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Aberrant plasticity in musculoskeletal pain: a failure of homeostatic control?

Tribikram Thapa[1], Thomas Graven-Nielsen[2], Siobhan M. Schabrun[3]

[1]Western Sydney University, School of Science and Health, Penrith, NSW, 2751, Australia.

[2]Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark.[3]Neuroscience Research Australia, Randwick, Sydney, Australia.

Corresponding author
Dr. Siobhan Schabrun
Neuroscience Research Australia
Margarete Ainsworth Building
Barker Street Randwick Sydney NSW 2031
Australia
Phone: +61 2 9399 1075
Email: s.schabrun@neura.edu.au

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ABSTRACT

Objective: Aberrant synaptic plasticity is hypothesised to underpin chronic pain. Yet, synaptic plasticity regulated by homeostatic mechanisms have received limited attention in pain. **Methods:** We investigated homeostatic plasticity in the human primary motor cortex (M1) of 21 healthy individuals in response to experimentally induced muscle pain for several days. Experimental pain was induced by injecting nerve growth factor into the muscle belly of the right extensor carpi radialis brevis muscle. Pain and disability were monitored until day 21. Homeostatic plasticity was induced on day 0, 2, 4, 6, and 14 in the left M1 using anodal transcranial direct stimulation (tDCS) applied for 7-min and 5-min, separated by a 3-min rest period. Motor evoked potentials (MEP) to transcranial magnetic stimulation assessed the homeostatic response. **Results:** On days 0 and 14, MEPs increased following the first block of tDCS ($P<0.004$), and decreased following the second block of tDCS ($P<0.001$), consistent with a normal homeostatic response. However, on days 2 ($P=0.07$) and 4 ($P=0.7$), the decrease in MEPs after the second block of tDCS was attenuated, representing an impaired homeostatic response. **Conclusion:** Findings demonstrate altered homeostatic plasticity in the M1 with the greatest alteration observed after four days of sustained pain. **Significance:** This study provides longitudinal insight into homeostatic plasticity in response to the development, maintenance, and resolution of pain over the course of 14 days.

Keywords: Homeostatic plasticity, Musculoskeletal pain, Non-invasive brain stimulation, Nerve growth factor

1. INTRODUCTION

Synaptic plasticity plays a key role in neural adaptation, and is fundamental to memory, learning, and recovery after injury or illness (Joseph 2013; Martin et al. 2000; Nudo 2013; Ziemann and Siebner 2008). A number of functional and structural mechanisms underpin synaptic plasticity in the human brain, including the dynamic expression of long-term potentiation (LTP) and long-term depression (LTD)-like changes in synaptic efficacy (Hebb 1949; Joseph 2013; Martin et al. 2000). Numerous studies suggest aberrant synaptic plasticity contributes to the development of chronic pain (Apkarian 2011; Apkarian et al. 2009; Apkarian et al. 2011; Baliki et al. 2011; Flor 2003; Flor 2008; Kuner and Flor 2017; May 2008; Morton et al. 2016). However, in addition to plasticity mechanisms that promote neural 'changeability', the human brain is governed by plasticity mechanisms that promote stability (Turrigiano 2012; Turrigiano 1999; Turrigiano 2006; Turrigiano and Nelson 2000). These 'homeostatic' mechanisms prevent overexpression of LTP and LTD based on the principle of a 'sliding synaptic threshold', such that high post-synaptic activity elicits a compensatory response that biases the synaptic threshold towards LTD, and low post-synaptic activity biases the synaptic threshold towards LTP (Karabanov et al. 2015; Muller-Dahlhaus and Ziemann 2015). Thus, homeostatic mechanisms are responsible for the control and regulation of synaptic plasticity. A disturbance in this mechanism could plausibly drive the aberrant synaptic plasticity observed in musculoskeletal pain conditions.

The induction and assessment of homeostatic plasticity in humans is typically achieved using non-invasive brain stimulation to 'prime' the response to a subsequent period of stimulation. In the primary motor cortex (M1), LTP- and LTD-like effects can be indexed using transcranial magnetic stimulation (TMS). For example, a single block of anodal transcranial direct current stimulation (tDCS) can induce an increase in the motor evoked potential amplitude to TMS and this response is thought to reflect the engagement of LTP-like processes (Fricke

et al. 2011). However, when preceded at short interval by a second block of anodal tDCS, this effect is reversed, and a reduction in motor evoked potential amplitude (LTD-like effect) is observed (Karabanov et al. 2015; Muller-Dahlhaus and Ziemann 2015). These effects are interpreted to reflect homeostatic plasticity such that a period of high LTP formation (excitation) causes the synaptic threshold to favour the induction of LTD.

Despite the importance of homeostatic mechanisms to healthy brain function, there has been limited investigation of this mechanism in pain. However, studies in several chronic pain states including migraine (Antal et al. 2008; Brighina et al. 2005; Brighina et al. 2002; Cosentino et al. 2014b), and low back pain (Thapa et al. 2018) suggest that homeostatic control is disturbed. Impaired homeostatic modulation is hypothesised to contribute to abnormally high cortical excitability, aberrant cortical reorganisation, increased pain perception, and sensorimotor dysfunction in these chronic pain conditions (Brighina et al. 2005; Brighina et al. 2002; Cosentino et al. 2014b; Thapa et al. 2018). While it is reasonable to assume that sustained pain may contribute to disruption of homeostatic control, there has been no longitudinal investigation of homeostatic plasticity in pain. How and when changes in homeostatic control develop in the transition to sustained pain or how they relate to the symptoms of pain is unknown. This information is essential to enhance our understanding of homeostatic plasticity in humans and to understand the impact of sustained pain on this fundamental neural mechanism.

Using a clinically-relevant, human pain model to induce progressively developing, sustained muscle pain over several days, homeostatic plasticity was investigated in the M1 using two successive blocks of anodal tDCS as pain developed, peaked, and resolved, over the course of 14 days. We hypothesised that several days of sustained pain would alter homeostatic plasticity (reduce the normal LTD-like response observed following two blocks of anodal tDCS in pain-free, healthy individuals (Fricke et al. 2011)) in the human M1.

2. METHODS

2.1 Participants

As there have been no previous studies of homeostatic plasticity and NGF (or indeed any type of experimentally-induced pain), there were insufficient data on which to base a power calculation. Our previous reliability study showed that 10 subjects was sufficient to observe a homeostatic response in healthy individuals in the absence of pain using an identical tDCS protocol (Thapa and Schabrun 2018) and previous studies examining the effect of NGF-induced pain on corticomotor excitability and intracortical inhibition have used samples ranging from 12-28 (De Martino et al. 2019; De Martino et al. 2018; Schabrun et al. 2016; Seminowicz et al. 2019; Summers et al. 2019). In this exploratory study, we enrolled twenty-one, right-handed, healthy individuals (mean ± standard deviation age: 23 ± 4 years, 12 males, 9 females). Handedness was assessed using the Edinburgh handedness questionnaire (Oldfield 1971), and a transcranial magnetic stimulation (TMS) safety screening questionnaire was completed prior to study commencement (Keel et al. 2001). Individuals with a history of any neurological, psychiatric or musculoskeletal condition were excluded. All participants received written and verbal description of experimental procedures and provided written informed consent consistent with the Declaration of Helsinki. Experimental procedures were approved by the local human research ethics committee (H10184).

2.2 Experimental Design

Homeostatic plasticity was induced in M1 using two blocks of anodal tDCS applied for 7-min and 5-min respectively, separated by a 3-min rest period (Fricke et al. 2011; Thapa and Schabrun 2018). Corticomotor excitability was monitored by recording 15 motor evoked potentials (MEPs) to single pulse transcranial magnetic stimulation (TMS) i) before tDCS, ii)

between the two tDCS blocks (used to evaluate the *plasticity* response to a single block of anodal tDCS), and iii) 10-min after the last tDCS block (used to evaluate the *homeostatic* response; Fig. 1A). We have previously shown that the homeostatic response induced using this tDCS protocol, and assessed using 15 MEPs, is reliable in healthy individuals at intervals of 2, 7 and 14 days (ICC=0.67, 95% CI 0.12 to 0.91) (Thapa and Schabrun 2018). In that study, we observed the greatest homeostatic response 10 minutes following the tDCS protocol. Thus, this timepoint was selected to assess the homeostatic response in the current study. Progressively developing, sustained muscle pain was induced by repeated injection of nerve growth factor (NGF) into the belly of the right extensor carpi radials brevis muscle (m. ECRB) at the end of the experimental session on days 0, 2, and 4 such that on Day 0, all assessments were completed prior to the induction of pain. Homeostatic responses were assessed on days 0, 2, 4, 6, and 14. Pain severity, disability, and sleep quality were assessed every second day from day 1 to day 21 by an on-line diary (Fig. 1B).

2.3 Induction of M1 homeostatic responses

Homeostatic responses were elicited in M1 using two blocks of anodal tDCS. This protocol has been used previously to investigate M1 homeostatic responses in both healthy and clinical populations (Fricke et al. 2011; Thapa et al. 2018; Thapa and Schabrun 2018). A battery driven, ramp controlled, constant current stimulator (DC-Stimulator Plus, NeuroConn, Ilmenau, Germany) was used to deliver anodal tDCS at an intensity of 1 mA. Current was ramped up and down over 10 seconds at the start and end of stimulation (Nitsche et al. 2008). Rubber electrodes, placed in sodium-chloride soaked sponges (5 x 7 cm), were positioned over the left M1 hot-spot corresponding to the right m. ECRB (anode; see below for hot-spot determination), and over the contralateral supraorbital region (cathode). Two adjustable rubber straps were used to fix the electrodes to the head.

2.4 Monitoring of corticomotor excitability

Single-pulse transcranial magnetic stimulation (TMS) was used to monitor corticomotor excitability in response to the first and second block of anodal tDCS. Transcranial magnetic stimulation (TMS) was performed (Magstim 200, Magstim Co., Ltd., Dyfed, UK) with a monophasic current waveform. A 70 mm figure-of-eight coil was positioned over the left hemisphere at a 45º angle from the sagittal plane. The optimal coil position was determined by locating the site at which the maximum muscle response from the relaxed right m. ECRB was evoked (termed the 'hot-spot'). A soft-tip pen was used to mark the hot-spot on the scalp for TMS coil and tDCS electrode re-positioning within and between sessions. On days not attending the laboratory for testing, participants were requested to precisely re-mark their hotspot using a mirror and a soft-tipped pen or if required, with assistance from a second person. Surface electromyography (EMG) was recorded from the right m. ECRB using disposable, surface electrodes (Ag-AgCl, Noraxon dual electrodes, inter-electrode distance 2.0 cm). Surface electrodes were placed 5 cm distal, and 1 cm lateral from the lateral epicondyle along a line from the lateral epicondyle to the midline of the wrist. The ground electrode was placed over the ipsilateral acromion. EMG signals were amplified (1000), bandpass filtered 20-1000 Hz and sampled at 2000 Hz (CED 1401 AD, Cambridge Electronic Design, Cambridge, United Kingdom) using Signal software (CED, version 5.08 x 86). All signals were stored on a computer for offline analysis. TMS intensity was adjusted at baseline on each day of testing to produce an average MEP of ~0.5 mV peak-to-peak amplitude in 15 trials (Burns et al. 2016; Cosentino et al. 2014b; Schabrun et al. 2016). A further 15 trials were recorded at the baseline TMS intensity between the two tDCS blocks to evaluate the response to the first tDCS protocol (*plasticity* response), and at 10-min follow-up to evaluate the response to the second tDCS protocol (*homeostatic* response).

2.5 Induction and assessment of sustained muscle pain

After cleaning the skin with alcohol, a dose of 5 µg (0.2 ml) sterile, recombinant human nerve growth factor (NGF; Lonza Australia Pty Ltd) was given as a bolus injection into the muscle belly of right m. ECRB on days 0, 2, and 4 using a 0.5-ml syringe with a disposable needle (31 Gauge). The injection site was located 5 cm distal, and 1 cm lateral from the lateral epicondyle along a line from the lateral epicondyle to the midline of the wrist (Bergin et al. 2015).

An online diary was used to assess pain intensity, muscle soreness, disability and sleep quality every second day from day 1 to day 21. The diary consisted of: i) an 11-point pain numerical rating scale (NRS) anchored with 'no pain' at zero and 'worst pain possible' at 10 to assess pain intensity, ii) a modified 7-point Likert scale anchored with 'a complete absence of soreness' at zero and 'severe muscle soreness, stiffness or weakness that limits the ability to move' at 6 to assess muscle soreness (Hayashi et al. 2013; Schabrun et al. 2016), iii) the patient-rated tennis elbow evaluation questionnaire (PRTEEQ) to assess disability (maximum score of 50 where higher scores represent greater disability), and iv) an 11-point NRS anchored with 'extremely poor sleep (shallow, unrefreshing)' at zero and 'excellent sleep (deep, refreshing)' at 10 to assess sleep quality.

2.6 Statistical analyses

For all analyses, SPSS software for windows, version 22 was used. A one-way repeated measures analysis of variance (ANOVA) was performed to examine differences in the i) TMS intensity used to elicit MEPs of 0.5 mV, and ii) the amplitude of the mean MEP, recorded at baseline with factor *day* (0, 2, 4, 6, and 14). To examine the change in the MEP amplitude in response to tDCS, a two-way repeated measures ANOVA was performed on raw data with factor *day* (0, 2, 4, 6, and 14), and *time* (baseline, between, 10-min). As the magnitude of the

homeostatic response is likely to be influenced by the amount of facilitation achieved following the first block of anodal tDCS, data reflecting the *plasticity* (time-point 'between'), and *homeostatic* (time-point '10-min') responses were also analysed as ratio values (plasticity-ratio=$MEP_{between}/MEP_{baseline}$, homeostatic-ratio=$MEP_{10\text{-}min}/MEP_{between}$, respectively). Ratio data in percentages were analysed using a one-way repeated measures ANOVA with factor *day*.

A one-way repeated measures ANOVA was used to explore changes in pain NRS scores, muscle soreness (Likert), sleep NRS scores, and disability (PRTEEQ) with factor *day* (1, 3, 5….21). Shapiro-Wilk tests were used to assess normality. Data that violated normality were log transformed. If normality was violated after transformation, a Friedman repeated measures ANOVA on ranks was conducted. The Greenhouse-Geisser method was used to correct for non-sphericity. Post-hoc tests were performed using either the Wilcoxon signed-rank test or Bonferroni t-tests adjusted for multiple comparisons. Significance was set at $P<0.05$.

3. RESULTS

3.1 TMS intensity and MEP amplitude at baseline

There was no significant difference in the TMS intensity required to elicit average MEPs of 0.5 mV peak-to-peak, or in the amplitude of the mean MEP recorded at baseline between days (TMS intensity: $\chi^2(4)=6.2$, $P=0.2$; MEPs at baseline: $F_{4,80}=2.1$, $P=0.09$; Table 1).

3.2 NGF-induced pain, muscle soreness, disability, and sleep quality

Pain NRS scores ($\chi^2(11)=152.5$, $P<0.001$; Fig. 2A) and Likert muscle soreness scores ($\chi^2(11)=171$, $P<0.001$; Fig. 2B) increased at day 1 (pain: z=-3.5, $P<0.001$, soreness: z=-3.8, $P<0.001$) and remained elevated from day 5 to day 15 (pain NRS scores: all z>-2.9, $P<0.004$,

Likert soreness: z>-3.4, P<0.001) compared with day 0. Similarly, disability (χ^2(11)=163.7, P<0.001; Fig. 2C) was increased at day 1 (z=-3.7, P<0.001) and remained elevated from day 5 to day 15 (overall: z>-2.9, P<0.003) compared with day 0. There was no change in sleep quality NRS scores across days ($F_{11,220}$=1.9, P=0.1; Fig. 2D).

3.3 The homeostatic response in M1 is altered after 2 days of sustained muscle pain

The progressive development of sustained muscle pain altered the MEP response to single and double tDCS (raw data; time x day interaction: $F_{8,160}$=3.5, P<0.001; Fig. 3). A single block of anodal tDCS increased MEP amplitudes on days 0, 2, 4, and 14 (post-hoc baseline vs. between; P<0.004; *plasticity response-raw data*; Fig. 3). The MEP amplitude was not significantly increased following single tDCS on day 6 (post-hoc baseline vs. between: P=0.09; *plasticity response-raw data*). However, examination of the ratio data for the *plasticity response* revealed that the increase in MEP amplitude was not significantly different across days ($F_{4,80}$=1.7, P=0.1).

Following the second block of anodal tDCS, MEP amplitudes were reduced, consistent with a normal *homeostatic* response, on days 0, and 14 (raw data; post hoc between vs. 10 min; P<0.001). However, there was no significant reduction in MEP amplitudes on days 2, 4, and 6 (raw data; post hoc between vs. 10 min; day 2: P=0.07, day 4: P=0.7, day 6 P=0.5; Fig. 3). These findings were supported by analysis of the ratio data for the *homeostatic* response which was different across days ($F_{4,80}$=4.0, P=0.005). Compared with days 0 and 14, the ratio was smaller on day 2 (P=0.027), and day 4 (P=0.022) with a similar tendency observed on day 6 (P=0.076). On day 4, the MEP amplitude was *increased* following the second block of anodal tDCS (Fig. 3). The temporal profile for the development of pain relative to changes in *homeostatic* response is presented in Fig 4.

4. DISCUSSION

This study is the first longitudinal investigation of homeostatic plasticity in the transition to sustained musculoskeletal pain. We demonstrate that M1 homeostatic plasticity is altered in response to the development and resolution of sustained muscle pain. Specifically, the homeostatic response was disrupted after two days of progressively developing muscle pain, with the greatest impairment observed at day 4. These unique findings have relevance for our understanding of the maladaptive plasticity hypothesis in pain which has focussed almost exclusively on synaptic plasticity mechanisms. Altered homeostatic control could plausibly explain the aberrant synaptic plasticity reported in chronic pain and may contribute to the pathogenesis of this condition, providing new avenues for understanding and treatment.

A wide range of neuronal inputs are known to result in the induction of LTP- and LTD-like synaptic plasticity (Classen et al. 2004; Stefan et al. 2000). Despite this, the impact of sustained periods of pain on homeostatic mechanisms that regulate synaptic plasticity is unknown. Preliminary studies in animal models of central pain syndrome (Wang and Thompson 2008) and neuropathic pain (Xiong et al. 2017) suggest a link between pain-induced hyperalgesia and altered homeostatic plasticity. In humans, studies of pain and homeostatic plasticity are restricted to patient populations with migraine (Antal et al. 2008; Brighina et al. 2011; Brighina et al. 2005; Cosentino et al. 2014b) and chronic low back pain (Thapa et al. 2018). These studies report altered M1 homeostatic plasticity that is hypothesised to contribute to excessive cortical excitability, enlarged cortical representations, and symptoms in these conditions. Notably, cyclic impairments in homeostatic control are associated with the initiation, continuation, and termination of pain in individuals with migraine (Antal et al. 2008; Cosentino et al. 2014a; Cosentino et al. 2014b). However, where in the transition from acute to chronic pain altered homeostatic plasticity develops has not been investigated.

The current study is the first to examine whether sustained pain impacts the M1 homeostatic response. Repeated intra-muscular injection of NGF sensitises muscle nociceptors and dorsal horn neurons (Hoheisel et al. 2007) resulting in pain and dysfunction that mimic symptoms of chronic musculoskeletal pain conditions (Andersen et al. 2008). For example, injection of NGF induces comparable pain, hyperalgesia, and functional limitation to patients with chronic lateral elbow pain of ~26 weeks duration (Bergin et al. 2015). Using this model, the present data provide the first evidence that several days of sustained pain is sufficient to alter the M1 homeostatic response. Our data demonstrate an increase in MEP amplitude following a single block of anodal tDCS that did not differ in magnitude across days (plasticity response - ratio data), suggesting the development, persistence and resolution of pain did not influence the *plasticity* response. Although the MEP amplitude was not significantly increased on day 6 when the raw data were examined, this discrepancy likely reflects low statistical power given the p-value (p=0.09) rather than a true difference in the plasticity response on this day. However, future studies with greater statistical power are needed to confirm this finding. In contrast, examination of both the raw and ratio data revealed an impaired *homeostatic* response on days 2, 4 and 6 when pain was present. The *homeostatic* response was normal when pain was absent on days 0 and 14. Thus, consistent with studies in chronic migraine, the temporal profile of the altered homeostatic response mimicked the trajectory of pain development. Specifically, two days of sustained muscle pain altered the M1 homeostatic response, with the greatest disturbance in homeostatic control observed around the time of greatest pain severity on day 4. As pain resolved, so too did the alteration in homeostatic plasticity, returning to normal at day 14 (see Figure 4).

The functional relevance of altered homeostatic plasticity in response to several days of sustained muscle pain requires further investigation. One possibility is that altered homeostatic plasticity in the early stages of pain represents an adaptive response that prevents

memory encoding of pain-driven synaptic patterns of activity. Evidence from human and animal studies suggest that high levels of LTP, as would be expected if homeostatic mechanisms fail to bias synaptic thresholds toward LTD, impairs subsequent learning (Kang et al. 2011; Rioult-Pedotti et al. 2000). For example, the learning of a motor skill in humans results in high LTP formation that has been shown to interfere with the learning of subsequent motor skills (Shadmehr and Brashers-Krug 1997). Similarly, spatial learning is impaired following high levels of hippocampal LTP in animals (Moser et al. 1998). Some support for this hypothesis can be drawn from studies that report impaired motor learning in people with acute (Sterling et al. 2001), and chronic pain (Boudreau et al. 2010; Kang et al. 2011). Alternatively, it is tempting to speculate that altered homeostatic plasticity represents an impairment that if maintained over weeks to months (i.e. when pain does not resolve as expected) allows consolidation of maladaptive patterns of synaptic plasticity that underpin sensorimotor symptoms and dysfunction in clinical conditions. Indeed, studies in focal hand dystonia suggest that prolonged periods of afferent input in the absence of effective homeostatic control lead to excessive synaptic strengthening that consolidates unwanted movement patterns (Kang et al. 2011; Quartarone and Pisani 2011). In the context of chronic musculoskeletal pain, movement dysfunction has been hypothesised to contribute to chronicity of symptoms by altering the load on surrounding tissues, presumably resulting in a prolonged alteration of afferent input (Hodges 2011; Hodges and Tucker 2011).

This study is not without limitations. First, homeostatic plasticity was examined using an excitatory priming protocol only. This approach was selected as previous studies in chronic pain have reported impaired M1 homeostatic plasticity characterised by a failure to reduce the MEP amplitude (slide the threshold towards LTD-like effects) following two blocks of excitatory anodal tDCS (Antal et al. 2008; Kang et al. 2011). Further research is required to understand the impact of sustained pain on homeostatic mechanisms induced using inhibitory

priming protocols. Second, the impact of sustained pain on homeostatic plasticity in other brain regions known to play a key role in pain perception (i.e., primary somatosensory cortex, dorsolateral prefrontal cortex) or within intracortical facilitatory and inhibitory networks was not investigated. Further work is needed to comprehensively disentangle the influence of sustained pain on homeostatic plasticity in humans. Third, homeostatic responses were induced using 5 x 7 cm sodium-chloride soaked sponges, which have been shown to stimulate brain regions outside M1 (Datta et al. 2010; Ho et al. 2016; Thair et al. 2017). Whether the effects observed in the current study are local to M1 is unknown. Future work should seek to induce homeostatic plasticity using more focal forms of stimulation. Fourth, the present study explored homeostatic plasticity using an experimental pain model. Although intramuscular injection of NGF is thought to mimic the development of sustained clinical pain, studies in clinical pain populations are needed to replicate our findings and determine the relevance of altered homeostatic plasticity to chronic pain. Finally, this study did not include a pain-free control group. Although our previous reliability study (Thapa and Schabrun 2018) suggests the altered homeostatic response observed in response to pain is unlikely to be explained by an effect of time, future studies should include a control group to further our understanding of M1 homeostatic plasticity in individuals with and without pain.

5. CONCLUSION

This study provides unique insight into the influence of progressively developing, sustained pain for several days on homeostatic plasticity in the human M1. Impaired homeostatic plasticity developed in parallel with the pain trajectory – manifesting after two days of sustained pain and returning toward baseline as pain resolved at day 14. Altered homeostatic control could plausibly explain the aberrant synaptic plasticity reported in chronic pain and

may contribute to the pathogenesis of this condition, providing new insight into the maladaptive plasticity hypothesis in chronic pain.

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FIGURE LEGENDS

Figure 1. A) Transcranial direct current stimulation (tDCS) protocol: two blocks of anodal tDCS (the first of 7-min duration and the second of 5-min duration, separated by a 3-min rest period) were applied to the primary motor cortex (M1) contralateral to the right (painful) extensor carpi radialis brevis (ECRB) muscle. Fifteen motor evoked potentials (elicited using transcranial magnetic stimulation) were recorded at baseline, during the 3-min rest period, and 10-min after the last block of tDCS. **B)** M1 plasticity was assessed and induced at the beginning of each experimental session on days 0, 2, 4, 6, and 14. Nerve growth factor (NGF) was injected into the belly of the right ECRB muscle on days 0, 2, and 4. Every alternate day, from day 1 to day 21, participants completed an online diary consisting of an 11-point pain numerical rating scale, a modified 7 point Likert muscle soreness scale, the patient rated-tennis elbow evaluation questionnaire (PRTEEQ), and an 11-point sleep numerical rating scale.

Figure 2. Mean ± standard error (N=21) for (**A**) pain intensity (numerical rating scale scores), (**B**) muscle soreness (Likert scale scores), (**C**) disability (Patient Rated Tennis Elbow Evaluation Questionnaire score), and (**D**) sleep quality (numerical rating scale scores). Pain intensity, muscle soreness, and disability increased at day 1 and remained elevated at day 15 compared with day 0 (*P<0.004).

Figure 3. Mean + standard error (N=21) for motor evoked potential amplitude normalised to baseline after the first ('between'), and second block of tDCS ('10-min)' on days 0, 2, 4, 6, and 14. *Significant increase in MEP amplitude following the first block of tDCS (P<0.004) or #significant decrease in MEP amplitude following the second block of tDCS (P<0.001).

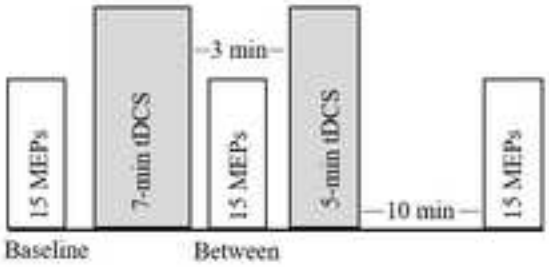
Figure 4. Mean + standard error (N=21) for pain scores (closed circles) and the homeostatic response (percent change of the MEP amplitude after the second block of tDCS relative to the MEP amplitude immediately after the first block of tDCS; closed triangles) demonstrating the

temporal profile of the change in homeostatic regulation (values < 0 % represent a normal homeostatic response) relative to the development of sustained pain.

Table 1. Mean ± standard deviation (N=21) for i) transcranial magnetic stimulation (TMS) intensity (percent of maximum stimulator output, MSO) required to evoke a motor evoked potential (MEP) of 0.5 mV peak-to-peak amplitude at baseline and ii) MEP amplitude recorded at baseline (prior to tDCS), on each day.

Cortical measures	**Day 0**	**Day 2**	**Day 4**	**Day 6**	**Day 14**
TMS (% MSO)	41 ± 6	41 ± 6	43 ± 9	42 ± 7	42 ± 6
MEP (mV)	0.48 ± 0.1	0.46 ± 0.1	0.49 ± 0.1	0.50 ± 0.1	0.55 ± 0.1

A



B

	Day																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
On-line diary		X		X		X		X		X		X		X		X		X		X		X
Assessment and induction of plasticity	X		X		X		X								X							
NGF injection	X		X		X