Anodal transcranial direct current stimulation of the motor cortex induces opposite modulation of reciprocal inhibition in wrist extensor and flexor

Alexandra Lackmy-Vallée,1 Wanalee Klomjai,1,2 Bernard Bussel,5 Rose Katz,1,2,3,4 and Nicolas Roche3,4,5

1Sorbonne Universités UPMC Université Paris 06, ER 6, F-75005, Paris, France; 2Faculty of Physical Therapy, Mahidol University, Nakonpathom, Thailand; 3Université de Versailles-Saint-Quentin, EA 4497, Garches, France; 4APHP Groupe Hospitalier Pitié-Salpêtrière—Service de Médecine Physique et Réadaptation, Paris, France; and 5APHP Hôpital Raymond-Poincaré—Service d’Explorations Fonctionnelles, Garches, France

Submitted 9 April 2013; accepted in final form 11 June 2014

Anodal transcranial direct current stimulation of the motor cortex induces opposite modulation of reciprocal inhibition in wrist extensor and flexor. J Neurophysiol 112: 1505–1515, 2014. First published June 11, 2014; doi:10.1152/jn.00249.2013.—Transcranial direct current stimulation (tDCS) is used as a noninvasive tool to modulate brain excitability in humans. Recently, several studies have demonstrated that tDCS applied over the motor cortex also modulates spinal neural network excitability and therefore can be used to explore the corticospinal control acting on spinal neurons. Previously, we showed that reciprocal inhibition directed to wrist flexor motoneurons is enhanced during contralateral anodal tDCS, but it is likely that the corticospinal control acting on spinal networks controlling wrist flexors and extensors is not similar. The primary aim of the study was to explore the effects of anodal tDCS on reciprocal inhibition directed to wrist extensor motoneurons. To further examine the supraspinal control acting on the reciprocal inhibition between wrist flexors and extensors, we also explored the effects of the tDCS applied to the ipsilateral hand motor area. In healthy volunteers, we tested the effects induced by sham and anodal tDCS on reciprocal inhibition pathways innervating wrist muscles. Reciprocal inhibition directed from flexor to extensor muscles and the reverse situation, i.e., reciprocal inhibition, directed from extensors to flexors were studied in parallel during voluntary movement in order to achieve a coordinated contraction and relaxation of the antagonist muscles. Consistent with animal data, it has been shown in humans that pathways mediating reciprocal inhibition in flexor and extensor motoneurons are disynaptic, and that motoneurons and inhibitory interneurons have received similar segmental and descending control (Cavallari et al. 1984; Cowan et al. 1986; Day et al. 1984; Rothwell et al. 1984; Shindo et al. 1984). At the level of the elbow and ankle, extensor and flexor muscles operate as real antagonists, so interneurons relaying reciprocal inhibition to flexor and extensor motoneurons are identified as Ia inhibitory interneurons. At the wrist level, extensor carpi radialis (ECR) and flexor carpi radialis (FCR) operate not only as antagonists but also as agonists in wrist abduction, so interneurons mediating reciprocal inhibition to α-motoneurons innervating extensor and flexor muscles differ from those mediating Ia inhibition at the ankle and elbow levels. The interneurons mediating reciprocal inhibition between FCR and ECR are not inhibited by Renshaw cells (Aymard et al. 1995) but receive input from both Ia and Ib afferent fibers (Wargan et al. 2006). It has been hypothesized that interneurons mediating reciprocal inhibition between wrist flexors and extensors probably share more characteristics with Ib than Ia inhibitory interneurons. Regarding their descending control, Ib inhibitory interneurons receive a powerful controlling input from the reticulospinal tract and thus are influenced by both contralateral and ipsilateral descending control (Crosby et al. 1962). Moreover, Illert et al. (1981) have shown that forelimb muscle motoneurons receive a concomitant descending control from both cortices. In humans, the role of the ipsilateral motor cortex is still under debate, although recent studies (Bradnam et al. 2011; McCambridge et al. 2011) suggest that ipsilateral transcranial direct current stimulation (tDCS) influences arm motor control. In humans, reciprocal inhibition directed from ECR to FCR and its descending control from the contralateral hand motor cortex have been studied after changes in excitability of contralateral hand motor cortex induced by transcranial magnetic stimulation (TMS) (Cowan et al. 1986; Rothwell et al. 1984) and by tDCS (Roche et al. 2009). To our knowledge, the control of transmission of the reciprocal inhibition from FCR to ECR has not yet been described in humans. This is mainly due to the difficulty of evoking an H reflex in extensor muscles in humans. Previous results suggest that corticospinal control is asymmetric between flexor and extensor ankle motoneurons in humans.

Address for reprint requests and other correspondence: A. Lackmy-Vallée, ER6 UPMC Univ. Paris 6, Service MPR, Hôpital Pitié-Salpêtrière, 47, bd de l’Hôpital, 75651 Paris Cedex 13, France (e-mail: alexandra.lackmy@upmc.fr).
(Crone et al. 1987). Although the presence of an ECR H reflex is rarely seen, we were able to recruit a sample of healthy subjects exhibiting an H reflex in ECR at rest with sizable amplitude allowing the study of reciprocal inhibition directed to ECR motoneurons. The aims of this study were therefore primarily to explore the effects of contralateral anodal tDCCS on the reciprocal inhibition directed from FCR to ECR, in order to compare corticospinal control acting on interneurons mediating reciprocal inhibition from flexors to extensors and vice versa, and, second, to explore the possible effects of anodal tDCCS applied over the ipsilateral motor cortex on pathways that mediate reciprocal inhibition in flexor and extensor wrist muscles.

Materials and Methods

Subjects

Thirty-two healthy subjects ranging from 22 to 60 yr (mean 31.1 ± 9.9 yr; 20 women and 12 men, 9 left-handed and 23 right-handed) were included in this study. However, not all the subjects participated in every experiment. Among them only nine subjects had an H reflex in ECR stable enough at rest to explore reciprocal inhibition directed from ECR to FCR. All subjects gave written informed consent before participating in the experiments. This study was performed according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the local ethical committee (CPP Ile de France 6-Pitié-Salpêtrière).

Transcranial Direct Current Stimulation of Motor Cortex

tDCCS was applied with a neuroConn DC-STIMULATOR (neuroConn, Ilmenau, Germany) via two conductive rubber electrodes placed in saline-soaked sponges (5 × 7 cm). TMS elicited by Magstim 200 (Magstim, Whitland, UK) was used to determine the position of the anode positioned over the hand motor cortex. The cathode was placed over the supraorbital region. Three conditions of tDCCS were tested: 1) the active contralateral condition: anode placed over the contralateral hand motor cortex and cathode positioned over the ipsilateral supraorbital area; 2) the active ipsilateral condition: anode over the ipsilateral hand motor cortex and cathode over the contralateral supraorbital area; and 3) the sham condition: electrodes were placed in the same position as in active conditions, but current was activated for only 120 s at the beginning and 30 s at the end of sham stimulation in order to mimic the sensations of ramp-up and ramp-down current perceived in the active conditions. In the experiments involving the ECR H reflex, because of the difficulty of evoking a test H reflex in ECR, the sham condition was performed in only one configuration with the anodal tDCCS electrode placed over the contralateral motor cortex. We were careful to respect a minimum delay of 48 h between the different tDCCS conditions, and on average the delay between two recording sessions was ~1 wk. Moreover, in the present study, active and sham contralateral tDCCS were tested only on reciprocal inhibition directed from FCR to ECR. Effects induced by contralateral tDCCS on reciprocal inhibition directed from ECR to FCR were obtained from our previous study (Roche et al. 2009).

In all conditions, current intensity was fixed at 1.75 mA. Current flowed continuously for 20 min for both active conditions and for 150 s in the sham condition, since Nitsche and Paulus (2000) had previously reported that a duration of at least 3 min of tDCCS was necessary to induce aftereffects. The current was ramped up or down over the first and last 8 s of stimulation. The experimental procedure was identical to that used by Roche et al. (2009).

Measurement of Spinal Network Excitability

Electromyogram recordings. Subjects were seated in a comfortable reclining armchair with the shoulder slightly abducted at ~60°, the elbow semiflexed, and slight pronation of the forearm. The distal part of the upper limb was supported by an armrest in order to exclude any active maintenance of wrist posture, and the subjects were asked to relax the arm. Electromyographic (EMG) activity was recorded from FCR and ECR muscles with bipolar surface electrodes positioned on the muscle belly. EMG activity was displayed in a wide analysis window beginning 100 ms before and ending 200 ms after the test stimulus. To ensure that subjects relaxed their wrist muscles, background EMG activity was measured a posteriori, in the 100-ms pretrigger windows with rectified EMG activity. EMG activity was sampled at 2 kHz, amplified (×5,000–10,000), and band-pass filtered (250 Hz–3 kHz) with a Digitimer D360 amplifier (Digitimer, Welwyn Garden City, UK). In all subjects, experiments were performed on the dominant upper limb based on patient self-reports.

Electrical stimuli. Transcutaneous electrical stimulation (rectangular pulses of 1-ms duration every 3 s) was applied to radial and median nerves via 3-cm-diameter hemispherical bipolar electrodes. The electrodes were placed near the cubital fossa to stimulate the median nerve and at the spiral groove to stimulate the radial nerve.

Test Stimuli

FCR H reflex. The FCR H reflex was evoked by electrical stimuli applied to the median nerve. First, the maximum direct motor response (Mmax) was first determined and the unconditioned H reflex was adjusted to 10–20% of Mmax in each subject (mean value 14.17 ± 2.5% Mmax). The amplitude of the unconditioned FCR H reflex corresponded to 50% of the maximum amplitude of H reflex in the majority of cases. It was kept constant throughout the experiment, since the H-reflex sensitivity to facilitation or inhibition can vary with its unconditioned amplitude (Crone et al. 1990).

ECR H reflex. The ECR H reflex was evoked by radial nerve stimulation. As mentioned above, it is difficult to elicit the H reflex in ECR (Pierrot-Deseilligny and Burke 2012). Thirty-two subjects participated in this study, but only nine subjects had an ECR H reflex large enough at rest to show changes in reciprocal inhibition. As above, the Mmax response was determined first and the unconditioned ECR H reflex was adjusted to 5–15% of Mmax in each subject and was kept constant throughout the experiment (mean value 6.62 ± 1.4% Mmax).

Conditioning Stimuli

Conditioning stimuli were applied to the nerve innervating the antagonist muscle, i.e., that counteracting the muscle in which the test H reflex was evoked. The intensity of the conditioning stimuli was adjusted to activate group I fibers but was below the activation threshold of Renshaw cells in order to prevent recurrent inhibition (Aymard et al. 1997). This implies that the conditioning stimuli did not evoke either a direct motor response (which may have an influence by antidromic activation of Renshaw cells) or an H reflex (which may have influence by orthodromic activation of Renshaw cells). The motor threshold was determined by the oscilloscope display of EMG. The average intensity of conditioning stimuli was 0.88 ± 0.02 motor threshold (MT) for the radial nerve and was 0.66 ± 0.02 MT for the median nerve (Table 1). The interstimulus interval (ISI) was the time interval between test and conditioning stimuli for which the level of reciprocal inhibition was maximal. By convention, a positive ISI corresponds to a conditioning stimulus preceding the test stimulus. The ISI was determined with 0.5-ms steps in the range ~3 ms to +1 ms for reciprocal inhibition from ECR to FCR (Day et al. 1984) and

J Neurophysiol • doi:10.1152/jn.00249.2013 • www.jn.org
pressed as a percentage of unconditioned H value.

formed on raw data, i.e., with amount of reciprocal inhibition ex-

means that inhibition was larger than the baseline and a negative

baseline period (t

RI from FCR to ECR
Sham
Contralateral
Ipsilateral
RI from ECR to FCR
Sham
Ipsilateral

Values are mean ± SE data obtained in baseline period in all subjects. Two-way repeated-measures ANOVAs were performed to compare effects of time period [baseline, period 1, period 2, and after transcranial direct current stimulation (post-tDCS)] and effects of tDCS condition (sham, active contralateral, and active ipsilateral) on amount of reciprocal inhibition (RI) and unconditioned H-reflex amplitudes. M\textsubscript{max}, maximum motor response; FCR, flexor carpi radialis; ECR, extensor carpi radialis.

−1 ms to +3 ms for reciprocal inhibition from FCR to ECR (Wargon et al. 2006). The mean ISI was 0.12 ± 0.6 ms for reciprocal inhibition from ECR to FCR and 0.60 ± 0.8 ms for reciprocal inhibition from FCR to ECR. The ISI was kept constant throughout the experiments.

Experimental Procedure

A randomized, sham-controlled tDCS study was performed. During the experiments, the subjects were blind to the conditions of tDCS. The active contralateral condition, the active ipsilateral condition, and the sham condition were randomly alternated. Each condition was performed on the subjects on different days to avoid carryover effects.

Once all parameters (unconditioned H-reflex amplitude, conditioning stimulation intensity, and ISI) were set, the baseline inhibition (without tDCS) was determined over the first 10 min, being defined as the baseline period. Next, the tDCS electrodes were attached. The anode was placed over the hand motor cortex for 20 min for both active (ipsilateral and contralateral conditions) and sham tDCS. These 20 min were divided into two 10-min periods: period 1 and period 2. The tDCS electrodes were removed immediately after the end of the stimulation. The amount of reciprocal inhibition was also measured for 10 min after the end of stimulation in order to evaluate posteffects (see diagram in Fig. 1). In each period (baseline, period 1 tDCS, period 2 tDCS, and post-tDCS), the amount of inhibition was assessed with three series of 40 H reflexes (20 conditioned H reflexes and 20 unconditioned H reflexes). Conditioned and unconditioned H reflexes were evoked every 3 s and randomly alternated.

Analysis

Parameter definitions. The background EMG activity was evaluated by calculating the pretrigger root mean square (rms) of rectified EMG activity in the 100-ms window preceding the test stimulation. The average H-reflex size was determined from peak-to-peak amplitudes expressed as a percentage of M\textsubscript{max}. The amount of reciprocal inhibition was defined as [(unconditioned H value − conditioned H value)/unconditioned H value] × 100. The mean amount of reciprocal inhibition from each period was normalized as a percentage of the baseline inhibition measured over the baseline period (t\textsubscript{b}) according to the equation [(inhibition − inhibition\textsubscript{b})/inhibition\textsubscript{b}] × 100. Therefore when the amount of reciprocal inhibition is normalized to the baseline, a positive value means that inhibition was larger than the baseline and a negative value means the inhibition was weaker than the baseline.

The normalized amounts of reciprocal inhibition were only used in the graphics representing group data. Statistical analyses were performed on raw data, i.e., with amount of reciprocal inhibition expressed as a percentage of unconditioned H value.

Statistical analysis. For individual data, a two-way ANOVA was performed with time period (baseline, period 1 tDCS, period 2 tDCS, and post-tDCS) as the first factor and tDCS condition (sham, active contralateral, and active ipsilateral tDCS) as the second factor. When reciprocal inhibition from FCR to ECR was investigated, as subjects were examined in all three conditions we first compared data from active contralateral tDCS to those obtained from sham tDCS and then we performed another analysis to compare data from active ipsilateral tDCS to those obtained from sham tDCS. To evaluate effects induced by active ipsilateral tDCS on reciprocal inhibition from ECR to FCR, we compared the modulations observed from sham
and active ipsilateral tDCS conditions only, since active contralateral tDCS had already been tested on another group of subjects in our previous study (Roche et al. 2009).

A two-way ANOVA was performed on unconditioned H reflexes (with time period and condition as factors) to ensure that the modulations of reciprocal inhibition resulted from tDCS, not from the variation of the H reflex over time. Moreover, 1) as the amplitude of the unconditioned H reflex may affect the conditioned H reflex (Crone et al. 1990) and 2) as the mean amplitude of the unconditioned H reflex in ECR was smaller than that in FCR (mean value of unconditioned ECR H reflex was 6.62 ± 1.4% M_max, compared with mean value of unconditioned FCR H reflex of 14.17 ± 2.5% M_max, cf. Table 1), a linear regression between the ratio ECR unconditioned H reflex/FCR unconditioned H reflex and the ratio ECR conditioned H reflex/FCR conditioned H reflex was calculated in order to ensure that modulations induced by tDCS were not affected by unconditioned H-reflex amplitude.

Group data were analyzed by using two-way repeated-measures ANOVAs to determine effects of tDCS and time period on reciprocal inhibition. Moreover, to determine whether modulations of reciprocal inhibition from ECR to FCR observed in left-handed subjects during active ipsilateral tDCS were different from those observed in right-handed subjects, a two-way ANOVA was performed with period (baseline, period 1 tDCS, period 2 tDCS, post-tDCS) as the first factor and handedness (left-handed subjects, right-handed subjects) as the second factor. Note that both two-way ANOVAs were performed on raw data using the amount of reciprocal inhibition expressed as a percentage of unconditioned H reflex rather than on normalized data (reciprocal inhibition expressed as % of baseline inhibition). To ensure that background EMG activity was constant over time and regardless of tDCS condition, a two-way ANOVA (testing tDCS condition and time period as factors) was performed on rms.

When the F value was significant, post hoc pairwise comparisons were performed with the Newman-Keuls test. Degrees of freedom calculated by statistical analyses are indicated in parentheses after the F value. Significance was taken at P < 0.05. Mean data are shown as means ± SE. Statistical analysis was performed with SigmaPlot 11.0 software.

RESULTS

Figure 2 illustrates a representative example of unconditioned and conditioned reflexes obtained over the baseline period in the same subject. Note that reciprocal inhibition induced in the FCR H reflex (Fig. 2, C and D) is larger than that in the ECR H reflex (Fig. 2, A and B). Table 1 summarizes the mean unconditioned ECR and FCR H-reflex amplitudes: for each tDCS condition (sham, contralateral, ipsilateral) and period (baseline, during tDCS, after tDCS), no significant change of unconditioned test reflex amplitude was observed.

Background EMG Activity

To ensure that all experiments were conducted at rest, we assessed the background EMG activity by calculating the rms of rectified EMG activity in the 100-ms pretrigger window. The mean rms was calculated for experiments testing reciprocal inhibition directed from FCR to ECR (mean rms = 0.103 ± 0.025 mV) and for experiments testing reciprocal inhibition directed from ECR to FCR (mean rms = 0.109 ± 0.0143 mV). Statistical analysis with tDCS condition as first factor (sham, active ipsilateral, and active contralateral) and time period as second factor attested that background EMG activity was constant over time and that it was not affected by tDCS for experiments exploring reciprocal inhibition directed from ECR to FCR [F(1,152) = 1.868, P_condition × period = 0.177; F(3,152) = 0.853, P_period = 0.471; F(6,88) = 0.0198, P_condition × period = 0.996] and experiments testing reciprocal inhibition directed from ECR to FCR [F(1,152) = 0.00561, P_condition = 0.940; F(3,152) = 0.350, P_period = 0.789; F(3,152) = 0.00369, P_condition × period = 1.000].
Sham Conditions

Figure 3A shows individual data obtained in nine subjects in whom it was possible to evoke a stable ECR H reflex at rest (5 right-handers, 4 left-handers). The amount of reciprocal inhibition directed from FCR to ECR was plotted against the four time periods (baseline, period 1 tDCS, period 2 tDCS, post-tDCS). The amount of reciprocal inhibition did not change significantly over time: the mean level of inhibition as a percentage of unconditioned H reflex for each time period was 34.63% in the baseline period, 35.44% in period 1 tDCS, 15.32% in period 2 tDCS, and 14.16% in post-tDCS.

Figure 3C shows individual data obtained in 20 subjects (17 right-handers, 3 left-handers). The amount of reciprocal inhibition directed from ECR to FCR did not change significantly over time: the mean level of inhibition as a percentage of unconditioned H reflex in ECR for each time period was 17.80% in the baseline period, 15.32% in period 1 tDCS, 14.43% in period 2 tDCS, and 13.7% in post-tDCS.

Figure 3 shows individual data obtained from nine subjects (5 right-handers, 4 left-handers). The amount of reciprocal inhibition of the ECR H reflex is plotted against the four time periods (baseline, period 1 tDCS, period 2 tDCS, post-tDCS). In nine subjects, the level of inhibition was lower in both periods 1 and 2 compared with baseline. During the post-tDCS period, the level of inhibition increased in seven subjects compared with period 2. The average reciprocal inhibition expressed as a percentage of unconditioned H reflex in the nine subjects was 16.55% ± 1.7% in the baseline period, 7.23% ± 3.1% in period 1 tDCS, 3.91% ± 3.1% in period 2 tDCS, and 10.92% ± 2.4% in post-tDCS.

Active tDCS Applied Over Contralateral Hand Motor Cortex

Reciprocal inhibition from FCR to ECR. Figure 3B shows individual data obtained from nine subjects (5 right-handers, 4 left-handers). The amount of inhibition of the ECR H reflex is plotted against the four time periods. In nine subjects, the level of inhibition was lower in both periods 1 and 2 compared with baseline. During the post-tDCS period, the level of inhibition increased in seven subjects compared with period 2. The average reciprocal inhibition expressed as a percentage of unconditioned H reflex in the nine subjects was 16.55% ± 1.7% in the baseline period, 7.23% ± 3.1% in period 1 tDCS, 3.91% ± 3.1% in period 2 tDCS, and 10.92% ± 2.4% in post-tDCS.

Figure 4A shows group data with the amount of reciprocal inhibition from FCR to ECR normalized to a percentage of its baseline value obtained from sham and active contralateral tDCS conditions. Two-way repeated-measures ANOVA showed an effect of time period (baseline, period 1 tDCS, period 2 tDCS, post-tDCS) and a significant interaction between time period and tDCS condition. Furthermore, there was a significant interaction between time period and tDCS condition. The average reciprocal inhibition expressed as a percentage of unconditioned H reflex in the nine subjects was 16.55% ± 1.7% in the baseline period, 7.23% ± 3.1% in period 1 tDCS, 3.91% ± 3.1% in period 2 tDCS, and 10.92% ± 2.4% in post-tDCS.
analyses attested that the amount of reciprocal inhibition was significantly depressed during both periods (period 1 tDCS and period 2 tDCS) when active tDCS was applied over the contralateral motor cortex: the mean values of inhibition evaluated in period 1 and period 2 were lower than those obtained in the baseline period (Newman-Keuls analysis, baseline vs. period 1 tDCS $P = 0.001$, baseline vs. period 2 tDCS $P < 0.001$). Moreover, at the end of tDCS application the amount of inhibition increased but was not restored to its baseline value (baseline vs. post-tDCS $P = 0.028$).

Note that the mean levels of reciprocal inhibition recorded during the baseline period were similar in sham and active tDCS conditions. Mean inhibition as a percentage of unconditioned H-reflex was 17.80 ± 2.9% in the sham condition compared with 16.55 ± 1.7% with contralateral stimulation (Table 1). Moreover, the mean values of unconditioned H-reflex amplitudes were not affected by condition or time period $[F_{(1,8)} = 0.0487, P_{condition} = 0.831; F_{(3,24)} = 1.878, P_{period} = 0.160; F_{(3,24)} = 0.451, P_{condition \times period} = 0.719]$. Mean values of unconditioned H-reflex amplitude were similar during the baseline period (7.08 ± 1.5% $M_{max}$ in sham condition compared with 7.46 ± 1.3% $M_{max}$ with contralateral stimulation) (Table 1).

Reciprocal inhibition from ECR to FCR. The effects of active contralateral tDCS on reciprocal inhibition directed from ECR to FCR were described in our previous study (Roche et al. 2009). The data presented in Fig. 4B were extracted from our previous findings for comparison with the results obtained from the present study of reciprocal inhibition directed from FCR to ECR (Fig. 4A). Reciprocal inhibition was enhanced in periods 1 and 2 compared with that observed in the baseline period and the sham condition (Fig. 4B) (2-way repeated-measures ANOVA [4 periods, baseline, period 1 tDCS, period 2 tDCS, post-tDCS] and 2 conditions (sham, active contralateral tDCS)); $F_{(1,12)} = 2.085, P_{condition} = 0.174; F_{(3,36)} = 3.323, P_{period} = 0.03; F_{(3,36)} = 8.078, P_{condition \times period} < 0.001$).

Reciprocal inhibition was restored to near-baseline levels after the end of stimulation for the active tDCS condition (Newman-Keuls analysis: baseline vs. period 1 tDCS $P = 0.04$, baseline vs. period 2 tDCS $P < 0.001$, baseline vs. post-tDCS $P = 0.38$).

Influence of unconditioned H-reflex size on amount of reciprocal inhibition. It can be seen that the size of the unconditioned ECR H reflex was lower than that of the unconditioned FCR H reflex (cf. Table 1). Therefore, to ensure that the size of the unconditioned H reflex did not impact on the results, two ratios, unconditioned ECR H reflex/unconditioned FCR H reflex and conditioned ECR reflex/conditioned FCR H reflex, were calculated. A linear regression was performed to determine whether the difference between ECR and FCR reflexes altered the amount of reciprocal inhibition (data not shown). The analysis revealed a significant linear correlation ($P < 0.001; R^2 = 0.957$), indicating that the level of reciprocal inhibition in ECR and FCR muscles would be equal if unconditioned H reflex evoked in ECR and FCR were similar.

Active tDCS Applied Over Ipsilateral Motor Cortex

Reciprocal inhibition from FCR to ECR. Reciprocal inhibition directed from FCR to ECR was tested in eight subjects as it was not possible to perform the experiment with active ipsilateral tDCS in one subject. The mean levels of inhibition,
expressed as a percentage of unconditioned H-test reflex, were 17.46 ± 3.9% in the baseline period, 15.65 ± 4.8% in period 1 tDCS, 14.28 ± 5.1% in period 2 tDCS, and 14.13 ± 5.3 in post-tDCS. The group data illustrated in Fig. 5A show the mean values of reciprocal inhibition evaluated in the active ipsilateral tDCS condition and in the sham condition. Statistical analyses showed that in both conditions (active ipsilateral tDCS and sham tDCS) the amount of reciprocal inhibition directed from FCR to ECR did not induce any statistically significant modification. Results obtained with anodal stimulation strongly resemble those obtained with sham stimulation. This finding is in accordance with previous results (Aymard et al. 1995; Day et al. 1984; Wargon et al. 1997).

Reciprocal inhibition from ECR to FCR. First, reciprocal inhibition directed from ECR to FCR was compared during sham (Fig. 3C) and anodal ipsilateral (Fig. 3D) stimulation in 20 subjects. As a percentage of unconditioned test H reflex, the mean levels of inhibition were 27.74 ± 2.7 in the baseline period, 27.9 ± 3.1 in period 1 tDCS, 29.82 ± 3.4 in period 2 tDCS, and 28.08 ± 3.0 in post-tDCS. The level of inhibition for group data is illustrated in Fig. 5B, which shows that ipsilateral tDCS did not induce any statistically significant modification. Results obtained with anodal stimulation strongly resemble those obtained with sham stimulation. This was confirmed by statistical analysis indicating that time period had no effect on the amount of inhibition in sham and active ipsilateral tDCS conditions [2-way ANOVA [2 conditions (sham, active ipsilateral tDCS) × 4 time periods (baseline, period 1, period 2, post-tDCS)]: 0.04; F(1,19) = 2.954, Pcondition = 0.104; F(3,57) = 1.829, Pperiod = 0.152; F(3,57) = 0.0868, Pcondition × period = 0.967]. Moreover, the mean values of unconditioned H-reflex amplitudes were not affected by condition or time period [F(1,19) = 1.329, Pcondition = 0.268; F(3,57) = 0.589, Pperiod = 0.626; F(3,57) = 0.128, Pcondition × period = 0.948]. Mean values of unconditioned H-reflex amplitude were similar during the baseline period (17.8 ± 3.2% Mmax in sham condition compared with 17.0 ± 1.9% Mmax with ipsilateral stimulation) (Table 1).

Subsequently, the number of subjects was increased in order to compare results obtained in right-handed and left-handed subjects. Thirty-one subjects were included, of which eight were left-handed (individual data are not shown). In the 23 right-handed subjects we found that the level of inhibition was increased compared with baseline in 9 subjects and decreased in 14 subjects in period 1. In period 2, the values were increased compared with baseline in 15 subjects and decreased in 8 subjects. Mean levels of inhibition as a percentage of unconditioned test H reflex were 24.02 ± 2.4% in the baseline period, 22.19 ± 2.7% in period 1 tDCS, 23.94 ± 2.7% in period 2 tDCS, and 23.62 ± 3.2% in post-tDCS. Moreover, for the eight left-handed subjects included in this series of complementary experiments, we found that the values were decreased compared with baseline in seven subjects in period 1 and in four subjects in period 2. Mean level of inhibition as a percentage of unconditioned test H reflex was 26.77 ± 4.0% in the baseline period, 22.01 ± 5.4% in period 1 tDCS, 25.41 ± 6.6% in period 2 tDCS, and 25.14 ± 4.9% in post-tDCS. Group data are shown in Fig. 5C. Whatever the handedness, no significant modification was seen during or after anodal lateral tDCS [2-way ANOVA: F(3,116) = 0.252, Pperiod = 0.860; F(1,116) = 0.243, Pcondition = 0.623; F(3,116) = 0.044, Pcondition × period = 0.987].

Study Limitations: ECR and FCR H Reflex Methodological Considerations

Thirty-two healthy subjects were enrolled in this study. Among them, 32 had an FCR H reflex and only 9 of them had an ECR H reflex. This finding is in accordance with previous results (Aymard et al. 1995; Day et al. 1984; Wargon et al. 1997).
Furthermore, the amplitude of unconditioned reflexes is usually smaller in ECR than in FCR H. The conditioning stimulus, when applied to the radial nerve, does not usually evoke an H reflex in the ECR, while the conditioning stimulus applied to the median nerve usually evokes an FCR H reflex. To avoid evoking FCR H reflexes that might induce activation of Renshaw cells between FCR and ECR motoneurons (Aymard et al. 1995), a lower-average-intensity conditioning stimulus was applied to the median nerve than that applied to the radial nerve (cf. Table 1). This lower median nerve stimulating stimulus intensity probably contributes to the lower baseline level of reciprocal inhibition in ECR compared with FCR motoneurons. However, in 1995 Aymard et al. were able to find a subject in which the conditioning stimuli applied to the radial nerve and to the median nerve were identical. In this condition, the amounts of reciprocal inhibition were similar in both ECR and FCR reflexes (Aymard et al. 1995; Fig. 1). However, Crone et al. (1990) emphasized that the amount of inhibition also depends on the unconditioned H-reflex amplitude, and the size of the unconditioned ECR H reflex was lower than that of the FCR H reflex. It could be argued that the differential effects of tDCS on reciprocal inhibition directed from ECR to FCR and from FCR to ECR could be at least partly due to differences in FCR and ECR unconditioned reflex amplitude. However, we found a significant linear correlation between the ratio unconditioned ECR H reflex/unconditioned FCR H reflex and the ratio conditioned ECR H reflex/conditioned FCR H reflex, indicating that the level of reciprocal inhibition in ECR and FCR would be equal if unconditioned H reflexes evoked in ECR and FCR were similar. This confirms that the effects of anodal tDCS applied over the contralateral hand motor area cannot be explained by the difference in size of conditioned reflexes. Moreover, differences between unconditioned reflex amplitudes may impact on quantitative comparisons but would not influence the qualitative differences (facilitation vs. inhibition). Therefore, it is more likely that the reciprocal inhibition behavior following tDCS strongly reflects differences in the excitability of interneurons.

Among the 32 subjects having an FCR H reflex 23 were right-handed and 9 left-handed, whereas among the 9 subjects having an ECR H reflex only 4 were left-handed. Therefore, the comparison between left-handed and right-handed subjects was restricted to reciprocal inhibition directed from ECR to FCR.

DISCUSSION

The main finding of the present study is that anodal tDCS applied over the contralateral hand motor cortex increases reciprocal inhibition directed from wrist extensors to wrist flexors but decreases reciprocal inhibition from wrist flexors to wrist extensors.

Opposite Effects of Active tDCS Applied to Contralateral Hand Motor Cortex

Since the position and stimulation characteristics of the active tDCS electrode were identical, when we tested reciprocal inhibition from ECR to FCR and from FCR to ECR, hand motor cortex excitability was the same for both directions of reciprocal inhibitions. The most striking finding of the present series of experiments is that increasing the excitability of the same motor cortex area induces an increase of reciprocal inhibition from ECR to FCR but a decrease in reciprocal inhibition from FCR to ECR. Evidence from both animal and human studies (Priori et al. 1998; Purpura and McMurtry 1965) shows that anodal tDCS applied over the motor cortex decreases the membrane resting potential of cortical cells, which are spontaneously active in M1 (Evarts 1981). In our previous study (Roche et al. 2009), we proposed that the enhancement of the reciprocal inhibition from ECR to FCR following tDCS was due to an increase in efficiency of the descending volley reaching the interneurons that mediate reciprocal inhibition from ECR to FCR. To understand the opposing effects of interneurons that mediate reciprocal inhibition from ECR to FCR and vice versa following the same changes in motor cortex excitability, we propose to take into account the mutual inhibition between opposite-side interneurons that mediate reciprocal inhibition; for example, an FCR interneuron that mediates reciprocal inhibition from FCR to ECR also inhibits the interneuron that mediates reciprocal inhibition from ECR to FCR as depicted in Fig. 6. In animal studies, mutual inhibition between opposite-side interneurons has been identified as a general central nervous system mechanism. Baldiisera et al. (1987) also demonstrated that mutual inhibition exists in humans at the wrist level. Thus we could propose two hypothetical mechanisms: 1) that the descending control acting on interneurons that mediate reciprocal inhibition is asymmetric and is concentrated on interneurons that mediate reciprocal inhibition from ECR to FCR (Fig. 6A) and 2) that the reciprocal inhibition at spinal level between ECR and FCR is asymmetric (Fig. 6B).

The first hypothesis is related to a study by Maertens de Noordhout et al. (1999) suggesting that the cortico-motoneuronal synaptic connections are stronger on wrist and finger extensor motoneurons than on flexor motoneurons. Lundberg and collaborators have shown in the cat that there is a corticospinal parallel control of motoneurons and of the corresponding reciprocal Ia interneurons, i.e., the interneurons that mediate reciprocal inhibition to antagonistic motoneurons (Lundberg 1970). Although the interneuron that mediates reciprocal inhibition at the wrist level exhibits characteristics different from those of Ia interneurons, it might thus be hypothesized that there is parallel descending control of ECR motoneurons and of interneurons that mediate reciprocal inhibition from ECR to FCR (and similarly of FCR motoneurons and interneurons that mediate inhibition from FCR to ECR). If the descending control acting on ECR and interneurons that mediate reciprocal inhibition from ECR to FCR is stronger than the descending control acting on FCR, the excitability of the interneurons that mediate inhibition from ECR to FCR (the ECR interneuron in Fig. 6) is increased more than that of the interneurons that mediate reciprocal inhibition from FCR to ECR (the FCR interneuron in Fig. 6). As a consequence, mutual inhibition between the two opposite-side interneurons is more strongly directed to the FCR interneuron than the ECR interneuron. Thus the net effect of the descending control and mutual inhibition is an increase in the excitability of the ECR interneuron. For the FCR interneuron, its descending control is weaker and leads to the reverse situation: the weaker facilitatory descending control is counteracted by greater mutual inhibition. Hence, this results in a decrease in FCR interneuron excitability.
The second hypothesis is shown in Fig. 6B. The descending control from the hand motor cortex projecting to the ECR and FCR interneurons is similar, but the reciprocal inhibition and the mutual inhibition at the spinal level are asymmetric. The reciprocal inhibition is more strongly directed from ECR interneurons to FCR motoneurons than in the reverse situation. This hypothesis is supported by results observed at the lumbar level indicating that reciprocal inhibition is asymmetric: the reciprocal Ia inhibition from extensors to flexors is more powerful than the reciprocal Ia inhibition from flexors to extensors (Crone et al. 1987; Eccles and Lundberg 1958). Moreover, the activity of spinal circuits is often asymmetric with regard to target motoneurons; for example, the monosynaptic reflex in FCR is stronger than that in ECR. In this case, when the mutual inhibition is more powerful on FCR interneurons than on ECR interneurons, the net effect on FCR interneurons is inhibitory whereas the effect on ECR interneurons is facilitatory.

However, the indirect methods we used do not allow us to choose between these two hypotheses. Furthermore, both asymmetric descending control and asymmetric reciprocal inhibition may coexist.

**Effects of tDCS Applied to Ipsilateral Hand Motor Cortex**

The possible effects of tDCS applied to ipsilateral hand motor cortex on spinal networks may originate from 1) ipsilateral connections to spinal neurons or 2) hemispheric connections from ipsilateral to contralateral homologous motor cortex areas.

Although the existence of uncrossed corticospinal tracts from motor cortex to spinal motoneurons has been established in healthy humans and higher primates (Kuypers 1981), the effects of activation of the ipsilateral cortex on spinal motoneurons are still under debate. A few papers have reported ipsilateral responses in distal muscles during strong voluntary contractions and high-intensity TMS (Wassermann et al. 1991; Ziemann et al. 1999). Bawa et al. (2004) concluded that, in general, forearm and hand muscles in healthy subjects did not show any ipsilateral motor evoked potentials. However, in poststroke patients, ipsilateral responses have been recorded (Alagona et al. 2001).

Interhemispheric connections between homologous cortical areas are well known and generally considered to be inhibitory, although the presence of excitatory interhemispheric connections has also been proposed (Bloom and Hynd 2005). Furthermore, several studies (for example, Hervé et al. 2005) suggest that ipsilateral control may be different in left-handed and right-handed subjects. In the present study, we were unable to find significant effects with ipsilateral anodal tDCS. If anything, it seems that a small decrease of reciprocal inhibition from ECR to FCR may be occurring in period 1, more marked in left-handed than in right-handed subjects.

**Functional Significance and Possible Therapeutic Applications**

Our findings have shown that an increase of excitability of contralateral hand motor cortex results in an increase of reciprocal inhibition to FCR motoneurons and, in contrast, a de-
crease of the reciprocal inhibition to ECR motoneurons. In other words, it seems likely that the function of this descending control is to facilitate ECR motoneuron excitability by reducing inhibitory influences and to depress the excitability of FCR motoneurons by enhancing inhibitory influences. Fine motor hand movements in humans (grasping, writing, typing, etc.) require ECR contraction to stabilize the wrist joint. The descending control acting on reciprocal inhibition is likely to contribute to the facilitation of ECR contraction. In this case, anodal tDCS may be useful for the treatment of patients with cortical lesions who show wrist extensor deficit. It might also be used to facilitate ECR contraction and promote control of hand movements. This therapeutic approach is based on papers published since 2008 (for review see Ayache et al. 2012) that indicate that anodal tDCS applied to the lesioned hemisphere may improve motor performance.

Recent studies performed in stroke patients suggested that cathodal or dual tDCS may reduce spasticity in upper limb muscles (Vandermeeren et al. 2013; Wu et al. 2013). Cathodal tDCS was not tested in the present study, but our findings that reveal an increase of reciprocal inhibition directed from ECR to FCR suggest that contralateral anodal tDCS may be used to reduce flexor spasticity in stroke patients. Indeed, Nakashima et al. (1989) showed that reciprocal inhibition between wrist muscles is decreased in spastic patients. Therefore, it may be hypothesized that a reinforcement of reciprocal inhibition directed to flexor motoneurons may limit the hyperexcitability of the FCR stretch reflex.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. G. W. Max Westby and to A. Hudson, Australian research fellow in Neurosciences, for having scrutinized the manuscript and to G. Bard for collating references and getting the text into presentable order.

GRANTS

This study was supported by grants from Sorbonne Universités UPMC Paris 6 (MESR, Ministère de l’Enseignement Supérieur et de la Recherche), Institut National de la Santé et de la Recherche Médicale (INSERM), Assistance Publique-Hôpitaux de Paris (AP-HP), Institut pour la Recherche sur la Moelle Épinière (IRME 2012A00407-36), and Agence Nationale de la Recherche (ANR-12-JSV4-0007-01).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


Nakashima K, Rothwell JC, Day BL, Thompson PD, Shannon K, Marsden CD. Reciprocal inhibition between forearm muscles in patients with writer’s