upper limb spasticity was evaluated using the Modified Ashworth scale (MAS), which tests resistance to passive movement with varying degrees of velocity. It is a point scale from 0 to 4. *Score 0* indicates no increase in tone, *score 1* indicates slight increase in tone giving a catch when the limb is moved in flexion or extension, *score 2* means more marked increase in tone but limb easily flexed, *score 3* considerable increase in tone and passive movement difficult and *score 4* means limb rigids in flexion or extension.

Muscular strength was estimated with Medical Research council (MRC). The MRC scale uses a score of 0 to 5 to grade the strength of a particular muscle group in relation to the movement of a single joint. *Grade 0* means no contraction possible, *grade1* indicates flicker or trace of contraction, *grade 2* indicates active movement with gravity eliminated *grade 3* means active movement is possible against gravity *grade 4* indicates active movement against gravity and resistance *grade 5* means normal strength.

Table 2 Electrophysiological metrics estimated in study participants.

Maximum amplitude of the H-reflex (Hmax). Maximal amplitude of the direct motor response (Mmax) The ratio H_{\max} / M_{\max} and H reflex latencies (in ms) were indicated in this table. Individual values (not normalized) of reciprocal Inhibition (RI) in both arms were evaluated at baseline and during Per2. period following 20 minutes of transcranial direct current stimulation (tDCS) application. The inhibition values were calculated using the following equation: $(\text{conditioned H-reflex} - \text{unconditioned H reflex}) / \text{unconditioned H-reflex} \times 100$. Negative values mean H conditioned > H unconditioned, so facilitation rather than inhibition. Values are shown \pm standard error of the mean (SEM). N/A = not available.

Table 3. Influence of time on degree of Reciprocal Inhibition (RI)

The table sums up results of the descriptive analyses performed in the first step. To take into account missing data due to : 1) five participants attended to one session only, 2) in 6 subjects, it was not possible to evoke an H-reflex in the flexor carpi radialis (FCR) of the unaffected arm, thus the main factors influencing the degree of RI were investigated separately (side: contralateral or ipsilateral, condition: sham or anodal transcranial direct current stimulation (tDCS), time period baseline: Per1 tDCS, Per2 tDCS, Post tDCS). For each experimental, one-way ANOVA on repeated measures followed (if significant) by a post-hoc analysis using Newman-Keuls test were performed. The significance level was set at $p < 0.05^*$, when $p > 0.5$ non-significant (ns) was indicated.

Figure 1. Experimental Procedure

A: scheme showing the location of the transcranial direct current stimulation (tDCS) anode over the undamaged hemisphere and anatomical landmarks used. Overview of terms used to describe the upper limbs regarding to the stimulated hemisphere.

B: illustrative waveforms of conditioned and unconditioned H-reflexes in the flexor carpi radialis (FCR) from one stroke participant in the unaffected and affected arm.

Figure 2. Modulations of the degree of reciprocal inhibition (RI) induced by anodal tDCS in stroke participants

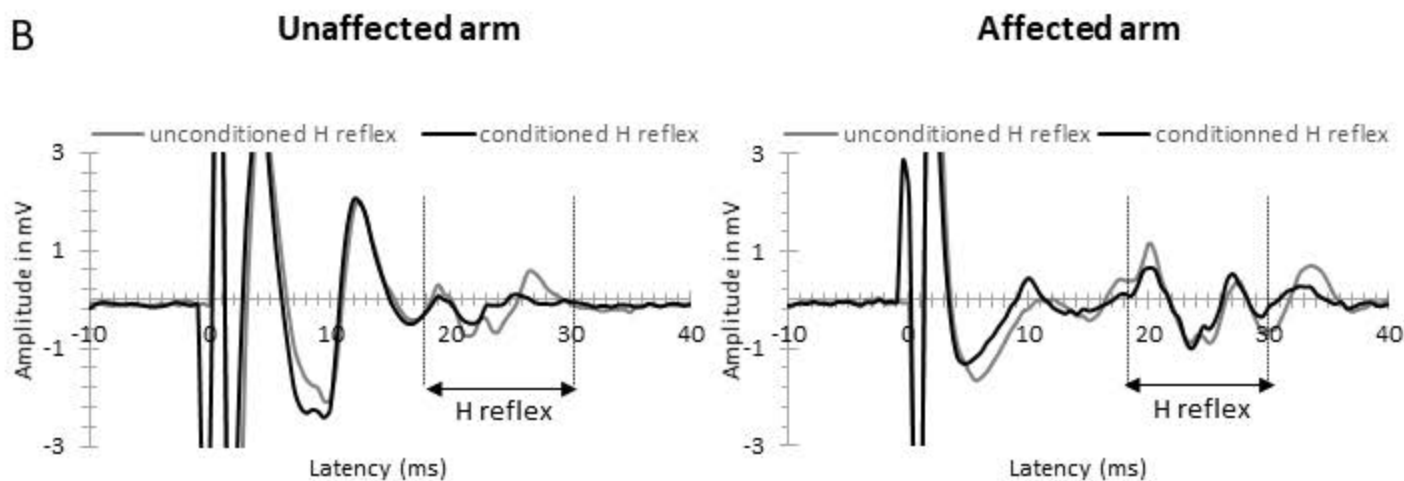
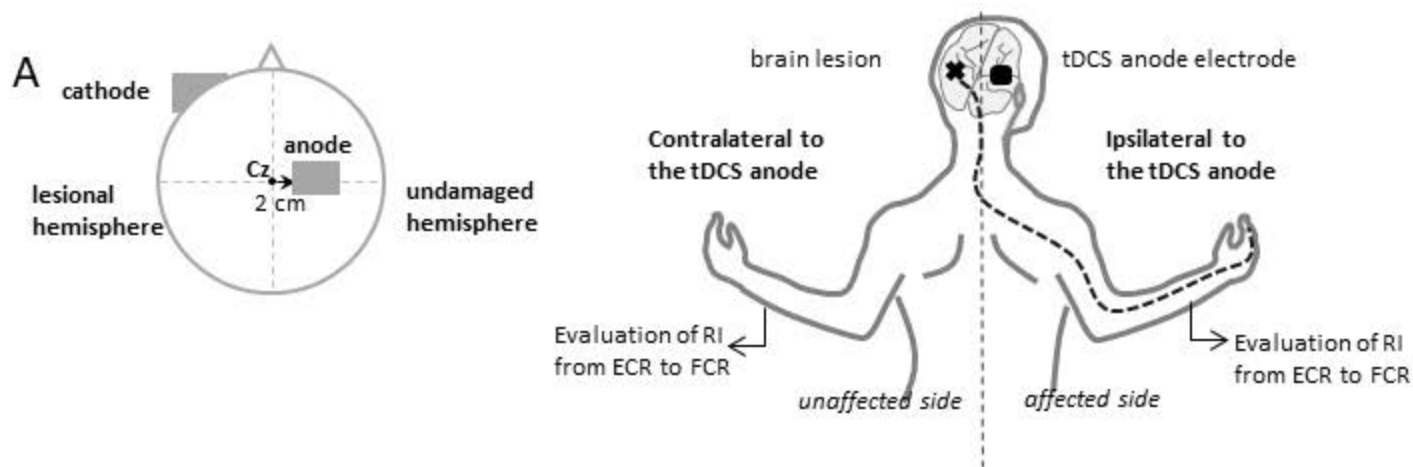
AB: Histograms representing the mean reciprocal inhibition (RI) estimated in the unaffected arm contralateral to the stimulated hemisphere. The time course of mean RI estimated in 12 participants in sham condition (A), and (B) those estimated in 16 participants with anodal transcranial direct current stimulation (tDCS). CD: Histograms representing the mean RI estimated in the affected arm ipsilateral to the stimulated hemisphere. The time course of mean RI estimated in 16 participants in sham condition (C), and (D) those estimated in 20 participants with anodal tDCS. Inhibitions were calculated with the following equation: $(\text{unconditioned H} - \text{H conditioned}) / \text{H test} \times 100$. Vertical bars represent the standard error of the mean (± 1 SEM). Group statistical comparisons were done with Repeated Measures Anova $*p < 0.05$.

Figure 3. Comparison of the degree of reciprocal inhibition (RI) induced by anodal tDCS in stroke participants vs. in healthy subjects

AD: Box plot charts showing distribution of the degree of reciprocal inhibition (RI) estimated in stroke participants (grey rectangles) of the present study and that estimated in healthy subjects (gridded white rectangle) included in our previous study during experiments with active anodal tDCS. (A) depicts the data estimated in the contralateral upper limb regarding the stimulated hemisphere and (B) those evaluated in the ipsilateral side. The top and bottom line of the box correspond to the 75th centile (top quartile) and 25th centile (bottom quartile), respectively, and the line in the box, to the 50th centile (median). The two bars extend from the 10th centile (bottom centile) to the 90th centile (top centile). The bar caps delimit outliers. The cross within the box indicates the arithmetical mean.

BC: Histograms representing the mean reciprocal inhibition (RI) estimated in the contralateral arm with respect to the stimulated hemisphere. The time course of mean RI estimated in 16 stroke participants (B), and (C) those estimated in 13 healthy subjects with anodal transcranial direct current stimulation (tDCS). EF: Histograms representing the mean RI estimated in the ipsilateral arm with respect to the stimulated hemisphere. The time course of mean RI estimated in 20 stroke participants (E), and (F) those estimated in 31 healthy subjects with anodal tDCS. For presentation convenience RI was normalized according to its baseline (t_0) value calculated with the following equation: $((RI_t - RI_{t_0}) / RI_{t_0} \times 100) + 100$. Vertical bars represent the standard error of the mean (± 1 SEM).

Scheme of experimental design



C **Chronology of the experiment**

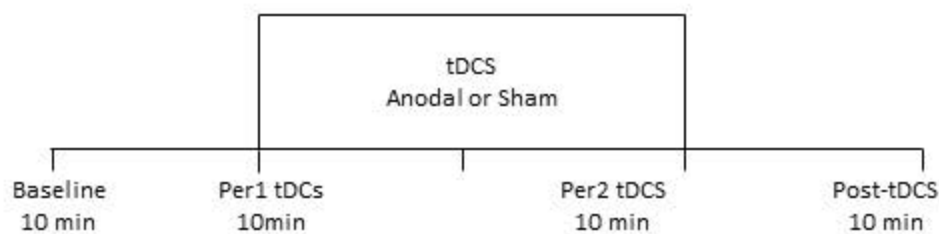
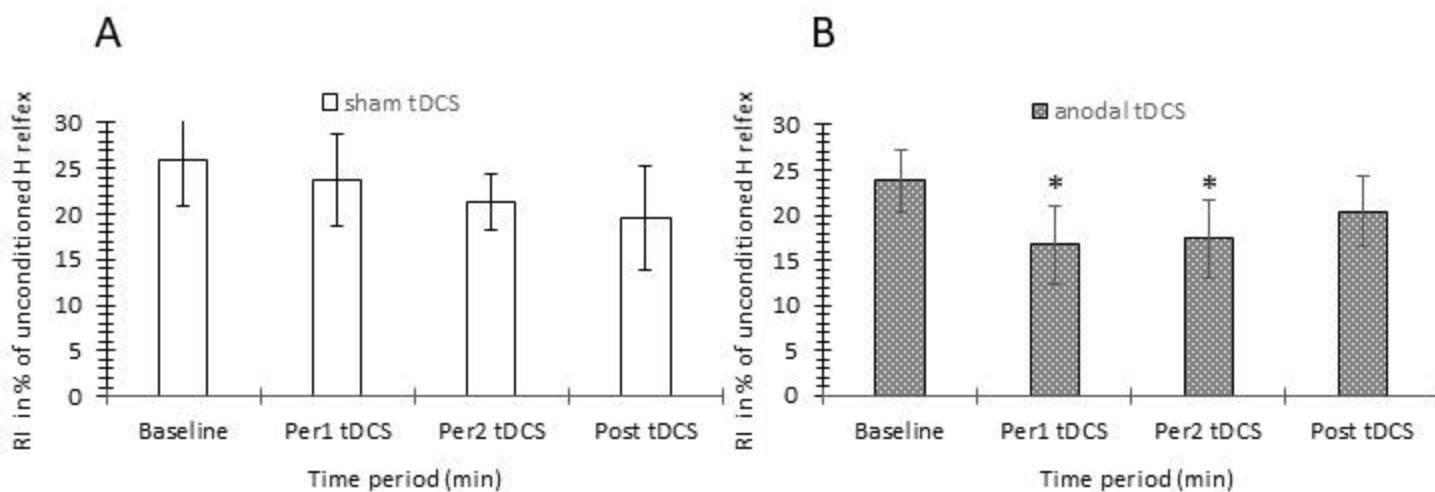


Figure 1

Contralateral to the tDCS anode



Ipsilateral to the tDCS anode

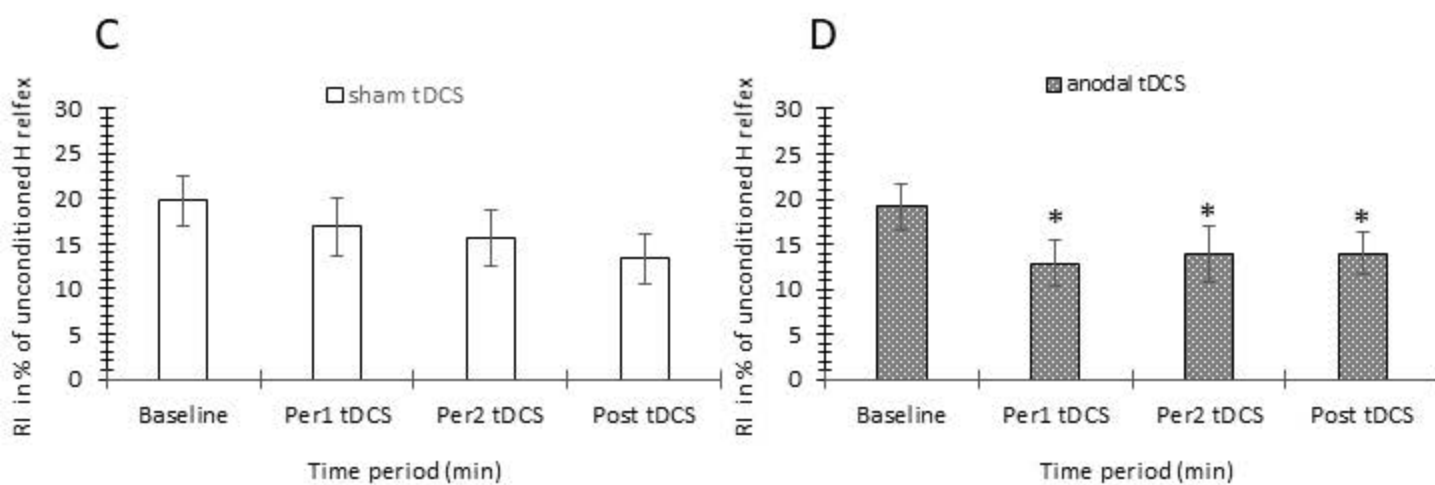
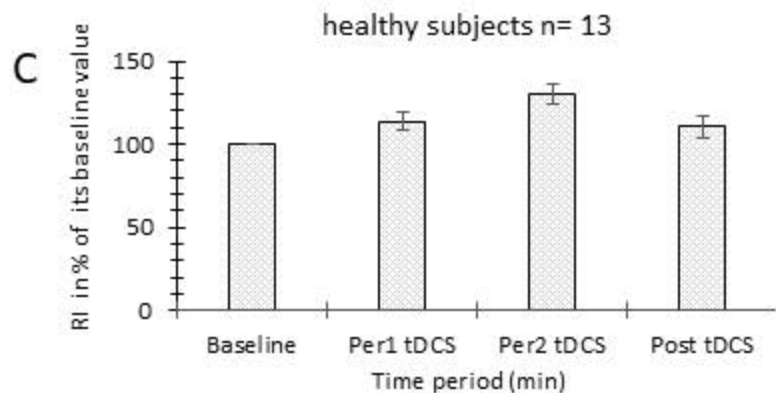
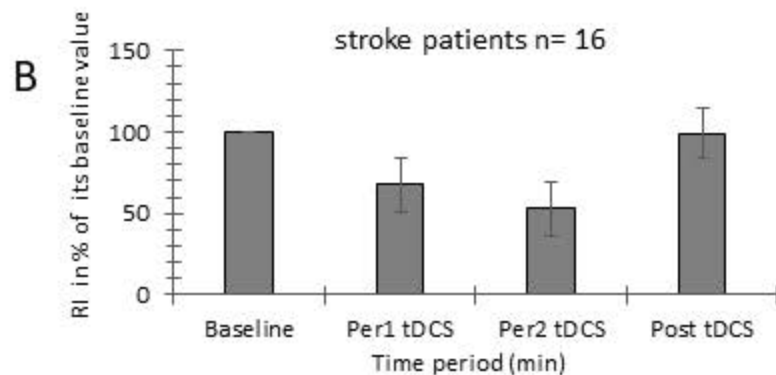
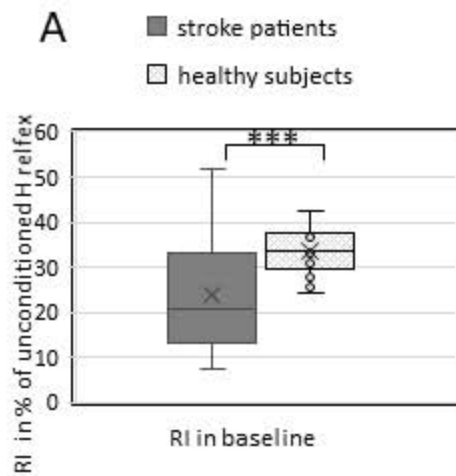


Figure 2

Contralateral to the tDCS anode



Ipsilateral to the tDCS anode

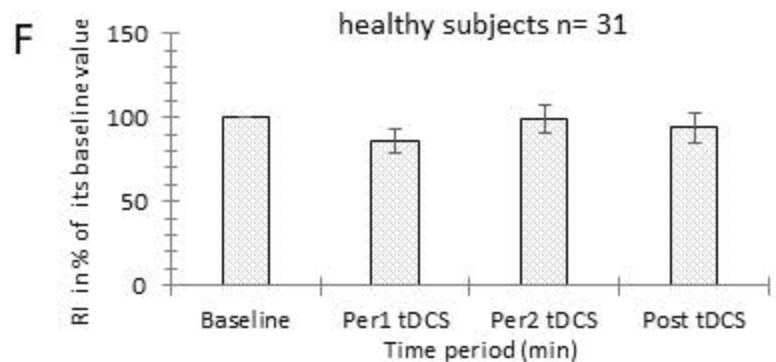
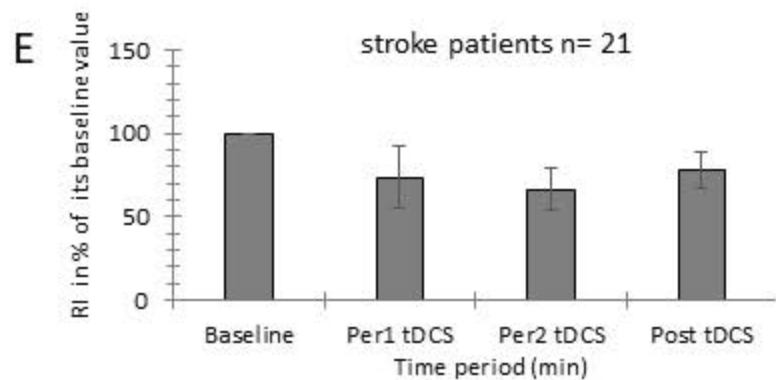
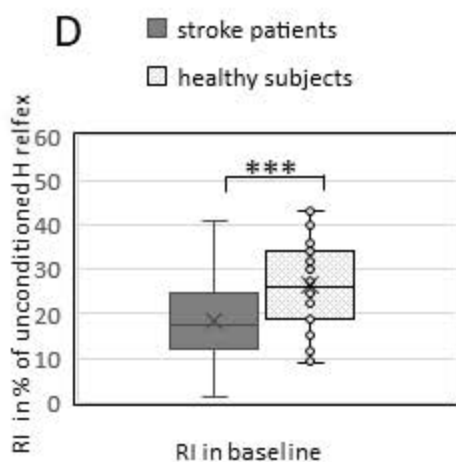


Figure 3

Participants	Sex	Age	Type	Site	Time post Stroke (months)	Paretic side	Affected arm					Unaffected arm				
							MRC Wrist flex	MRC Wrist ext	MRC Elbow flex	MRC Elbow ext	MAS	MRC Wrist flex	MRC Wrist ext	MRC Elbow flex	MRC Elbow ext	MAS
P1	M	41	hemorrhage	cortical	15.78	L	2	0	2	1	1	5	5	5	5	0
P2	F	55	ischemia	cortical	2	R	3	3	1	4	0	5	5	5	5	0
P3	M	63	hemorrhage	subcortical	9.65	R	0	0	2	2	1	5	5	5	5	0
P4	F	32	hemorrhage	subcortical	2.06	R	0	0	2	2	2	5	5	5	5	0
P5	M	47	hemorrhage	subcortical	2	L	0	0	2	2	0	5	5	5	5	0
P6	F	69	ischemia	subcortical	2.25	R	0	0	2	1	3	5	5	5	5	0
P7	M	60	ischemia	subcortical	0.5	L	0	0	2	1	1	5	5	5	5	0
P8	M	62	hemorrhage	cortical	6	L	0	0	0	0	0	5	5	5	5	0
P9	F	55	ischemia	cortical	1	L	2	2	2	2	0	5	5	5	5	0
P10	M	55	ischemia	subcortical	2.75	R	N/A	N/A	N/A	N/A	1	N/A	N/A	N/A	N/A	N/A
P11	M	62	ischemia	cortical	17	R	3	3	4	2	2	5	5	5	5	0
P12	M	28	ischemia	cortical	1	R	4	4	4	4	N/A	5	5	5	5	0
P13	M	59	ischemia	subcortical	1	L	0	0	0	0	1	5	5	5	5	0
P14	M	50	ischemia	subcortical	13	R	0	1	3	1	0	5	5	5	5	0
P15	M	66	hemorrhage	subcortical	75	R	4	3	4	4	2	5	5	5	5	0
P16	M	78	ischemia	subcortical	4.32	R	1	1	2	2	3	5	5	5	5	0
P17	M	46	ischemia	cortical	2.5	R	0	0	0	1	3	5	5	5	5	0
P18	M	56	hemorrhage	subcortical	47	R	3	3	4	4	2	5	5	5	5	0
P19	M	44	ischemia	cortical	1.13	L	4	4	4	4	1	5	5	5	5	0
P20	M	46	hemorrhage	subcortical	15	R	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P21	M	69	hemorrhage	subcortical	48	R	3	2	4	4	3	5	5	5	5	0

Table 2. Electrophysiological metrics estimated in stroke participants.

Participants Rank	paretic side H _{max} /M _{max}	paretic side H reflex latency	non- paretic side H _{max} /M _{max}	non paretic side H reflex latency	RI paretic side				RI non paretic side			
					sham Baseline	sham Per2	anodal Baseline	anodal Per2	sham Baseline	sham Per2	anodal Baseline	anodal Per2
P1	N/A	N/A	21.02	17	N/A	N/A	N/A	N/A	18.49	18.29	N/A	N/A
P2	52.34	19,2	20.64	21,3	14.21	15.66	12.60	17.77	43.08	24.98	27.01	10.55
P3	39.94	18,6	N/A	N/A	N/A	N/A	1.46	1.52	N/A	N/A	N/A	N/A
P4	62.61	16,9	41.18	17,4	11.29	11.14	16.71	23.71	24.91	23.77	38.215	41.55
P5	7.42	11	3.4	21,3	7.45	12.54	11.94	19.84	N/A	N/A	20.29	3.73
P6	75.74	15	77.24	13	23.68	23.27	10.85	-1.61	56.18	47.21	17.64	-0.49
P7	20.37	19	N/A	N/A	N/A	N/A	25.64	21.50	N/A	N/A	N/A	N/A
P8	35.88	17,6	19.18	21,6	43.05	46.12	38.15	50.98	N/A	N/A	9.31	5.23
P9	41.34	18	21.22	13	34.40	22.51	23.80	19.56	11.79	21.89	30.02	14.81
P10	53.70	18,9	22.82	17,63	20.68	23.21	15.14	7.52	16.17	14.99	33.70	40.79
P11	23.73	18,7	N/A	N/A	23.58	13.96	21.01	13.53	N/A	N/A	N/A	N/A
P12	4.77	20	21.31	22	N/A	N/A	12.97	0.45	N/A	N/A	31.52	21.86
P13	39.33	17	10.78	18,2	19.76	-1.85	18.99	3.55	36.35	27.01	42.07	32.29
P14	15.47	19,4	N/A	N/A	18.23	4.80	21.41	25.66	N/A	N/A	N/A	N/A
P15	25.19	19,6	54.90	18,7	7.06	3.18	14.79	-5.33	2.78	5.47	24.52	19.93
P16	65.42	17,6	50.49	20	12.02	15.92	28.00	28.84	52.41	26.10	50.93	54.94
P17	62.12	19	38.49	18	17.56	0.41	5.77	2.25	13.58	22.03	1.85	-1.51
P18	55.13	18,5	N/A	N/A	N/A	N/A	2.90	-0.37	N/A	N/A	7.52	-3.95
P19	30.41	18	23.74	17	24.04	18.06	33.87	25.856	25.03	17.14	4.92	7.95
P20	72.80	21	16.77	19,7	35.86	35.01	44.77	17.60	N/A	N/A	25.92	10.90
P21	89.17	18,5	43.60	16,8	3.50	7.14	22.38	5.93	9.36	6.46	15.16	20.7
Mean ± SEM	43.64 ± 5.3	18.07 ± 0.5	32.07 ± 5.3	18.28 ± 0.7	19.77 ± 2.7	15.69 ± 2.5	19.15 ± 2.5	13.94 ± 3.1	25.84 ± 5.0	21.27 ± 3.1	23.78 ± 3.5	17.41 ± 4.3

Table3. Influence of time period on degree of RI

Conditions	Side	Nb of participants	Statistics	Post Hoc Analysis
Sham tDCS	contralateral	12	ddl = 47, F = 1.164, P _{period} = 0.194	ns
Anodal tDCS	contralateral	16	ddl = 63, F = 3.001, P _{period} = 0.04*	baseline vs. Per1 tDCS, P = 0.048* baseline vs. Per2 tDCS, P = 0.05* baseline vs. Post tDCS, P = 0.202
Sham tDCS	ipsilateral	16	ddl = 63, F = 2.366, P _{period} = 0.084	ns
Anodal tDCS	ipsilateral	20	ddl = 79, F = 3.633, P _{period} = 0.018*	baseline vs. Per1 tDCS P = 0.021* baseline vs. Per2 tDCS P = 0.041* baseline vs. Post tDCS P = 0.016*