THE INTERACTION BETWEEN METAPLASTIC NEUROMODULATION AND FATIGUE IN MULTIPLE SCLEROSIS

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By Claire Xian

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ABSTRACT

Fatigue contributes substantially to decrements of quality of life in Multiple Sclerosis (MS) yet, available treatments demonstrate limited efficacy. Transcranial direct current stimulation (tDCS) is a form of non-invasive brain stimulation which presents promise in the management of fatigue, likely related to its capacity to modulate fatigue-related changes in corticospinal excitability. However, high variability limits its clinical application. There is some evidence for capitalising on homeostatic metaplasticity using tDCS as a way to boost outcomes however, this remains to be explored in fatigue in MS. We investigated the impact of cathodal tDCS (ctDCS) priming on anodal tDCS (atDCS)-induced corticospinal excitability and fatigue modulation in MS. 10 MS patients and 10 healthy controls completed a fatiguing exercise whilst receiving either ctDCS or sham (stDCS) primed atDCS to the motor cortex. We assessed change in maximal voluntary contraction force and motor evoked potential (MEP) amplitude across time. Force similarly declined during fatiguing exercise irrespective of group ($P \geq 0.15$) and neuromodulation ($P \geq 0.09$). However, force returned to baseline in controls post-exercise with ctDCS-atDCS ($P \geq 0.14$), highlighting a possible interaction between excitability modulation during, and recovery of force following exercise. In healthy controls, MEP facilitation brought about by stDCS primed atDCS ($P < 0.01$) and exercise alone ($P < 0.01$) was enhanced with ctDCS primed atDCS. This effect was absent in MS ($P \geq 0.13$) suggesting an impairment of metaplasticity mechanisms. These findings expand understanding of tDCS effects in MS and emphasize important considerations for optimising its therapeutic application.

INTRODUCTION

Multiple Sclerosis (MS) is an immune-mediated disease characterised by demyelination, inflammation, and neurodegeneration of the central nervous system[1]. MS patients characteristically exhibit elevated motor fatigue[2-4]; an abnormality which ultimately limits their ability to perform daily tasks and contributes to substantial decrements in quality of life.

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique involving the delivery, via electrodes, of low-intensity electrical current which can modulate corticospinal excitability in targeted motor regions[5-7]. Online tDCS effects are mainly related to a polarity-specific bidirectional modification of membrane resting potential[5, 8, 9]. Anodal tDCS (atDCS) increases excitability by causing subthreshold membrane depolarisation; whereas, cathodal tDCS (ctDCS) causes subthreshold hyperpolarisation and an excitability decrease[7, 9, 10]. Prolonged stimulation can induce lasting enhancement and diminution of synaptic efficacy[5], compatible with the persistent forms of neuroplasticity known as long-term potentiation (LTP) and long-term depression (LTD), respectively[10, 11].

It is well-documented that corticospinal excitability increases during single-joint fatiguing exercise, which is thought to reflect a boost in motor output to the muscle to compensate for the progressive difficulty in maintaining contractile force[12, 13]. Little is known about how tDCS interacts with motor fatigue in MS. However in healthy populations, tDCS delivered to the motor cortex has demonstrated efficacy, possibly by modulating fatigue related changes in corticospinal excitability[14-16]. Still, high variability and small sample sizes limit its clinical application. Inconsistency in tDCS effects can result from a multiplicity of intrinsic[17-20] and extrinsic variables[7]; hence, a number of questions remain as to how to apply tDCS in order to optimise its functional outcomes. Beyond the parameters of the stimulation itself, the direction and magnitude of effects critically depend on the level of synaptic activity at the time of stimulation, as well as the history of synaptic activity in the targeted region. All neural activity is subject to homeostatic regulation that ensures the stability of neural function[21, 22]. Accordingly, non-linear phenomena arise such that a stimulus which characteristically increases excitability can lead to decreased excitability if applied with another excitatory stimulus[23-25].

Metaplasticity is a form of neural activity regulation in which the induction of synaptic change is dependent on the history of activity at the synapse. It functions to keep synaptic activity within a dynamic range to support the integration of temporally spaced episodes of synaptic change[26]. The Bienenstock-Cooper-

Munro model[23] provides a theoretical solution to metaplasticity, proposing that the threshold for synaptic modification dynamically and bidirectionally adjusts as a function of prior activity. Specifically, prior LTD shifts the modification threshold to the left, making the induction of further LTD more difficult and LTP more likely. The opposite is observed with prior LTP[23, 26, 27]. Capitalising on metaplasticity, previous studies applying temporally separate periods of tDCS to the motor cortex have demonstrated efficacy for improving motor learning and skill acquisition in healthy individuals[28, 29]. Overall, lowering the modification threshold with ctDCS priming appears to be advantageous for boosting both functional and corticospinal excitability outcomes of subsequent atDCS[28, 29]. While understanding of how to best apply tDCS to exploit metaplasticity is incomplete, this data is encouraging. To date, the impact of metaplastic neuromodulation on motor fatigue has not been explored in MS.

The aim of this study was to investigate the interaction between metaplastic neuromodulation and fatigue in MS. We hypothesised that ctDCS primed atDCS applied concurrently with fatiguing exercise would enhance corticospinal excitability facilitation and reduce fatigue, compared to atDCS primed by sham stimulation (stDCS).

METHODS

Subjects

11 MS patients (10 relapsing remitting and one primary progressive MS) from the MS Society of South Australia and 10 healthy control subjects (matched for age, gender, handedness (Edinburgh Handedness Inventory[30]), and physical activity (International Physical Activity Questionnaire[31])) were recruited for participation in the study. Subjects were excluded for any contraindications to transcranial magnetic stimulation (TMS) such as metallic implants in the skull, cardiac pacemaker, pregnancy, and/or history of seizures/epilepsy. Patients using Selective Serotonin Reuptake Inhibitors (SSRI) (N = 3) were included in the study so that the sample would be representative of the population; anti-depressant use is frequent in MS[32]. Procedures were approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent.

Experimental setup and electromyography

Subjects were seated with the right elbow flexed approximately 90°, pronated forearm resting on a horizontal surface, and index finger abducted against a force transducer (MLP 100; Transducer Techniques, Temecula, USA). Forearm and wrist were restrained using a custom manipulandum (Fig. 1). Responses evoked from the right first dorsal interosseus (FDI) muscle were recorded using surface electromyography (EMG): two Ag-AgCl electrodes attached to the skin in a belly-tendon montage and two grounding straps around the forearm.

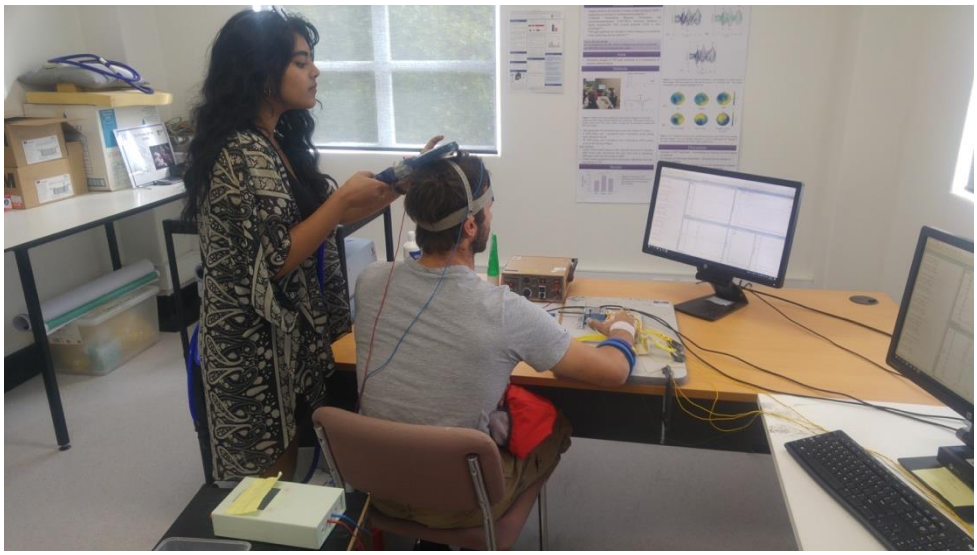


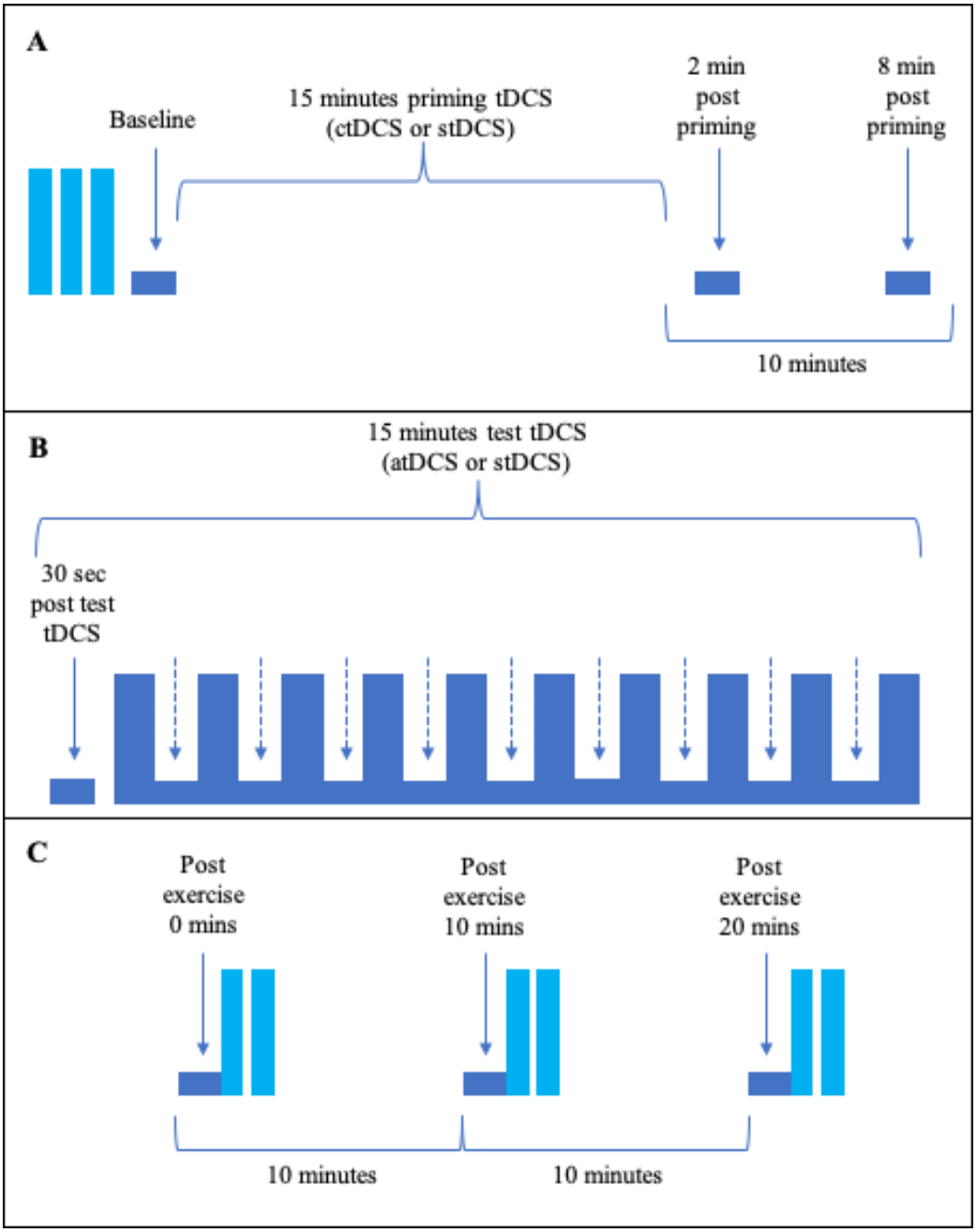
Figure 1. Experimental setup.

Experimental protocol

All subjects completed three sessions, held in the afternoon to control for diurnal influence (at the same time of the day for each participant across sessions) and separated by at least 48 hours to avoid any intervention carryover effects[5]. Experimenter and subject were blind to tDCS polarities, which were pseudo-randomised across sessions by a second experimenter.

Before fatiguing exercise, maximal voluntary contraction (MVC) force was determined by calculating the average force across three 3-5-second maximal FDI abduction tasks. Baseline measurements of corticospinal excitability involved 15 single TMS pulses and 3 peripheral nerve stimulations (PNS). Subjects then received ctDCS or stDCS priming at rest. Measurements (15 TMS and 3 PNS) were taken at 2 minutes and 8 minutes post priming to detect priming effects on corticospinal excitability. Test stimulation (atDCS or

stDCS) commenced 10 minutes following the conclusion of priming. This inter-stimulation interval was appropriate for ensuring test stimulation was performed during after-effects of priming[33]. Subjects were asked to describe any sensations from the tDCS at the beginning, middle, and end of all stimulation periods. Measurements (15 TMS and 3 PNS) were performed at 30 seconds following test stimulation commencement. Subjects then performed the fatiguing exercise involving 10 intermittent 30-second MVCs. Measurements (5 TMS and 1 PNS) were taken between sets (~30 seconds). Subjects were verbally instructed to start and stop contracting. Visual feedback of force and EMG output was displayed on a computer screen (Fig. 1) and verbal encouragement to perform maximally was provided throughout the exercise. Immediately succeeding exercise, post-exercise measurements (15 TMS, 3 PNS and two 3-5-second MVCs with 30 seconds rest between contractions) were completed. Post-exercise measurements were repeated at 10 minutes and 20 minutes following the conclusion of exercise to monitor fatigue recovery and tDCS after-effects.



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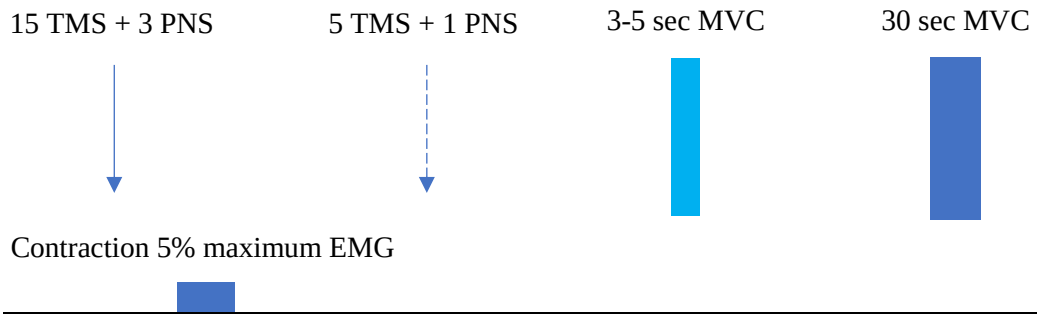


Figure 2. Experimental protocol schematic. (A) baseline and priming tDCS (B) fatiguing exercise and test tDCS (C) recovery

Transcranial Direct Current Stimulation

Current was induced by two 35cm² saline-soaked, sponge electrodes and delivered using a battery-powered direct current stimulator (NeuroConn DC Stimulator Plus, Germany). The active electrode was centred on the representational field of right FDI and reference electrode on the contralateral supraorbital region. This montage appears to be optimal for enhancing excitability of motor cortex[7]. All current was delivered at 1 mA intensity. atDCS and ctDCS were delivered for 15 minutes[5, 7, 10]. During stDCS, electrodes were positioned identically to real tDCS but stimulation was only delivered for 10 seconds (with 8-second ramp-up and ramp-down). This has demonstrated reliability as a sham protocol as subjects feel the initial sensations associated with real stimulation, but no corticospinal excitability changes are induced[34]. Three priming-test tDCS combinations were applied in separate sessions for all participants (i.e., stDCS-stDCS, stDCS-atDCS, and ctDCS-atDCS).

Transcranial Magnetic Stimulation

Measuring the amplitude of motor evoked potential (MEP), elicited by the delivery of single-pulse TMS, was used to evaluate corticospinal excitability. Stimuli were applied with a figure-of-eight coil (9cm external wing diameter) connected to a monophasic Magstim 200² magnetic stimulator (Magstim, Whitland, UK). The coil was held over the scalp at an angle of 45° to the sagittal plane, with the handle pointing laterally and posteriorly, to produce current flow in a posterior-anterior direction. All pulses were delivered to the representation of right FDI on left motor cortex while subjects contracted the muscle so it was active at 5% of maximum EMG (as established during baseline MVC trials). An active condition was preferred for practical ease; i.e., a lower stimulation intensity required to consistently produce MEP[35]. Hotspot was determined by mapping (at 50-60% of maximum stimulator output) for the region that corresponded to the largest amplitude MEP. Consistency of coil position was ensured by marking the tDCS electrodes and scalp with permanent marker and markings were continually checked throughout the protocol. The lowest stimulus intensity required to elicit a MEP distinguishable from background EMG signal in 3 of 5 trials, defined the active motor threshold (AMT). One primary progressive MS patient did not complete the experiment as their AMT exceeded maximum stimulator output and we were unable to obtain measurements. TMS intensity was set at 120% of AMT for all measurement blocks (MS: 63.3 ± 7.0%, CTRL: 62.7 ± 12.9% of maximum stimulator output)[35].

Peripheral Nerve Stimulation

Stimuli were delivered using a constant-current stimulator (DS7A; Digitimer, Hertfordshire, UK). A bipolar bar electrode probe was secured over the ulnar nerve at the wrist with cathode angled distally. The location was determined as the site which produced the largest compound muscle action potential (M-wave) in resting FDI at 10 mA of current. To establish the maximum M-wave (Mmax), stimulation intensity increased in increments of 5 mA until the amplitude of M-wave did not increase further. Test intensity was set at 120% of the intensity required to produce Mmax (MS: 26.6 ± 13.2 mA, CTRL: 22.4 ± 6.1 mA).

Data Analysis

MVC data were analysed manually using offline recordings on Spike2 software (Version 6.18). Peak force amplitude was measured during brief MVCs at baseline and post-exercise, and averaged across trials at each time point. Mean force (from initial peak to when subject was instructed to stop contracting) was measured for each of the 30-second MVCs throughout the exercise. Force data were expressed as a percentage of baseline force to account for baseline differences between groups.

EMG was amplified with CED 1902 (1000x) and band-pass filtered (20Hz high pass, 1kHz low pass) before digitization with a 1401 interface (Cambridge Electronic Design, UK) at 2kHz and stored offline. The root mean squared EMG was measured for all MVCs.

MEP and Mmax amplitudes were measured peak to peak in millivolts using offline recordings on Spike2 software. MEP trials were excluded from analysis if, within the 100 milliseconds prior to TMS pulse, voluntary EMG activity exceeded 0.1 millivolt in amplitude. MEP amplitude at individual time points were calculated as the average amplitude across all trials in the measurement block. MEPs were normalised to Mmax to reveal corticospinal changes and exclude changes at the level of the muscle. Values were expressed as a percentage of baseline.

Statistical Analysis

Statistical analyses were completed using IBM SPSS Statistics software (Version 24). Linear mixed model analyses with factors time, group (MS vs. CTRL), and neuromodulation (stDCS-stDCS vs. stDCS-atDCS vs. ctDCS-atDCS), were used to determine main effects and interactions. For all comparisons, a Shapiro-Wilk test confirmed normality of the data. Specific significant differences were identified with

Bonferroni's correction for multiple comparisons. Student t-tests (two-sampled, equal variance) were used for determining group differences in demographic characteristics at baseline. One-way analyses of variance were used for assessing differences between neuromodulation conditions in time of day, lab temperature, and humidity. All data in text and tables are expressed as mean ± SD and in figures as mean ± SEM. Significance was set at $P < 0.05$.

RESULTS

tDCS was tolerated by all subjects. No adverse reactions were reported except for one subject who described mild headache during ctDCS. Subjects experienced comparable sensations during real and sham stimulation. The sensations described included tingling, itchiness, warmth, prickling, and burning. Majority reported sensations when asked at the beginning of stimulation but, felt nothing when asked in the middle and at the end of stimulation. There were no differences between neuromodulation conditions in time of day (14:12pm ± 1.3 hrs; $P \geq 0.57$), lab temperature (22.4 ± 3.1°C; $P \geq 0.38$), or humidity (41.7 ± 9.6%; $P \geq 0.32$).

Characteristic	MS (N = 10)	CTRL (N = 10)
Age (years)	49.0 ± 13.6	46.8 ± 16.4
Sex (female)	6	6
Height (cm)	169.2 ± 12.4	169.7 ± 12.0
Weight (kg)	80.5 ± 15.7	72.2 ± 17.4
Handedness	0.6 ± 0.5	0.5 ± 0.6
Work index	2.5 ± 0.4	2.8 ± 0.8
Sport index	2.1 ± 1.4 *	4.2 ± 1.3
Leisure time index	2.5 ± 0.5	3.0 ± 0.8
AMT	52.7 ± 4.1	52.3 ± 9.9
SSRI use (N)	3	0
EDSS	3.5 ± 2.4	
FSS	5.7 ± 1.1	
mFIS	51.9 ± 10.9	
Disease duration (years)	7.1 ± 7.0	

Table 1. Demographic and clinical characteristics of patients and controls are summarised. Work, sport, and leisure time indices are sub-scales of International Physical Activity Questionnaire. Selective Serotonin Reuptake Inhibitor (SSRI). Expanded Disability Status Score (EDSS). Fatigue Severity Scale score (FSS). Modified Fatigue Impact Scale score (mFIS). *P < 0.05 compared to CTRL

Corticospinal excitability

There was a significant main effect of time ($P < 0.01$) on MEP amplitude normalised to Mmax, but not group ($P = 0.89$) nor neuromodulation ($P = 0.48$). There was a significant group x neuromodulation ($P < 0.01$) and time x neuromodulation interaction ($P = 0.04$), but no significant group x time nor group x time x neuromodulation interactions ($P \geq 0.38$).

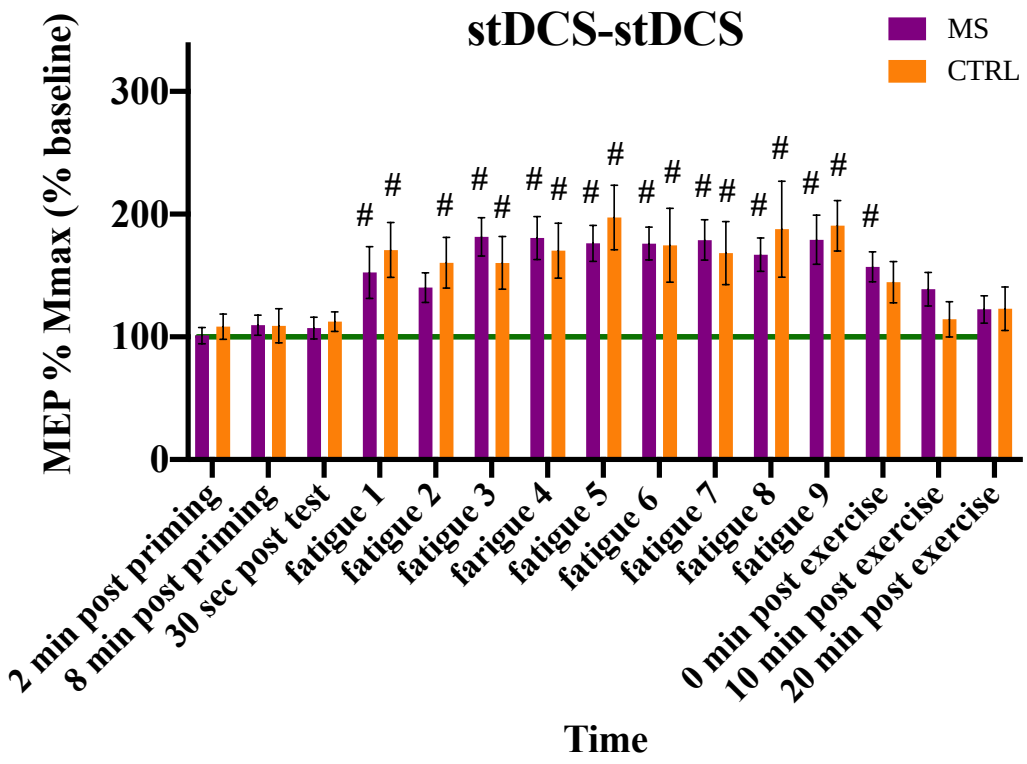
In stDCS-stDCS condition (Fig. 3A), throughout fatiguing exercise MEPs were facilitated compared to baseline ($P \leq 0.04$). The pattern of MEP facilitation over time was similar between groups ($P \geq 0.40$).

In stDCS-atDCS condition (Fig. 3B), slight MEP augmentation in MS and depression in CTRL was observed, compared to stDCS-stDCS. These changes were sufficient to produce significant group differences at time points fatigue 5, 7, 8, and 9 ($P \leq 0.04$) but differences between stDCS-atDCS and stDCS-stDCS conditions did not reach significance ($P \geq 0.07$), with the exception of a significant between condition difference at fatigue 5 in CTRL ($P = 0.04$).

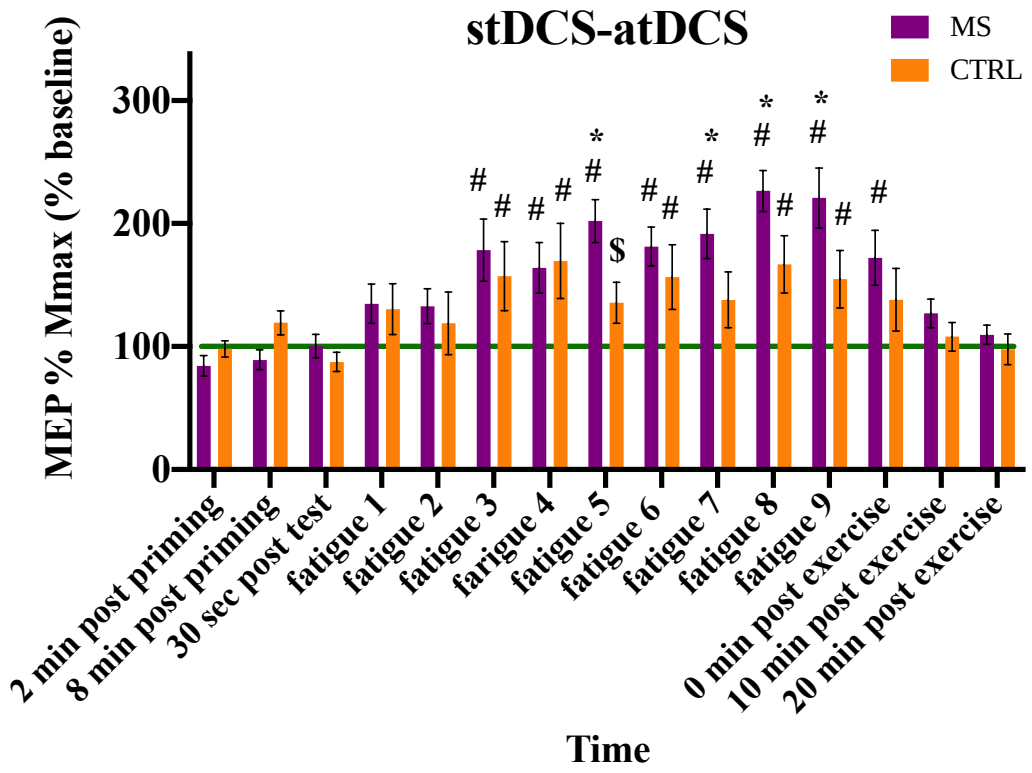
In ctDCS-atDCS condition (Fig. 3C), MEP significantly increased compared to stDCS-atDCS at fatigue 7, 8 and 9, and 0 min post-exercise in CTRL ($P < 0.01$). There was also significant MEP facilitation compared to stDCS-stDCS condition at fatigue 7 and 8, and 0 min post-exercise in CTRL ($P \leq 0.01$). This effect was absent in MS ($P \geq 0.13$). In both groups, MEP returned to baseline values by 10 minutes post-exercise, across all neuromodulation conditions ($P \geq 0.07$). ctDCS priming alone had no effect on MEP in either group, determined by no differences from baseline at 2 minutes and 8 minutes post priming ($P \geq 0.80$).

There were no main effects of time ($P = 0.42$), group ($P = 0.66$), or neuromodulation ($P = 0.62$), nor any significant interactions ($P \geq 0.09$), on background EMG activity during measurements.

(A)



(B)


stDCS-atDCS
MS
CTRL
MEP % Mmax (% baseline)
300
200
100
0
2 min post priming
8 min post priming
30 sec post test
fatigue 1
fatigue 2
fatigue 3
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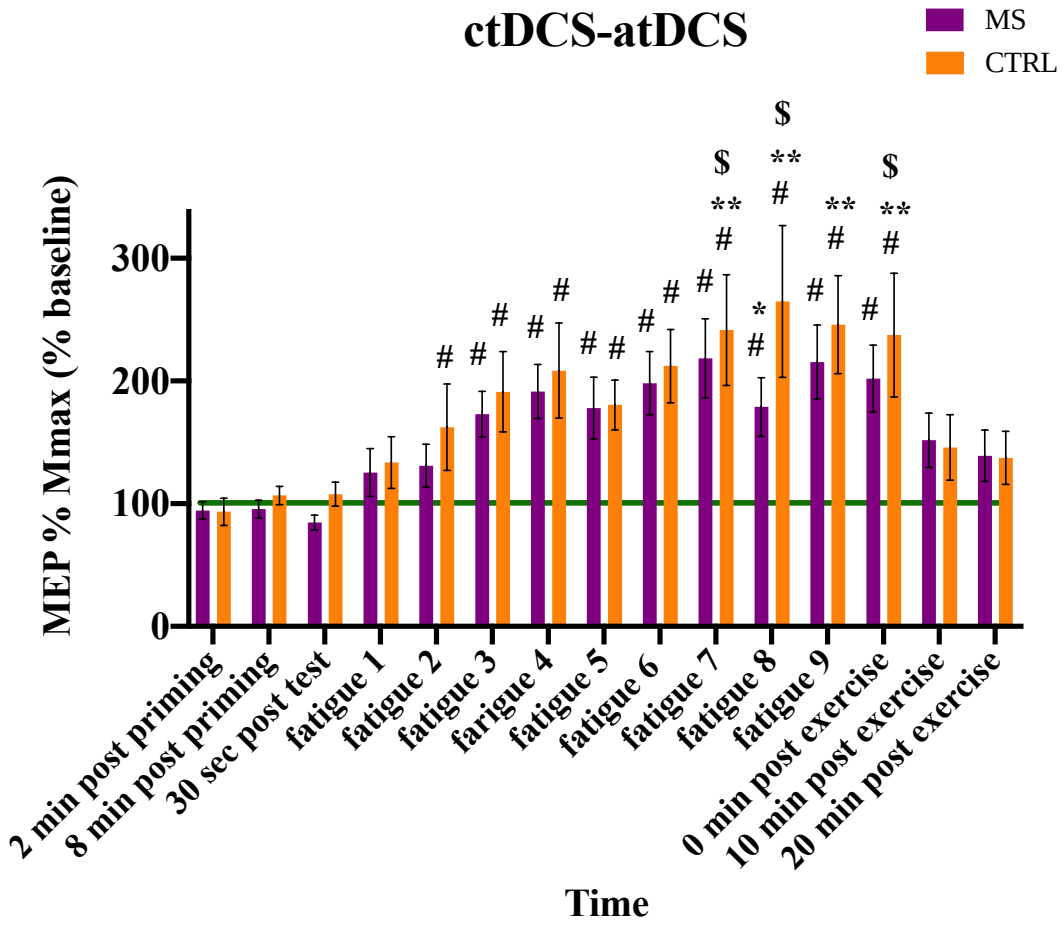
fatigue 5
fatigue 6
fatigue 7
fatigue 8
fatigue 9
0 min post exercise
10 min post exercise
20 min post exercise
Time

(C)


ctDCS-atDCS
MS
CTRL
MEP % Mmax (% baseline)
300
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30 sec post test
fatigue 1
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fatigue 6
fatigue 7
fatigue 8
fatigue 9
0 min post exercise
10 min post exercise
20 min post exercise
Time

Figure 3. Mean MEP normalised to Mmax (% baseline) are displayed for both groups over time, across neuromodulation conditions (A) stDCS-stDCS, (B) stDCS-atDCS, (C) ctDCS-atDCS

Purple columns represent MS; orange columns represent CTRL. Error bars indicate the mean ± SEM. Horizontal green line represents baseline at 100%.

# indicates significant difference from baseline ($P < 0.05$).

* indicates significant difference compared to CTRL ($P < 0.05$).

$ indicates significant difference compared to stDCS-stDCS condition ($P < 0.05$).

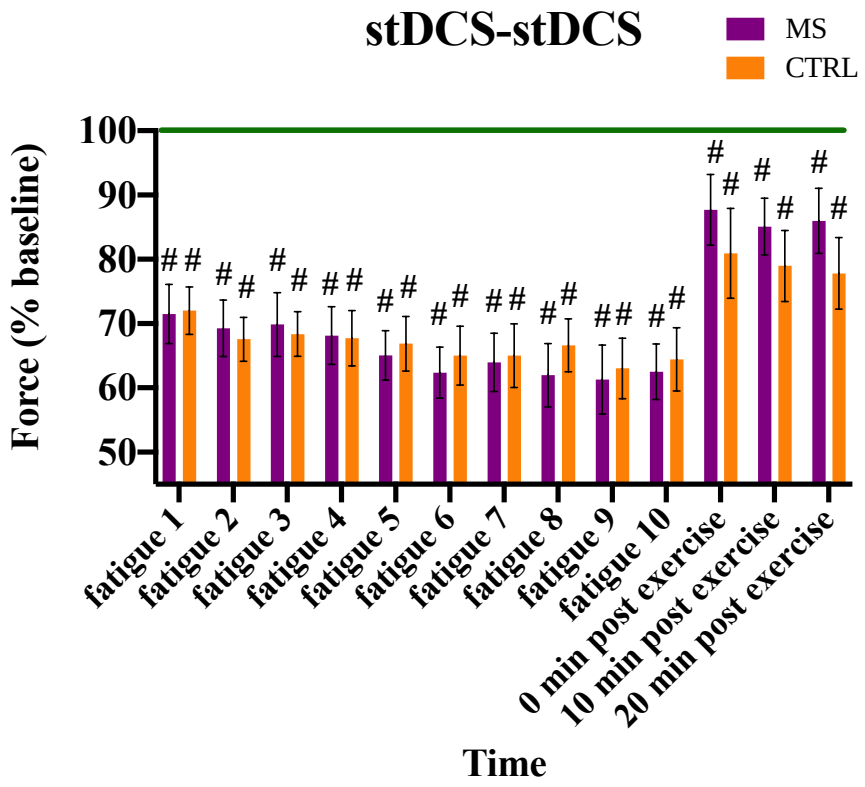
** indicates significant difference compared to stDCS-atDCS condition ($P < 0.05$).

Fatigue

There was a significant main effect of time on MVC force (% baseline) ($P < 0.01$) and an almost significant effect of group ($P = 0.06$) but, no significant effect of neuromodulation ($P = 0.76$) nor any significant interactions ($P \geq 0.09$).

During fatiguing exercise, MVC force progressively declined at a similar rate in both groups ($P \geq 0.15$), across all neuromodulation conditions ($P \geq 0.09$) (Fig. 4). There was some evidence for recovery of MVC force in CTRL following the conclusion of fatiguing exercise in the ctDCS-atDCS condition, with no difference from baseline at 0 or 20 min post-exercise ($P \geq 0.14$); whereas, it remained significantly attenuated in MS ($P < 0.01$) (Fig 4C). The group difference at 20 minutes post-exercise was significant ($P = 0.01$).

(A)


stDCS-stDCS
MS
CTRL
Force (% baseline)
100
90
80
70
60
50
fatigue 1
fatigue 2
fatigue 3
fatigue 4
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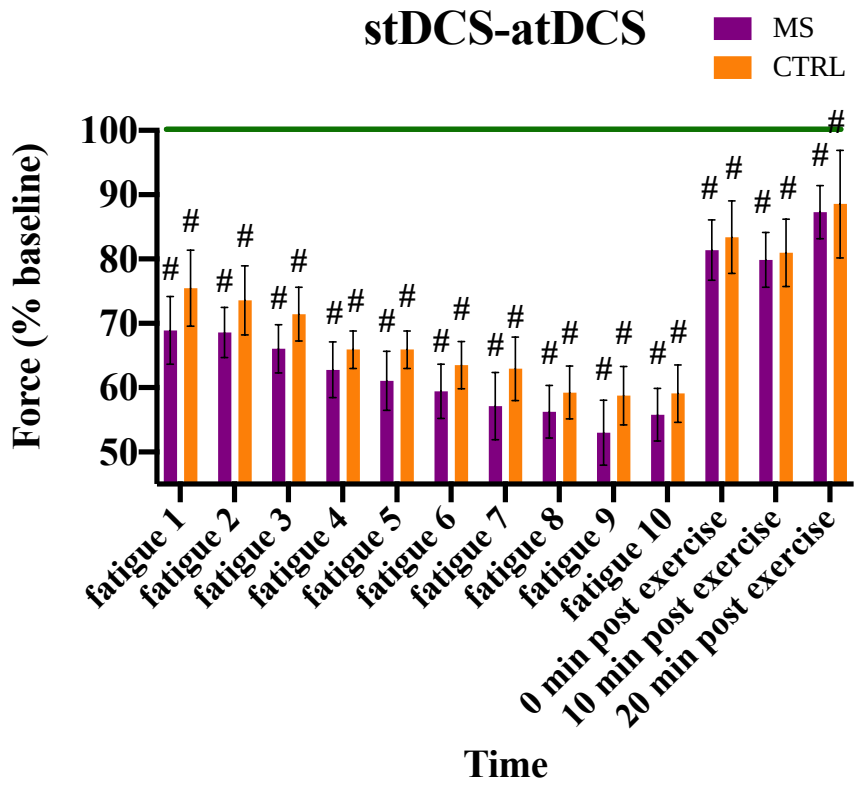
fatigue 9
fatigue 10
0 min post exercise
10 min post exercise
20 min post exercise
Time

(B)


stDCS-atDCS
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Force (% baseline)
100
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fatigue 1
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Time

(C)

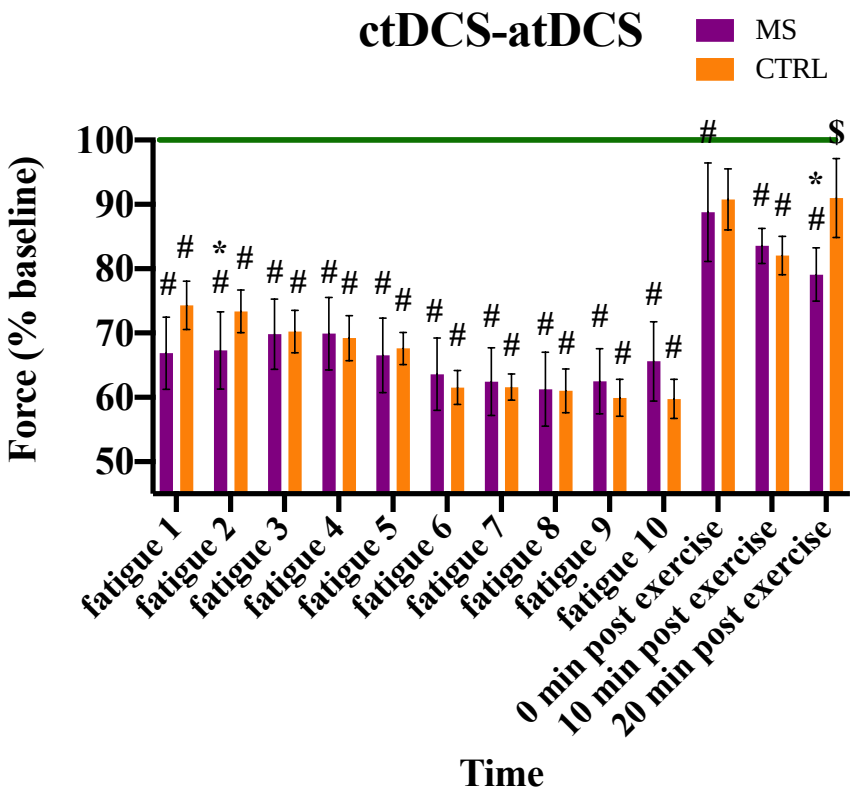

Figure 4. Fatigue results. MVC force (% baseline) is displayed for both groups over time, across neuromodulation conditions (A) stDCS-stDCS (B) stDCS-atDCS (C) ctDCS-atDCS.

Purple columns represent MS; orange columns represent CTRL. Error bars indicate the mean ± SEM.

Horizontal green line represents baseline at 100%.

* indicates significant difference compared to CTRL ($P < 0.05$).

# indicates significant difference from baseline ($P < 0.05$).

$ indicates significant difference compared to stDCS-stDCS condition ($P < 0.05$).

DISCUSSION

Main findings

To the best of our knowledge, this study is the first to examine the interaction between metaplastic neuromodulation and fatigue in MS. Notably, it provides novel data on differential tDCS-induced modulation of MEP in patients compared to healthy subjects, likely related to an impairment of homeostatic mechanisms which normally intervene to prevent dysregulated corticospinal excitability. Specifically, neither ctDCS-atDCS nor stDCS-atDCS were related to significant shifts in corticospinal excitability in MS patients, compared to the excitability facilitation induced by the exercise itself (stDCS-stDCS). Accordingly, no benefit was observed for fatigue during exercise. While we observed the expected enhancement of corticospinal excitability with ctDCS-atDCS in healthy subjects, this also did not translate to any effect for fatigue during exercise. Interestingly though, there looked to be a relationship between augmented excitability with ctDCS-atDCS and the recovery of MVC force following fatiguing exercise. This highlights an interaction which may be worth exploring in future work.

Effects of ctDCS and stDCS primed atDCS on corticospinal excitability

The enhancement of MEP observed in healthy subjects with concurrent application of ctDCS primed atDCS and exercise, confirms our hypothesis and further substantiates the notion that induction of excitability change is sensitive to the state of the network imposed by the history of synaptic activity[26, 27, 36, 37]. This is in accordance with seminal metaplasticity studies which showed the efficacy of tDCS priming in shaping the magnitude, direction, and duration of effects[36, 37]. A novel finding of the present study is the absence of such an effect in MS patients, implying an impairment of metaplasticity mechanisms and fostering speculation about aligning pathophysiology. Since the exercise itself generated identically facilitated MEP in both groups, group distinctions in tDCS effects were in response to the neuromodulation.

Our results do not illuminate which part of the complex excitability regulation machinery is implicated in MS however, the interference of gamma-Aminobutyric acid (GABA)-ergic inhibition is an attractive theory. In healthy motor cortex, tDCS-induced plasticity is dependent on changes at glutamatergic synapses, predominantly driven by N-methyl-D-aspartate receptor-dependent mechanisms[9, 10]. Though, GABAergic inhibition has also been shown to influence the direction and degree of effects[38, 39]. Pathological corticospinal

hyperexcitation is typically present in MS patients linked to elevated cerebral spinal fluid concentration of inflammatory cytokines, which are released during acute MS attacks[40-42]. Inflammatory cytokines have been shown to interfere with GABA-mediated inhibition and exacerbate glutamate-mediated excitation[43]. Since modulation of GABA-mediated inhibition and glutamate-mediated excitation are common to the mechanisms of both tDCS and the disease, the potential role of the associated synapses as a site for the failure of corticospinal excitability regulation in MS is highlighted. Even though no corticospinal excitability abnormalities were observed in patients at baseline, such abnormalities have typically been reported using different TMS measures of corticospinal excitability to those used in the present study, such as central motor conduction time and intracortical inhibition[3, 40, 41, 44]. Extensive investigation is required, involving the systematic manipulation of components of the metaplasticity machinery, in order to clarify this interpretation and determine the precise location of the fault.

Trends for the alteration of corticospinal excitability were observed with stDCS-atDCS but these did not reach significance. It is interesting to note however, that the trend-wise alterations occurred in opposing directions between groups. That is, with stDCS-atDCS we observed enhancement in patients and diminution in controls, of exercise-induced MEP facilitation. Although it is broadly accepted that atDCS at rest induces MEP facilitation denoting LTP-like changes in synaptic efficacy[5, 7, 9, 10], these changes are susceptible to reversal with behavioural engagement of the regions stimulated by the neuromodulation[25, 45]. For example, Thirugnasambandam et al.[24] reported an abolishment of MEP changes with combined atDCS and voluntary muscle contraction. In the present study, the trend towards attenuation of exercise-induced MEP facilitation in control subjects, may reflect homeostatic mechanisms initiated to prevent hyperexcitation and ultimately preserve favourable neuronal function[21, 22]. The contrasting trend towards MEP amplification in MS patients harmonises with the proposition that excitability regulation mechanisms are defective in this clinical group.

tDCS effects on fatigue

Force declined during exercise irrespective of group and neuromodulation. Since the shifts in corticospinal excitability were only slight in both groups with stDCS-atDCS, this may have been insufficient to counteract the progressive failure of the nervous system to drive the muscle maximally[12]. However, the significantly augmented excitability in healthy subjects with ctDCS-atDCS and lack of associated effect on force, obscures this interpretation. This implies that the development of fatigue during exercise may not rely

on corticospinal excitability changes. Abdelmoula et al.[16] arrived at a similar conclusion in their study on healthy subjects but, observed the inverse relationship; i.e., atDCS improved fatigue without affecting corticospinal excitability. A key protocol distinction that could account for this contrasting result is the delivery of atDCS prior to commencement of exercise. Accordingly, it could be that force is resistant to online interventions and tDCS-induced fatigue amelioration might critically depend on the timing of stimulation. Contrariwise, Williams et al.[14] applied atDCS during fatiguing exercise and reported improved performance. Mutually these experiments assessed fatigue by time to task failure in submaximal isometric contractions (rather than intermittent MVCs as in the present study). In view of this, it is possible that fatigue augmentation with tDCS is task specific and governed, at least in part, by exercise intensity.

While significant alterations of excitability with ctDCS-atDCS did not influence force during exercise, there was evidence for a delayed benefit. Specifically, following the conclusion of fatiguing exercise, controls showed no difference in force from baseline at 0 or 20 min post-exercise; whereas in MS, force remained attenuated. This highlights a potential interaction between the modification of excitability during and the recovery of force following exercise but, further exploration with a larger sample is required to clarify this. An explanation for this result could be that the pronounced increase in excitability sufficiently facilitated motor output to compensate for the loss of contractile force accompanying fatigue[12, 13]. However, the temporal dissociation between MEP increase and force benefit complicates this interpretation. Delayed functional effects on motor learning have been demonstrated with a tDCS priming paradigm by Christova et al.[28]. In this experiment, ctDCS-atDCS facilitated learning retention as indicated by performance improvement at retest two weeks later. Current understanding of the mechanisms underlying such delayed effects are incomplete. Though, LTP involves a cascade of synaptic strengthening processes that are thought to continue after the conclusion of the LTP inducing stimulus[11, 27]. Conceivably, positive tDCS effects on force were related to the less immediate parts of the LTP process, accounting for the delay. Alternatively, tDCS may have indirectly influenced networks that contribute to force production beyond the motor cortex via functional connections. This may have generated changes in pathways that do not contribute to responses elicited via TMS. Probing the timing of stimulation in relation to the fatiguing task provides direction for future research.

Limitations

Some limitations of the present study should be mentioned. First, it could be argued that metaplasticity was not generated in either group because the priming alone did not cause alteration of MEP. However, it has been demonstrated that subsequent synaptic modifications can occur even when priming stimulation does not cause detectable changes in excitability[19, 26]. Second, it is unclear whether effects with ctDCS-atDCS were explicitly related to the influence of ctDCS priming on MEP modulation by atDCS or on MEP modulation by the exercise itself. The inclusion of a ctDCS-stDCS condition would have resolved ambiguity around this interpretation. This provides an avenue for further investigation. Third, it is well known that innumerable variables can influence tDCS effects. Since hormones are potent regulators of plasticity[19], we should ideally have controlled for fluctuations associated with menstrual cycle in female subjects. We also did not exclude patients taking SSRIs which can influence the magnitude and direction of tDCS effects; increased extracellular serotonin has been shown to magnify MEP facilitation with atDCS and cause excitation with ctDCS[46]. However, we believe a major confounding effect of serotonin is improbable as we did not observe such magnification of MEP facilitation with atDCS nor excitation with ctDCS in patients and theoretically, controlling for this neurotransmitter should only have strengthened findings. Disease phase is another noteworthy factor which should be taken into account in future investigations since the release of inflammatory cytokines during relapse influence GABAergic processes which participate in the mechanisms of tDCS. In view of this, and the inadequate statistical power due to small sample size, generalising our results to the broader clinical population must be carried out conservatively.

Conclusions and significance

This study provides novel data on differential tDCS modulation of corticospinal excitability in MS patients compared to healthy subjects, likely attributed to a failure of regulatory mechanisms which normally intervene to keep excitability within a functional range. In healthy subjects, we observed the expected augmentation of corticospinal excitability with ctDCS-atDCS, alongside the recovery of MVC force to baseline following the conclusion of exercise. Our data underline the state dependency of tDCS effects, i.e., the direction and extent of tDCS effects may be more precisely predicted by taking into account both the history of corticospinal excitability and the level of excitability at the time of stimulation, rather than solely parameters of the stimulation. The distinct responses to metaplastic neuromodulation between groups justifies the importance of exploring tDCS effects in MS, emphasising that predicting outcomes using studies on

healthy subjects is problematic. The data also suggest that an impairment of corticospinal excitability regulation mechanisms may be of crucial relevance in the pathophysiology of MS. Understanding of these factors has implications in the optimisation of tDCS protocols that aim to reduce fatigue and improve motor function in MS.

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