

# Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia

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**The excitability of inhibitory circuits in patients with writer's cramp is reduced at multiple levels within the sensorimotor system, including the primary motor hand area (MI). Although this may play a major role in the pathophysiology of writer's cramp, it is still unclear what factors may cause the imbalance between inhibition and excitation to arise. One possibility is that homeostatic mechanisms that keep cortical excitability within a normal physiological range are impaired. In eight patients with writer's cramp and eight healthy age-matched controls, we combined low-frequency repetitive transcranial magnetic stimulation (rTMS) with transcranial direct current stimulation (TDCS) to probe regional homeostatic plasticity of the left MI. Confirming our previous study (Siebner *et al.*, *J Neurosci* 2004; 24: 3379–85), 'facilitatory' preconditioning of the MI with anodal TDCS enhanced the inhibitory effect of subsequent 1 Hz rTMS on corticospinal excitability. Conversely, 'inhibitory' preconditioning with cathodal TDCS reversed the after effect of 1 Hz rTMS, producing an increase in corticospinal excitability. The results were quite different in patients with writer's cramp. Following preconditioning with TDCS, 1 Hz rTMS induced no consistent changes in corticospinal excitability, indicating a loss of the normal 'homeostatic' response pattern. In addition, the normal inhibitory effect of preconditioning with cathodal TDCS was absent. The present data suggest that homeostatic mechanisms that stabilize excitability levels within a useful dynamic range are impaired in patients with writer's cramp. We propose that a faulty homeostatic response to acute increases in corticospinal excitability favours maladaptive motor plasticity. The role of homeostatic-like plasticity in the pathophysiology of task-specific dystonias warrants further study.**

**Keywords:** focal dystonia; homeostatic plasticity; repetitive transcranial magnetic stimulation; transcranial direct current stimulation; writer's cramp

**Abbreviations:** AMT = active motor threshold; FDI muscle = first dorsal interosseus muscle; ICF = intracortical facilitation; ISI = interstimulus interval; LTD = long-term depression; LTP = long-term potentiation, MEP = motor-evoked potential; PAS = paired associative stimulation; RMT = resting motor threshold; rTMS = repetitive transcranial magnetic stimulation; SICI = short-latency intracortical inhibition; TDCS = transcranial direct current stimulation

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## Introduction

Dystonia is a condition characterized by co-contraction of antagonist muscles and overflow of activity to muscles that are not usually involved in a task (Hallett, 1998). Several lines of evidence suggest that some forms of focal occupational dystonias such as musician's cramp are related to

maladaptive plasticity caused by overtraining (Rothwell and Huang, 2003).

Other studies suggest that some forms of task-specific dystonia such as writers' cramp, which develop after less intensive motor training, may be due to an intrinsic

abnormality of sensorimotor plasticity (Classen, 2003; Rothwell and Huang, 2003; Byl, 2004). In both cases, however, dystonia can be considered as a model of ‘maladaptive motor plasticity’ in humans.

In a previous paper we reported that there was an intrinsic abnormality in the mechanism underlying Hebbian-like associative plasticity in focal dystonia (Quartarone *et al.*, 2003). The model we used was ‘paired associative plasticity’ (PAS), described by Stefan and coworkers (Stefan *et al.*, 2000). In healthy subjects, repeated pairing of electrical stimulation of the median nerve followed 20–25 ms later by transcranial magnetic stimulation (TMS) of the hand area of motor cortex leads to a lasting increase in size of motor-evoked potentials (MEPs) in median nerve innervated hand muscles. The effect is thought to involve a form of long-term potentiation (LTP) at excitatory synapses activated by the TMS pulse (Stefan *et al.*, 2000; Wolters *et al.*, 2003). In patients with writer’s cramp, PAS-induced facilitation was greater and less focal than normal, leading to increases in MEPs in muscle innervated by ulnar as well as median nerve (Quartarone *et al.*, 2003). Since the mechanisms involved in producing PAS also appear to be related to some forms of motor learning (Ziemann *et al.*, 2004), we hypothesized that writer’s cramp patients have an increased tendency to strengthen sensorimotor associations, which might contribute to the abnormalities of movement control seen in dystonia.

As pointed out by many authors, the effectiveness of LTP-like processes must normally be regulated quite closely to stabilize neuronal activity within a useful physiological range (Sejnowski, 1977). The positive-feedback nature of LTP carries the risk of triggering an uncontrolled increase in synaptic effectiveness that becomes potentially destabilizing (Abbott and Nelson, 2000; Turrigiano and Nelson, 2000, 2004). Evidence suggests that this can be prevented by making the amount of LTP dependent on the level of activity in the postsynaptic neuron: the greater the ongoing activity, the less effective are processes leading to LTP, whilst processes leading to long-term depression (LTD) are enhanced. Conversely, the lower the activity of the postsynaptic neurons, the more effective are processes that lead to LTP. This is known as ‘homeostatic’ plasticity and is formalized in the model originally described by Bienenstock, Cooper and Munro (Bienenstock *et al.*, 1982). Given that modifications of synaptic strength must be carefully controlled, it is possible that a failure of these mechanisms could be one factor that maintains the increased excitability of PAS in patients with dystonia.

In the present paper we investigate whether patients with focal task-specific dystonia behave abnormally in a new model of homeostatic plasticity in human motor cortex. This model uses transcranial direct current stimulation (TDCS) to precondition the response of the motor cortex to a subsequent period of repetitive TMS (rTMS) (Siebner *et al.*, 2004). Preconditioning of the motor cortex with an excitatory stimulus (10 min of anodal TDCS) leads to an increase in the inhibitory effect of 1 Hz rTMS, whereas preconditioning

with a suppressive stimulus (10 min of cathodal TDCS) reverses the effect and leads to facilitation (Siebner *et al.*, 2004). Our question was therefore whether the after effects of 1 Hz rTMS can be modulated to the same extent in patients with arm dystonia. If not, then this may introduce a tendency that favours overactivity of LTP-like processes tested with PAS.

## Material and methods

### Participants

We studied eight patients with simple writer’s cramp (seven males; age range 30–72 years, mean age  $46 \pm 15$  years) and eight healthy, age-matched controls (seven males; age range 24–70 years, mean age  $42 \pm 15$  years). All participants were consistent right-handers according to the Edinburgh handedness inventory (Oldfield, 1971). All patients underwent extensive neurological examination in order to rule out patients with other obvious neurological diseases. The clinical data for dystonic patients are summarized in Table 1. Only patient 3 had previously been treated with intramuscular botulinum toxin injections. The last injection was 6 months prior to the experiment. The protocol was approved by our local ethics committee in accordance with the Declaration of Helsinki on the use of human subjects in experiments.

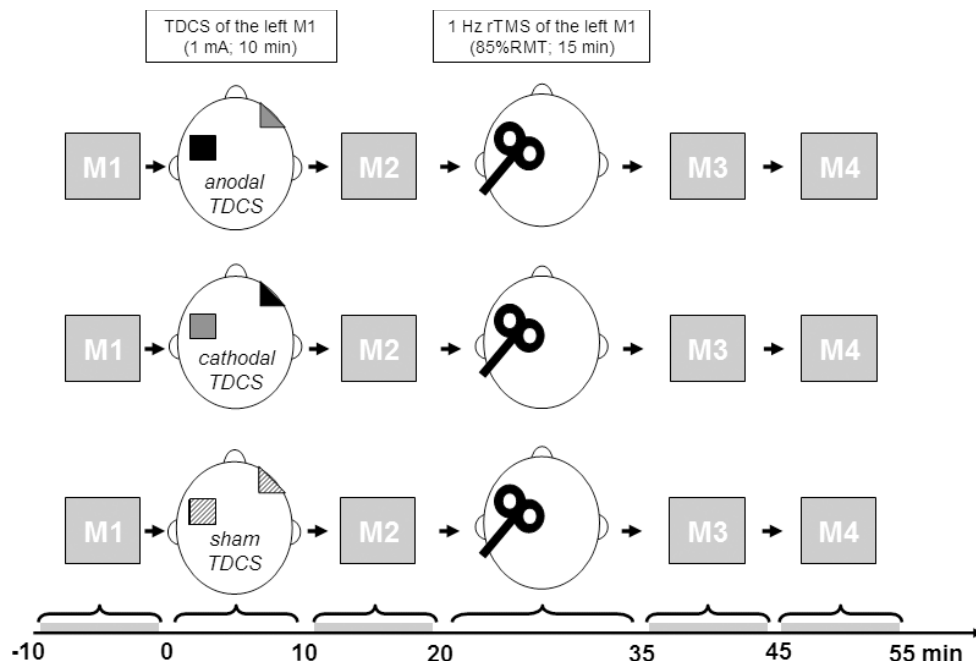
### Experimental design

In patients with writer’s cramp and healthy subjects we explored the effect of TDCS priming on the conditioning effect of 1 Hz rTMS on motor cortex excitability (Siebner *et al.*, 2004).

**Table 1** Clinical details of the eight patients with writer’s cramp

Patient No.	Age (years)	Sex	Duration of disease (years)	Dystonic pattern
1	30	M	4	Ulnar deviation and index extension
2	37	M	3	Ulnar deviation, wrist extension, elbow lifting
3	72	M	30	Ulnar deviation, 4th and 5th finger flexion, thumb extension
4	53	F	7	Ulnar deviation, wrist extension, 4th and 5th finger flexion
5	49	M	15	Ulnar deviation, arm abduction
6	60	M	20	Ulnar deviation, wrist extension, elbow lifting
7	38	M	0.5	Thumb adduction, wrist extension, elbow lifting
8	30	M	3	Ulnar deviation and index extension

M = male; F = female.



**Fig. 1** Experimental design. Ten minutes of anodal, cathodal or sham TDCS priming was given to the left primary motor hand area, on separate days. For anodal TDCS direct current ( $\pm 1$  mA) was given for 10 min via two large-sized electrodes placed over primary motor cortex and the contralateral frontal pole. For cathodal TDCS the montage was reversed. Ten minutes after tDCS preconditioning, 900 biphasic pulses of 1 Hz rTMS were given to the left primary motor cortex at 85% of rest motor threshold. Corticospinal excitability was measured before (M1), after tDCS (M2) and twice after rTMS (M3–M4).

All the participants received 15 min of subthreshold 1 Hz rTMS delivered to the primary motor cortex (Siebner *et al.*, 2004). This conditioning protocol sometimes produce a slight reduction of corticospinal excitability (Maeda *et al.*, 2000b). This protocol was preceded either by a 10 min period of priming with real TDCS to the left M1 using anodal (excitatory) or cathodal (inhibitory) polarity (Siebner *et al.*, 2004). In a control experiment on eight controls and five patients we tested the effects of a 10 min period of priming by sham stimulation on the after effects produced by 1 Hz rTMS over the left M1. Several measures of cortical excitability, such as MEP amplitude, short latency intracortical inhibition (SICI) and intracortical facilitation (ICF), were assessed using TMS, before TDCS (baseline, M1), after TDCS (M2) and twice after 1 Hz rTMS (M3–M4), see Fig. 1 (Siebner *et al.*, 2004). Each block of trials lasted ~8 min. To ensure comparability of our measurements across individuals, we always waited for 10 min before starting with a new intervention or the next block of measurements.

## Transcranial cortex stimulation

### Repetitive transcranial magnetic stimulation

Focal rTMS was given through a standard figure-of-eight shaped coil connected to a Magstim Rapid stimulator (Magstim Company, Whitland, Dyfed, UK). The mean loop diameters of the coil were 9 cm. The magnetic stimulus had a biphasic waveform with a pulse width of ~300  $\mu$ s. The junction region of the coil pointed backwards and laterally at a 45° angle away from the midline, approximately perpendicular to the line of the central sulcus. During the first phase of the stimulus, the current in the centre of the coil flowed toward the handle and the first phase of the biphasic stimulus induced a posterior–anterior current in the brain. The coil was placed tangentially to the scalp at the optimum scalp position that consistently

elicited the largest MEPs with the steepest slope in the right first dorsal interosseus (FDI) muscle ('motor hot spot'). In the present study we used an intensity of stimulation of 85% of resting motor threshold (RMT), as used in our previous study (Siebner *et al.*, 2004).

### Transcranial direct current stimulation

A constant direct current of 1 mA intensity was applied through saline-soaked sponge electrodes (surface 35 cm<sup>2</sup>). For cathodal stimulation the cathode was placed above the motor cortical representational field of the right FDI muscle, as revealed by TMS, and the anode above the contralateral orbita (Nitsche *et al.*, 2001). For anodal stimulation the montage was reverted (Nitsche *et al.*, 2001). Cathodal and anodal TDCS were delivered at least 1 week apart, through a battery-driven constant-current stimulator (DC stimulator; Rolf Schneider Electronic, Gleichen, Germany), using the same stimulation protocol, for a period of 10 min.

We also gave sham stimulation to the left M1. For sham TDCS the DC stimulator was switched on for only 5 s at the beginning of the sham stimulation and then turned off (Siebner *et al.*, 2004).

### Cortical excitability measurements

Surface electromyography was used to record MEPs in the relaxed FDI muscle. Changes in motor cortical excitability were probed using single-pulse TMS. Monophasic pulses were given to the left M1-HAND using a high-power Magstim 200 stimulator (Magstim Company) and a standard figure-of-eight coil, with external loop diameters of 9 cm. An identical coil position was used as for rTMS. The coil was positioned over the 'hot spot' for stimulation of the contralateral FDI muscle and the handle of the coil pointed 45° postero-laterally. The monophasic magnetic stimulus had a rise

time of  $\sim 100$   $\mu$ s, decaying back to zero over  $\sim 0.8$  ms. The coil current during the rising phase of the magnetic field flowed toward the handle. Thus, the induced current in the cortex flowed in a postero-anterior direction.

Before (M1), after TDCS (M2) and twice after rTMS at 1 Hz (M3–M4) we measured the RMT and active motor threshold (AMT), MEP amplitude at rest, and SICI and ICF in blocks of measurements (see Fig. 1). Within each block, we first measured motor thresholds. RMT was defined as the minimum intensity that could evoke a peak-to-peak MEP of 50 mV in at least five out of 10 consecutive trials in the relaxed FDI muscle. AMT was defined as the stimulator intensity capable to elicit a reproducible MEP of at least 200 mV in the tonically contracting contralateral FDI muscle in at least five of 10 consecutive trials.

After measurements of motor thresholds, corticospinal excitability, SICI and ICF were determined with single-pulse and paired-pulse TMS using the paradigm described by Kujirai *et al.* (1993). The intensity of the test stimulus (TS) was adjusted to elicit MEPs with a peak-to-peak amplitudes of 0.8–1.0 mV. The intensity of the conditioning stimulus was set at 80% of AMT. Stimulus intensities were determined immediately before the experiment and kept identical throughout the experiment (M1, M2, M3, M4). Interstimulus intervals (ISI) of 2 and 4 ms tested the magnitude of SICI, whereas longer ISI of 9–12 ms probed the strength of ICF. These ISIs were selected to ensure comparability with our previous work (Lang *et al.*, 2004; Siebner *et al.*, 2004). However, it should be noted that the restricted set of ISIs tested in the present study only provide limited information about the strength of SICI and ICF. The TS was also given alone to elicit unconditioned MEPs in the relaxed right FDI muscle, and the mean peak-to-peak amplitude of the unconditioned MEPs was used to assess corticospinal excitability. In each block of measurements, 20 unconditioned MEPs (TS alone) and 15 conditioned MEPs were recorded for each ISI, and stimulation conditions were intermingled in a pseudorandom order.

### Data acquisition and analyses

All subjects were seated in a comfortable reclining chair. Electromyographic (EMG) activity of the right FDI muscle was recorded with Ag-AgCl surface electrodes using a belly-tendon montage. EMG signals were amplified and filtered using a time constant of 3 ms and a high-pass filter set a 3 kHz (Neurolog System; Digitimer Ltd, Welwyn Garden City, UK). Signals were acquired at a rate of 5 kHz (CED 1401 laboratory interface; Cambridge Electronic Design, Cambridge, UK) on a personal computer for off-line analysis. Auditory (speakers) and visual (oscilloscope) feedback of EMG activity were given to the subjects to ensure complete relaxation.

For each block of measurements, the peak-to-peak amplitudes of each MEP (mV) were measured off-line and the mean MEP amplitudes were calculated for each stimulation condition, using NuCursor software (Sobell Research Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College of London, London, UK). The mean amplitude of the conditioned MEPs was normalized to the unconditioned MEP amplitudes for each block of measurement. The conditioned MEP amplitudes at an ISI of 2 and 4 ms were pooled and used as an index of SICI, while the conditioned MEP amplitudes at an ISI of 9 and 12 ms were pooled to characterize changes in ICF. Data are given as mean  $\pm$  SD.

For each measure of cortical excitability (unconditioned MEPs, SICI and ICF), we performed separate repeated-measures analysis

of variance (ANOVA). Our main focus was on stimulation-induced changes in corticospinal excitability, as a previous study has only shown a homeostatic response pattern for the unconditioned MEPs but not for SICI and ICF (Lang *et al.*, 2004; Siebner *et al.*, 2004). Between-groups comparison was performed using a three-way repeated-measures ANOVA with 'time of measurement' (before TDCS, after TDCS, after 1 Hz rTMS<sub>1</sub> and after 1 Hz rTMS<sub>2</sub>), 'type of priming' (anodal TDCS and cathodal TDCS) as within-subjects factors and 'group' (controls and patients) as between-subject factor. The Greenhouse–Geisser method was used if necessary to correct for non-sphericity. A *P* value of  $<0.05$  was considered significant. If the ANOVA revealed a significant difference between groups, two-way ANOVAs with 'time of measurement' (before TDCS, after TDCS, after 1 Hz rTMS<sub>1</sub> and after 1 Hz rTMS<sub>2</sub>) and 'type of priming' (anodal TDCS and cathodal TDCS) were performed within each group to explore time-dependent changes in mean MEP amplitude.

Conditional on a significant *F* value, we also performed paired-samples *t*-tests to explore the strength of main effects and the patterns of interaction between experimental factors. *Post hoc* pair-wise comparisons were based on *a priori* knowledge of the homeostatic response pattern as revealed by our previous studies (Siebner *et al.*, 2004). No correction for multiple comparisons was applied because pair-wise comparisons were only used to describe the temporal patterns underlying a significant main effect or interaction as revealed by the ANOVA.

### Results

None of the subjects reported adverse effects after TDCS or rTMS. RMT and AMT were unaffected by TDCS or rTMS in healthy controls and in dystonic patients (see Table 2 for raw data). However, the amplitude of unconditioned MEPs evoked by a suprathreshold stimulus was influenced by both forms of conditioning and is discussed in detail below (Fig. 2A and B).

A three-way repeated measures ANOVA using the mean MEP amplitude as dependent variable, showed no main effects of the factors group [ $F(1,14) = 1.3$ ;  $P > 0.1$ ], time of measurement [ $F(3,42) = 2.1$ ;  $P = 0.1$ ] or type of priming [ $F(1,14) = 1.9$ ;  $P = 0.1$ ]. There was, however, an interaction between the factors type of priming and time of measurement [ $F(3,42) = 3.3$ ;  $P = 0.02$ ]. This interaction indicates that the type of priming produced different temporal patterns of changes in MEP amplitude. In addition, we found an interaction between type of priming, time of measurement and group [ $F(3,42) = 6.9$ ;  $P < 0.001$ ] showing that the modulatory effects of the two types of interventions (cathodal TDCS followed by 1 Hz rTMS versus anodal TDCS followed by 1 Hz rTMS) on mean MEP amplitudes differed between patients and controls.

### Changes in corticospinal excitability in healthy subjects

In healthy subjects, the pattern of changes in mean MEP amplitude closely resembled the pattern of conditioning effects reported by Siebner *et al.* (2004). For the unconditioned MEP amplitude, two-way repeated measures ANOVA showed no

**Table 2** Mean ( $\pm$ SD) values for each measure of corticospinal excitability; corticospinal excitability was assessed before (M1), after tDCS (M2) and twice after rTMS (M3–M4)

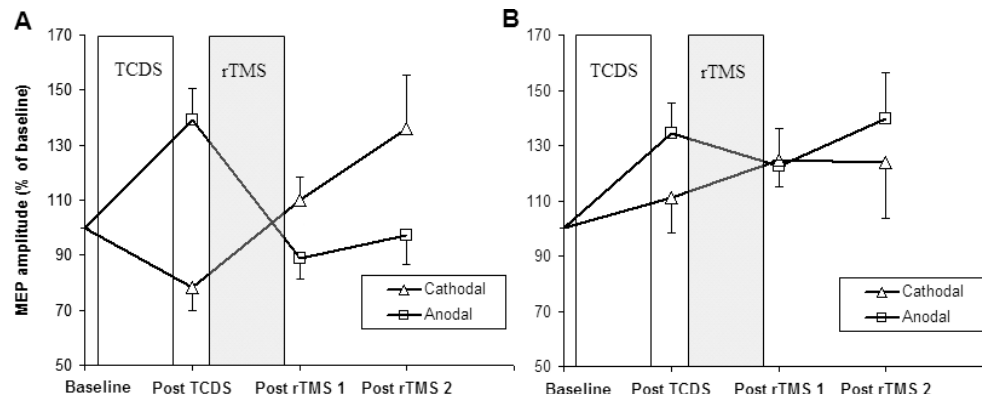
	Preconditioning	M1	M2	M3	M4
<b>Patients (n = 8)</b>					
MEP amplitude (mV)	anodal tDCS	0.9 $\pm$ 0.5	1.1 $\pm$ 0.5	1.2 $\pm$ 0.9	1.3 $\pm$ 0.7
	cathodal tDCS	0.9 $\pm$ 0.5	0.9 $\pm$ 0.4	1.1 $\pm$ 0.4	0.9 $\pm$ 0.4
	sham tDCS (n = 5)	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.9 $\pm$ 0.3
RMT (%)	anodal tDCS	46 $\pm$ 8	46 $\pm$ 8	46 $\pm$ 8	46 $\pm$ 8
	cathodal tDCS	46 $\pm$ 7	46 $\pm$ 8	46 $\pm$ 8	46 $\pm$ 7
	sham tDCS (n = 5)	47 $\pm$ 6	47 $\pm$ 5	46 $\pm$ 6	46 $\pm$ 5
AMT (%)	anodal tDCS	37 $\pm$ 6	37 $\pm$ 5	37 $\pm$ 6	37 $\pm$ 6
	cathodal tDCS	37 $\pm$ 6	36 $\pm$ 6	37 $\pm$ 5	37 $\pm$ 6
	sham tDCS (n = 5)	37 $\pm$ 5	37 $\pm$ 4	37 $\pm$ 5	37 $\pm$ 5
SICI (%)	anodal tDCS	58 $\pm$ 19	70 $\pm$ 24	66 $\pm$ 21	62 $\pm$ 21
	cathodal tDCS	62 $\pm$ 22	65 $\pm$ 39	41 $\pm$ 31	60 $\pm$ 24
	sham tDCS (n = 5)	55 $\pm$ 23	69 $\pm$ 37	88 $\pm$ 39	77 $\pm$ 27
ICF (%)	anodal tDCS	116 $\pm$ 30	122 $\pm$ 39	119 $\pm$ 49	118 $\pm$ 36
	cathodal tDCS	126 $\pm$ 43	131 $\pm$ 40	118 $\pm$ 45	119 $\pm$ 36
	sham tDCS (n = 5)	140 $\pm$ 18	153 $\pm$ 42	160 $\pm$ 74	176 $\pm$ 79
<b>Healthy controls (n = 8)</b>					
MEP amplitude (mV)	anodal tDCS	0.9 $\pm$ 0.3	1.2 $\pm$ 0.5	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3
	cathodal tDCS	0.8 $\pm$ 0.3	0.6 $\pm$ 0.2	0.8 $\pm$ 0.3	1.1 $\pm$ 0.6
	sham tDCS	0.9 $\pm$ 0.3	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2	0.9 $\pm$ 0.4
RMT (%)	anodal tDCS	48 $\pm$ 8	47 $\pm$ 8	48 $\pm$ 8	48 $\pm$ 8
	cathodal tDCS	48 $\pm$ 8	47 $\pm$ 7	47 $\pm$ 8	48 $\pm$ 8
	sham tDCS	47 $\pm$ 6	47 $\pm$ 6	48 $\pm$ 5	47 $\pm$ 6
AMT (%)	anodal tDCS	39 $\pm$ 6	39 $\pm$ 6	39 $\pm$ 6	38 $\pm$ 6
	cathodal tDCS	38 $\pm$ 6	39 $\pm$ 5	38 $\pm$ 6	38 $\pm$ 6
	sham tDCS	38 $\pm$ 5	38 $\pm$ 5	39 $\pm$ 5	39 $\pm$ 5
SICI (%)	anodal tDCS	36 $\pm$ 14	43 $\pm$ 19	42 $\pm$ 16	46 $\pm$ 18
	cathodal tDCS	41 $\pm$ 23	44 $\pm$ 14	45 $\pm$ 14	46 $\pm$ 27
	sham tDCS	45 $\pm$ 17	49 $\pm$ 15	49 $\pm$ 15	50 $\pm$ 16
ICF (%)	anodal tDCS	141 $\pm$ 40	134 $\pm$ 39	123 $\pm$ 55	135 $\pm$ 88
	cathodal tDCS	125 $\pm$ 26	133 $\pm$ 29	123 $\pm$ 23	122 $\pm$ 42
	sham tDCS (n = 5)	136 $\pm$ 45	199 $\pm$ 79	204 $\pm$ 35	144 $\pm$ 52

RMT/AMT = resting/active motor threshold, given in % of maximum stimulator output; SICI/ICF = short-latency intracortical inhibition/intracortical facilitation, given in % of the unconditioned test response.

main effect of time of measurement [ $F(3,21) = 2.46$ ;  $P = 0.09$ ] or type of priming [ $F(1,7) = 0.4$ ;  $P > 0.1$ ], but a significant interaction between the factors time of measurement and type of priming [ $F(3,21) = 8.5$ ;  $P < 0.001$ ]. TDCS produced a bidirectional change in corticospinal excitability (Fig. 2A, Table 2). Ten minutes of anodal TDCS raised the level of corticospinal excitability, whereas 10 min of cathodal TDCS decreased corticospinal excitability [pre-TDCS versus post-TDCS; anodal TDCS:  $t(1,7) = -3.4$ ,  $P = 0.01$ ; cathodal TDCS:  $t(1,7) = 2.6$ ,  $P = 0.03$ ]. A subsequent session of 1 Hz rTMS reversed the excitability changes that had been induced by TDCS priming. When given after ‘facilitatory’ anodal TDCS, 1 Hz rTMS was caused a reduction in corticospinal excitability for at least 20 min after the end of rTMS [post-TDCS versus post-rTMS<sub>1</sub>:  $t(1,7) = 3.2$ ,  $P = 0.01$ ; post-TDCS versus post-rTMS<sub>2</sub>:  $t(1,7) = 2.99$ ,  $P = 0.02$ ]. When 1 Hz rTMS followed ‘inhibitory’ cathodal TDCS, 1 Hz rTMS increased corticospinal excitability [post-TDCS versus post-rTMS<sub>1</sub>:  $t(1,7) = -3.1$ ,  $P = 0.01$ ; post-TDCS versus post-rTMS<sub>2</sub>:  $t(1,7) = -3.2$ ,  $P = 0.01$ ].

### Changes in corticospinal excitability in patients with writer’s cramp

The after effects of TDCS and rTMS on mean MEP amplitudes are illustrated in Fig. 2B. Patients showed a different pattern of changes in corticospinal excitability relative to healthy controls (Fig. 2, Table 2). In contrast to the healthy subjects, the two-factorial within-group ANOVA revealed no significant interaction between the factors time of measurement and type of priming [ $F(3,21) = 1.3$ ;  $P = 0.2$ ]. There was also no main effect of time of measurement [ $F(3,21) = 2.3$ ;  $P = 0.1$ ] or type of priming [ $F(1,7) = 1.4$ ;  $P > 0.1$ ]. Only anodal but not cathodal TDCS produced an after effect on MEP amplitudes. In analogy to healthy volunteers, anodal TDCS resulted in a lasting facilitation of corticospinal excitability [pre-TDCS versus post-TDCS; anodal TDCS:  $t(1,7) = -2.7$ ;  $P = 0.03$ ]. In contrast, cathodal TDCS failed to induce consistent changes in MEP amplitude in the patient group [pre-TDCS versus post-TDCS; cathodal TDCS:  $t(1,7) = -0.8$ ;  $P = 0.43$ ]. In addition, neither anodal nor cathodal TDCS had a priming effect on subsequent rTMS conditioning.



**Fig. 2** Changes in MEP amplitude induced by transcranial stimulation of left primary motor cortex in healthy controls and patients with writer's cramp. **A** illustrates the mean amplitude of MEPs following single pulse TMS at different times before and after the two type of conditioning in healthy controls. There was a 'facilitatory' response to anodal TDCS preconditioning that was reversed by a subsequent period of 1 Hz rTMS. Conversely, 'inhibitory' preconditioning with cathodal TDCS resulted in an opposite after effect of 1 Hz rTMS, which led to an increase in corticospinal excitability. **B** plots the changes in corticospinal excitability in writer's cramp patients after the two type of conditioning. Patients showed an abnormal responsiveness to TDCS and rTMS. (i) Only anodal TDCS produced a normal facilitatory effect on corticospinal excitability, whereas 'inhibitory' cathodal TDCS had no after effect on corticospinal excitability. (ii) Regardless of the type of preconditioning, rTMS had no consistent effect on corticospinal excitability. In particular, although anodal TDCS produced a 'normal' facilitatory response, subsequent 1 Hz rTMS did not reverse the increase in excitability produced by anodal preconditioning. MEP amplitudes are given as a percentage of the MEP size at baseline. Error bars represent the standard error of the mean.

Regardless of the type of priming, 1 Hz rTMS exerted no consistent after effects on MEP amplitudes. Importantly, 1 Hz rTMS failed to reverse the MEP facilitation that had been induced by previous anodal TDCS (Table 2, Fig. 2B).

In a subgroup of five patients, we additionally tested non-specific effects of TDCS preconditioning using a sham procedure (Table 2). One-way ANOVA showed that MEP amplitudes were unchanged after sham TDCS and were also unaffected after the subsequent period of 1 Hz rTMS [main effect of time of measurement:  $F(3,12) = 1.3$ ;  $P = 0.3$ ].

### No changes in paired-pulse excitability

Table 2 lists the relative strength of mean SICI and ICF for each block of measurements. Separate three-way repeated-measures ANOVA were calculated using the mean SICI or ICF as dependent variable. ANOVAs only revealed a main effect of group for SICI, indicating that the relative strength of SICI at an ISI of 2 and 4 ms was different in patients compared with healthy controls [ $F(1,14) = 7.7$ ;  $P = 0.01$ ]. This was because mean SICI was attenuated in patients relative to healthy controls (Table 2). The mean values of SICI for patients and volunteers across all four measurements were, respectively,  $70 \pm 37$  and  $42 \pm 18$  (unpaired  $t$ -test  $t = -3.7$ ;  $P < 0.0003$ ). There was no main effect of group for ICF [ $F(1,14) = 0.25$ ;  $P = 0.6$ ]. Both ANOVAs revealed no main effects of time of measurements or type of priming nor an interaction between the factors ( $P > 0.1$ ). In contrast to the ANOVA of unconditioned MEP amplitudes, ANOVA showed no interaction between type of priming, time of measurement and group for SICI [ $F(3,42) = 1.8$ ;  $P = 0.1$ ] or ICF [ $F(3,42) = 0.7$ ;  $P = 0.5$ ]. Taken together, the relative strength of SICI and ICF was unchanged throughout the experiments in both healthy controls and patients with writer's cramp.

### Discussion

The present results confirmed the presence of 'homeostatic-like' plasticity in the left M1 of healthy human subjects: LTD-like after effects of a period of 1 Hz rTMS were enhanced after a period of excitatory (anodal) preconditioning, whereas LTP-like effects were enhanced after a period of suppressive (cathodal) preconditioning. Neither effect was seen in patients with dystonia: anodal preconditioning failed to convert the after effect of 1 Hz rTMS into clear inhibition, whereas cathodal preconditioning not only had no effect on 1 Hz rTMS, but also failed itself to have any lasting effect on MEP amplitudes.

### Homeostatic-like plasticity in human motor cortex

In a previous paper we argued that preconditioning effects of TDCS on the long-term response to subsequent 1 Hz rTMS were due to 'homeostatic' regulation of synaptic efficacy (Siebner *et al.*, 2004). Changes in amplitude of MEP after rTMS were thought to reflect changes in the efficacy of synaptic relays in the MEP pathway (Siebner and Rothwell, 2003). The fact that the direction of these changes could be reversed by preconditioning with anodal or cathodal TDCS suggested a mechanism resembling the Bienenstock–Cooper–Munro (BCM) model of homeostatic plasticity (Bienenstock *et al.*, 1982). Though the exact physiological mechanisms remains to be clarified, this homeostatic mechanism determined whether the net result was excitatory or inhibitory. If correct, then lack of such effects in patients with dystonia implies that these mechanisms of synaptic stabilization no longer function effectively. In particular, the lack of MEP depression after anodally primed 1 Hz rTMS is compatible with the idea that

the motor cortex of patients with dystonia may strengthen synaptic connections even when levels of background excitability are high, perhaps leading to positive reinforcement of strong pathways. Since the cellular mechanisms that regulate homeostatic plasticity are still under investigation, we can only speculate in very general terms about the nature of the disorder that could cause this to happen. However, it may, for example, reflect a failure of mechanisms that integrate overall levels of synaptic activity and hence determine the BCM-like effects on plasticity, or the mechanisms that are then used to regulate synaptic efficacy.

The lack of any effect of cathodal preconditioning on MEPs was intriguing, particularly in view of the clear effect of anodal TDCS. This cannot be due to a general problem in producing long-term inhibitory effects (Siebner *et al.*, 1999). For instance, Edwards *et al.* (2004) recently reported that the 'theta burst' form of rTMS led to greater inhibitory after effects on MEPs in dystonia than in normal subjects. One possible reason for the failure of cathodal TDCS to do the same relates to the fact that 'phasic' rTMS activates cortical neurons directly, whereas 'tonic' TDCS can only bias the firing frequency and membrane potential of already active cells. Data from animal experiments suggest that cathodal conditioning produces its effects by a combination of reducing ongoing levels of discharge and hyperpolarizing neuronal membranes. In animal studies of synaptic plasticity, hyperpolarizing a postsynaptic neuron increases the likelihood of synapses undergoing LTD. This, combined with a decrease in basal levels of discharge, could be the trigger for induction of synaptic depression by cathodal TDCS and lead to smaller test MEPs. If cell membrane potentials are already hyperpolarized in patients with dystonia at rest compared with healthy subjects, then the effects of cathodal TDCS might be reduced; in contrast, anodal TDCS, which depolarizes neurons and increases cell discharge, would function as usual.

Since there was no immediate cathodal effect on MEP amplitude, we have no evidence for a general decrease in excitability that could favour the 'homeostatic' mechanism. Thus we cannot at this stage make firm conclusions about the response of homeostatic mechanisms to inhibitory preconditioning. Either way, patients have a reduced efficiency of mechanisms that might reverse plasticity in motor cortex.

### Paired-pulse cortical excitability

In agreement with our previous study (Siebner *et al.*, 2004), TDCS priming and rTMS conditioning produced no specific after effects on SICI and ICF over and above the changes of unconditioned MEPs. This finding suggests that the present paradigm probed homeostatic-like plasticity regulating the excitability of the corticospinal output neurons. However, the present data cannot exclude subtle additional effects of transcranial stimulation on paired-pulse excitability, because only two ISIs each were used to probe SICI (2 and 4 ms) and ICF (9 and 12 ms). It is also worth recalling that we did not re-adjust the stimulus intensity after each intervention to match the

mean amplitude of the unconditioned MEPs across the four blocks of measurements. Therefore, the changes in MEP amplitude may have obscured subtle effects on SICI and ICF.

In line with previous studies (Ridding *et al.*, 1995; Siebner *et al.*, 1999), the amount of SICI was reduced in patients with writer's cramp, indicating cortical disinhibition. It remains to be clarified whether there is a link between deficient intracortical inhibition and faulty homeostatic-like plasticity in the M1. It is conceivable that a reduction of the inhibitory influence on corticospinal output neurons drives abnormal homeostatic plasticity. Alternatively, a lack of homeostatic mechanisms may cause a state of disinhibition.

### Relevance of results to previous studies in dystonia

Previously, we reported that PAS led to excessive and non-focal facilitation of MEPs in patients with arm dystonia. We argued that this 'intrinsic abnormality' might be the result of enhanced plasticity at central synapses and that it might contribute to the motor symptoms of dystonia by excessive reinforcement of unwanted sensorimotor interactions (Quartarone *et al.*, 2003). Moreover, in line with these results, Edwards *et al.* (2004) recently reported that long lasting after effects of rTMS were enhanced in patients with primary dystonia. In a PET study, we showed that 1 Hz rTMS of premotor cortex decreased metabolic activity at both the site of stimulation and connected areas at a distance, and that this effect was larger in patients with focal hand dystonia than in healthy controls (Siebner *et al.*, 2003).

The present data extend the concept of aberrant plasticity in dystonia by showing that in writer's cramp patients there may be abnormalities in the 'set point' of processes that control levels of plasticity, perhaps causing them to become fixed at an abnormal default level. The fact that anodal preconditioning leads to no homeostatic effect on 1 Hz rTMS suggests that in dystonic patients the effectiveness of LTP-like processes would not be reduced during periods of high activity. This could lead to reinforcement of redundant synaptic connections during periods of high motor activity, and to consolidation of movement combinations that are not wanted. Moreover, not only may dystonic patients 'overlearn', but they may have a problem in reversing learning. This is supported by the fact that cathodal TDCS failed to produce depressive effects on cortical excitability in dystonic patients. Animal studies suggest that the primary mechanism of action of cathodal TDCS is to hyperpolarize the resting membrane potentials in cortical neurons, resulting in secondary reduction of spontaneous discharge rates (Bindman *et al.*, 1962, 1964; Gartside, 1968). The failure to reduce cortical excitability with cathodal TDCS indicates that corticospinal excitability is no longer modulated by neuronal activity levels in motor cortex. This then might also contribute to a failure to activate mechanisms that reverse learning-dependent plasticity.

The fact that the motor 'memory' of a recently learned simple ballistic movement can be disrupted by rTMS of

primary motor area (M1) suggests that M1 is involved in the early stages of some types of motor learning (Muellbacher *et al.*, 2002; Baraduc *et al.*, 2004). The mechanisms probably involve changes in efficiency of synaptic connections. Ziemann *et al.* (2004) showed that motor learning reduced the effectiveness of associative LTP-like plasticity but enhanced the induction of LTD-like plasticity. The implication is not only that synaptic changes in M1 are relevant for behavioural learning, but also that their effectiveness follows a 'homeostatic' rule. This careful regulation of synaptic strength could reduce behavioural interference between overlapping motor tasks, avoiding the consolidation of movement combinations that are not wanted. In dystonia, failure of these regulatory mechanisms might lead to consolidation of abnormal motor engrams containing redundant information, so that when a desired movement is selected there is a spread of activation to muscle that need not be involved in the task. Our results are in keeping with the abnormalities of motor cortex excitability preceding movement in patients with dystonia that are due to an altered release or running of motor programmes (Gilio *et al.*, 2003).

Since we only examined the after effects of TDCS and rTMS conditioning on corticospinal excitability at rest, the present study provides no information about conditioning effects of transcranial cortex stimulation on corticospinal excitability during voluntary contraction. Therefore, it remains to be shown whether the present approach can also characterize homeostatic-like plasticity in the 'activated' corticospinal motor system.

In conclusion, the present data suggest an intrinsic abnormality of homeostatic mechanisms that regulate Hebbian-like plasticity within human motor cortex of patients with writer's cramp. This might lead to the reinforcement of unwanted synaptic connections particularly during practice of a motor task and result in unfocused muscle contraction and dystonia.

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