Disrupted Cortical Homeostatic Plasticity Due to Prolonged Capsaicin-induced Pain

Geraldine Wittkopf, Priscilla; Boye Larsen, Dennis; Gregoret, Luisina; Graven-Nielsen, Thomas

Published in:
Neuroscience

DOI (link to publication from Publisher):
10.1016/j.neuroscience.2023.09.011

Publication date:
2023

Document Version
Early version, also known as pre-print

DISRUPTED CORTICAL HOMEOSTATIC PLASTICITY DUE TO PROLONGED CAPSAICIN-INDUCED PAIN

Priscilla Geraldine Wittkopf[1+], Dennis Boye Larsen[1+], Luisina Gregoret[1], Thomas Graven-Nielsen[1*]

[1] Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University, Aalborg, Denmark, Selma Lagerløfs Vej 249, 9260 Gistrup, Denmark

+ shared first authorship

Original paper for: Clinical Neurophysiology

Pages (text): 20

No. of figures: 5

No. of tables: 0

Running title: Prolonged pain disrupts homeostatic plasticity

Disclosures: Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). Declarations of interest: None. All authors have approved the final article.

***Corresponding Author:**

Prof. Thomas Graven-Nielsen, PhD, DMSc.

Center for Neuroplasticity and Pain (CNAP)

Department of Health Science and Technology

Faculty of Medicine, Aalborg University

Selma Lagerløfs Vej 249, 9260 Gistrup, Denmark

Phone: +45 9940 9832, Fax: +45 9815 4008

E-mail: tgn@hst.aau.dk

ABSTRACT

Homeostatic plasticity (HP) regulates cortical excitability (CE) stability but is disrupted in persistent pain conditions. This study investigated how prolonged experimental pain affects HP and if pain relief modulates disrupted HP. Twenty-four healthy participants were randomised into a PainRelief or NoPainRelief group and attended four sessions; two sessions on consecutive days, separated by two weeks. Transcranial magnetic stimulation motor-evoked potentials reflecting CE and quantitative sensory testing (QST) measures were recorded. A capsaicin (pain condition) or placebo (control condition) patch was applied to the hand. HP was induced by cathodal-cathodal transcranial direct current stimulation (HP1) with CE assessment before and after. The PainRelief group had ice applied to the patch, while the NoPainRelief group waited for five minutes; subsequently another HP induction (HP2) and CE assessment were performed. After 24 hrs with the patch on, HP induction (HP3), QST, and CE recordings were repeated. Capsaicin reduced CE and the pain condition showed disrupted homeostatic responses at all time points (HP1: showed CE inhibition instead of facilitation; HP2 & HP3: lack of CE facilitation). Conversely, homeostatic responses were induced at all time points for the placebo condition. Capsaicin pain disrupts HP which is not restored by ice-induced pain relief. Future research may explore the prevention of HP disruption by targeting capsaicin-induced nociception but not pain perception.

Keywords: Pain, capsaicin, Homeostatic plasticity, Plasticity, Transcranial magnetic stimulation, Transcranial direct current stimulation.

INTRODUCTION

Chronic pain remains a major healthcare issue affecting a large proportion of the adult population (Breivik et al., 2006). In some persistent pain conditions, altered excitability of sensorimotor cortices has been associated with pain severity and pain duration (Kregel et al., 2015; Massé-Alarie et al., 2016), but it is less clear how and why these changes manifest, but may be related to less flexible neuroplastic adaptations (Thapa et al., 2018).

Synaptic plasticity is a fundamental neural mechanism, which strengthens (long-term potentiation, LTP) or weakens (long-term depression, LTD) synaptic transmission (Abbott and Nelson, 2000). However, the positive feedback nature of these mechanisms poses a risk to the stability of the system, which necessitates control mechanisms regulating excessive facilitation or inhibition of neural activity, also known as homeostatic plasticity (Abbott and Nelson, 2000; Cooper and Bear, 2012). Homeostatic plasticity is based on a 'sliding-threshold' of bidirectional synaptic plasticity theory, depending on postsynaptic activity (Pozo and Goda, 2010; Cooper and Bear, 2012). For example, in the case of high post-synaptic activity, a synaptic modification threshold slides towards a homeostatic response favouring LTD induction (Abraham, 2008; Cooper and Bear, 2012; Karabanov et al., 2015). Homeostatic plasticity is investigated using a priming-test design, in which the priming alters cortical excitability and the test stimulus triggers and captures the homeostatic response (Karabanov et al., 2015). Two blocks of non-invasive brain stimulation such as repetitive transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS) of the primary motor cortex, with an interval of no stimulation in-between blocks, can induce a corticomotor homeostatic response as reflected by TMS-induced motor-evoked potentials (MEPs) (Karabanov et al., 2015; Hassanzahraee et al., 2018; Thapa et al., 2018; Wittkopf et al., 2021a, 2021b). In healthy participants, a block of inhibitory stimulation (cathodal tDCS) primed by another block of inhibitory stimulation yields a homeostatic response, as reflected by increased MEPs (Wittkopf et al., 2021b, 2021a). Conversely, this homeostatic plasticity response is disrupted in several pain conditions such as migraine (Antal et al., 2008), dystonia (Quartarone et al., 2008; Quartarone and Pisani, 2011), and chronic low back pain (Thapa et al., 2018). Disruption of homeostatic plasticity may extend beyond the focal affected area and is not restricted to the cortical representations of affected muscles (Quartarone et al., 2008; Thapa et al., 2018), as seen in e.g. migraineurs (Antal et al., 2008). In addition, a recent study identified disruption of homeostatic plasticity in chronic low back

pain participants as assessed by MEPs of the first dorsal interosseous muscle (Thapa et al., 2018). Interestingly, no association was found between disruption in homeostatic plasticity and pain intensity nor duration (Thapa et al., 2018). The authors hypothesised that disruption of homeostatic plasticity may develop in sub-acute or early stages of pain and progress over time. This was later demonstrated in an experimental muscle pain model with pain persisting over several days, where the homeostatic response was impaired after four days of sustained musculoskeletal pain (Thapa et al., 2021). Currently it is unknown if homeostatic plasticity disruption occurs only due to sustained musculoskeletal pain, if other types of prolonged pain can affect homeostatic regulation, or if the homeostatic response can be restored by pain relief.

The aim of the present study was to assess the cortical homeostatic response during prolonged capsaicin-induced skin pain (24 hrs) in healthy subjects, to investigate if 1) the transition from acute to prolonged cutaneous pain critically interferes with measures of homeostatic plasticity, and 2) if such interferences are negated by pain relief.

EXPERIMENTAL PROCEDURES

Participants

A sample size calculation was conducted using α of 0.05, β of 0.80 and an estimated effect size (partial $\eta^2 = 0.18$) based on MEPs amplitude analyses of a previous study (Wittkopf et al., 2021b) using cathodal-cathodal tDCS. The sample size was calculated based on a 2x2x4 mixed model analysis of variance (ANOVA), with one between-subject factor (two groups), and two within-subject factors (two levels of condition and four levels of time), resulting in a needed sample size of 20 participants. Differences in study designs were considered and recruitment of 24 healthy, right-handed participants was targeted. In a cross-over design, all subjects participated in a Pain condition and a Control condition and were randomized into a PainRelief group (N=12, four females, mean ± SD age 26.2 ± 4.6 years) or a NoPainRelief group (N=12, four females, 25.2 ± 2.0 years). Participants were requested not to take part in the study if they did not consider themselves healthy, had history of or present neurological disorder or mental illness, were experiencing pain or sensory disturbances, were taking any medication either prescribed or purchased over the counter, were known to be pregnant, had a dermatological condition, had history of epilepsy, had metal implants in the head or jaw, or had allergy to capsaicin. During the study visit, volunteers were formally screened for

eligibility using the Edinburgh Handedness Inventory and the TMS and tDCS standardised safety questionnaires (Oldfield, 1971; Rossi et al., 2011). All participants received written and verbal information, provided written consent, and were reminded that they could withdraw consent at any time and without reason. The study was carried out at Center for Neuroplasticity and Pain (CNAP), Aalborg University, Aalborg, Denmark. Ethical approval was received from the local ethics committee (VN-20190069), and the study was conducted in accordance with the declaration of Helsinki and pre-registered at ClinicalTrials.gov (NCT04485689).

Experimental protocol

A randomized, cross-over (Pain vs Control condition), and between-subjects repeated-measures design including two groups (PainRelief vs NoPainRelief) was used to evaluate the effects of pain and subsequent pain relief on homeostatic plasticity (**Fig. 1**). All participants took part in two experimental conditions (Pain vs Control), separated by two weeks, and each condition was tested on two consecutive days (Day0, Day1).

Within each condition, participants were seated comfortably with their hands and arms at rest. Quantitative sensory testing (QST) assessments were recorded and followed by placement of electromyographic electrodes at the right hand first dorsal interosseous (FDI) muscle for recording corticomotor excitability, as reflected by TMS-evoked MEPs. On Day 0, baseline corticomotor excitability was assessed before capsaicin (7x7 cm^2, 8%, Qutenza®, Grunenthal GmbH, Germany; Pain condition) or placebo (Control condition) patches were applied to the dorsum of the right hand, where they remained for 24 hrs (i.e., including Day0 and Day1 recordings). The placebo patches (Demo patch, Astellas) are identical to the capsaicin patches, except for the active ingredient capsaicin. Another round of QST measures and corticomotor excitability were recorded 30 mins after patch application (**Fig. 1;** POST-PATCH). As capsaicin reduces corticomotor excitability compared to placebo (Boudreau et al., 2007), the percentage of maximum stimulator output was adjusted until the post-capsaicin patch MEP amplitudes matched those of the pain-free baseline assessment (10% amplitude deviation allowed). This was done to ensure that the isolated effect of pain on homeostatic plasticity was tested, and not simply the reduction in cortical excitability due to the sustained nociception. This adjusted TMS intensity was used for the subsequent assessment of MEPs on Day0. Corticomotor excitability was assessed before and

at time points: 0-, 15-, 30-, and 45-minutes post homeostatic plasticity induction (**Fig. 1;** HP1, HP PARADIGM). The PainRelief group then had ice applied to the patch, while the NoPainRelief group remained at rest, after which another block of homeostatic plasticity induction was applied (**Fig. 1;** HP2), and corticomotor excitability was recorded following the same procedure described above (**Fig. 1;** HP PARADIGM). Twenty-four hours after patch application (Day1), participants returned and QST measures were recorded, and homeostatic plasticity was induced (**Fig. 1;** HP3) followed by corticomotor excitability measures (**Fig. 1;** HP PARADIGM).

Pain intensity was scored on a 0-10 numerical rating scale (NRS, 0 – no pain, 10 – maximum pain imaginable) every 10 min after patch application for 3 hrs during the first session (**Fig. 1;** from BASELINE until after HP2). Immediately after the last MEP recording (i.e., time point 45 mins) of the first homeostatic plasticity induction (Fig. 1; HP1) , ice was applied on the top of the patch (for both, Pain and Control conditions) for 5 minutes in the PainRelief group, while participants in the NoPainRelief group waited for five minutes before data collection continued with or without ice until end of the two hours (ice was removed during MEP recordings for each time point and reapplied during the intervals). Pain intensity was collected hourly, three hours after the experimental assessments on Day0, and hourly three hours before and two hours during the Day1 recordings to clarify if the capsaicin patch induced prolonged pain.

Assessment of corticomotor excitability

Bipolar Ag/AgCl electrodes (Neuroline 720, Ambu A/S, Copenhagen, DK) were mounted on the muscle belly of the right first dorsal interosseous (FDI) muscle, with an approximate 20-mm interelectrode distance. The reference electrode was placed at the styloid process of the ulna. The electromyography data were pre-amplified (gain: 1,000x), analogue band-pass filtered (5 Hz−1 kHz) and sampled at 5 kHz by a 16-bit data acquisition card (NI6122, National Instruments, Austin, Texas).

All TMS methods are described in accordance to the guidelines on TMS methodology reporting (Chipchase et al., 2012). A magnetic stimulator (Magstim 200^2, Magstim Company, Whitland, UK) was used to deliver monophasic pulses, using a focal figure-of-8 coil (D702, Magstim Company, Whitland, UK). To induce a posterior-anterior current, the coil handle was placed pointing backwards and laterally at a 45° angle to the sagittal plane over the hot

spot of the FDI muscle. An interstimulus interval of five to seven seconds was used. Each participant was fitted with a neoprene cap (FOC.US V3, Brain Control Ltd. London, UK). The cap ensured standardised orientation and location of the delivery of TMS pulses and was used to determine the hot spot and resting motor threshold (RMT) for the FDI muscle. The optimal scalp position was determined using 50% of the maximum stimulator output and was defined as the site that yielded the highest and most consistent peak-to-peak MEP amplitudes in three trials. The hotspot was marked with a pen on the cap. The RMT was determined based on the stimulator output intensity needed to evoke MEPs of ≥50 μV in the FDI muscle in 5 out of 10 trials with the muscle at rest (Rossini et al., 2015). A stimulation intensity of 120% of RMT was used for the MEPs at the baseline MEPs measurement. Peak-to-peak MEPs were shown online by custom-made LabView software (Mr. Kick III, Aalborg University, Aalborg, Denmark). A window of 100 ms before TMS stimulation was used to confirm that no contraction or tension (≥ 50 μV) of the muscle was present before the stimulation. The peak-to-peak amplitude was extracted for each MEP and averaged across 10 sequential MEPs recorded at each time point. The averaged MEPs were used for analysis.

Induction of homeostatic plasticity by transcranial direct current stimulation

Homeostatic plasticity was induced in the left primary motor cortex using cathodal tDCS applied for 7 minutes followed by an interval of 3 minutes of no tDCS and another block of 5 minutes of cathodal tDCS (Fricke et al., 2011; Thapa and Schabrun, 2018; Wittkopf et al., 2021b). A constant current of 1 mA was transmitted through the tDCS system (FOC.US V3, Brain Control Ltd. London, UK), using two 3.14 cm^2 Ag/AgCl gelled electrodes (Pistim, Neuroelectrics, Barcelona, Spain) placed into holes of the neoprene cap (NE056 Headcap R, Neuroelectrics, Barcelona, Spain) corresponding to the international 10/10 EEG system. The cathode was placed at C3 (primary motor cortex) and return electrode placed at Fp2.

Thermal sensitivity

A 3x3 cm^2 (9 cm^2) contact thermode (Medoc Advanced Medical Systems, Ramat Yishay, Israel) was used to assess the thermal sensitivity on the skin above the carpal bones (proximal relative to the patch) and on the anterior part of the forearm (midpoint between the medial epicondyle and the pisiform bone). Each stimulus series began at 32°C, and detection and pain thresholds were assessed, respectively. The temperature decreased and

increased in ramps, and the participant's cold detection threshold (CDT) and warm detection threshold (WDT) were recorded and expressed as the temperature where the first sign of change was detected and indicated by them pressing a button. Cold pain thresholds (CPT) and heat pain thresholds (HPT) were assessed by the participants indicating the moment the thermal sensation first became painful. The measurements were repeated 3 times, within the temperature range of 0 to 50°C, and the average was used for analysis.

Mechanical pain threshold

The mechanical pain threshold (MPT) was determined at the skin above the carpal bones (proximal relative to the patch) and on the anterior part of the forearm (midpoint between the medial epicondyle and the pisiform bone) using a set of 7 weighted pinprick stimulators (PinPrick; MRC Systems GmbH, Heidelberg, Germany) that exerts forces between 8 and 512 mN on a contact area of 0.25-mm tip diameter. Five threshold determinations were made, each with a series of ascending and descending stimulus intensities. The final threshold was defined as the geometric mean of the 5 suprathreshold and subthreshold readings (Rolke et al., 2006).

Statistics

Data are presented as mean and standard error of the mean. Statistical analyses were performed using SPSS version 26 for Windows (Chicago, IL, USA). Data distribution was assessed using the Shapiro-Wilk test. If data exhibited a non-normal distribution across several time points, all data were log-transformed (base 10) and used for subsequent analyses. As earlier evidence from our group (Wittkopf et al., 2021b) has delineated the temporal effects of the current HP method on MEP effects, HP effect was reflected as the pooled average MEPs from time 0 min, 15 min, and 30 min. For each homeostatic plasticity induction (**Fig. 1**; HP1, HP2, HP3), a three-way mixed model analysis of variance (ANOVA) was conducted on MEPs with a between Group factor (PainRelief, NoPainRelief) and two within-subject factors *Condition* (Pain and Control) and *Time* (pre-HP, pooled average 0 min, 15 min, 30 min MEPs). Thermal and mechanical sensitivity were analysed using a four-way mixed model analysis of variance (ANOVA) with a between factor Group (PainRelief, NoPainRelief) and within-subject factors *Condition* (Pain and Control), *Time* (baseline, 30 mins post patch, 24 hrs post patch), and *Site* (hand and arm). A Greenhouse–Geisser

correction was used if Mauchly's test showed that sphericity could not be assumed. Adjustments were made for multiple post-hoc comparisons using the Bonferroni correction. Statistical significance was accepted for $p<0.05$ and effect sizes reported as partial η^2 for omnibus tests, and Cohen's d for posthoc comparisons.

RESULTS

Capsaicin successfully induced pain progressively during the first 120 minutes post-patch application. A time × group interaction was found for pain NRS scores during the Pain condition ($F_{3.7,77.2}$=12.56, p<0.0005, partial η^2=0.374). Simple main effects analyses showed that the PainRelief and NoPainRelief group both reported increased pain 30 mins after capsaicin patch application up until 120 mins after (all comparisons between 10 up until 120 minutes; significance and effect sizes in **Tables S1-S2**; **Fig. 2;**). When ice was applied, the PainRelief group had a drop in perceived pain intensity, as compared to the NoPainRelief group (all comparisons up until 190 minutes; significance and effect sizes in **Table S3**; **Fig. 2**). Moreover, the pain intensity was recorded for three hours after the Day0 recordings, and three hours before and two hours during the Day1 recordings (**Fig. 3**).

Mechanical and thermal pain sensitivity

There were no significant differences in mechanical pain sensitivity, WDT and HPT between capsaicin and placebo patch at any time point (data not shown). There was a significant main effect of site when analysing both WDT ($F_{1,22}$=5.96, p=0.023, partial η^2=0.213) and HPT ($F_{1,22}$=9.93, p=0.005, partial η^2=0.311) indicating a higher sensitivity to warm temperature on the arm when compared to the hand.

Corticomotor excitability before assessment of homeostatic plasticity

A significant condition × time interaction was found for the baseline and post patch MEPs ($F_{1,22}$=5.28, p=0.031, partial η^2=0.194). Simple main effects analysis showed that MEPs were reduced post-patch application compared to baseline, only in the pain condition (p=0.0004, Cohen's *d*=0.86), but not the control condition (p=0.52, Cohen's *d*=0.601; **Fig. 4**).

Homeostatic plasticity

When analysing the first homeostatic response (HP1), a significant condition × time interaction was found ($F_{1,22}$ =24.71, p=0.000056, partial η^2=0.529). Simple main effects analysis revealed that the control condition showed a homeostatic response, reflected by an increase in MEP amplitudes (average across time 0-, 15- and 30-min post-HP) compared with pre-HP1 (p=0.000067, Cohen's d=0.86) while the pain condition had a decrease in MEPs (p=0.013, Cohen's d=0.70) (**Fig. 5, HP1**). In the second assessment of the homeostatic response (HP2) after PainRelief or NoPainRelief, a significant condition × time interaction was found ($F_{1,22}$ =5.71, p=0.026, partial η^2=0.206). Simple main effects analysis revealed that the control condition showed a homeostatic response, reflected by an increase in MEP amplitudes compared with pre-HP2 (p=0.00029, Cohen's d=0.76) while this was not evident in the pain condition (p=0.56; **Fig. 5, HP2**). Interestingly, the Group factor was not significant (p=0.13, partial η^2=0.1), suggesting that there were no significant effects by PainRelief or NoPainRelief during HP assessment.

For the HP3 on Day1, a significant condition × time interaction was found ($F_{1,22}$=7.32, p=0.013, partial η^2=0.25), with simple main effects demonstrating a homeostatic response in the control condition (p=0.00039, Cohen's d=0.63) when compared to pre-HP3, but not in the pain condition (p=0.78) (**Fig. 5, HP3**).

DISCUSSION

The present study provides evidence that the capsaicin patch induced moderate pain for up to 24 hours and reduced corticomotor excitability 30 minutes post patch application. The placebo patch did not affect the homeostatic response, which was present after homeostatic plasticity induction. Capsaicin disrupted the homeostatic response and this disruption persisted up to 24 hours. The application of ice on top of the capsaicin patch reduced the pain intensity substantially but did not reinstate the homeostatic response.

Prolonged pain induced by capsaicin patches

High-concentration capsaicin patches produce a model of prolonged pain that can be investigated for up to 24 hours (Malmberg et al., 2004; Lo Vecchio et al., 2018). Capsaicin has been shown to activate primarily the polymodal A-delta fibre sensitive to mechanical and heat stimuli with rapid onset and the polymodal C-fibre responding to mechanical and heat stimuli (Arendt-Nielsen and Andersen, 2005; Henrich et al., 2015). Application of

capsaicin patches can be used to investigate the process of sensitisation, as primary and secondary hyperalgesia develop, indicating that peripheral and central sensitisation mechanisms are involved (Malmberg et al., 2004; Arendt-Nielsen and Andersen, 2005; Lo Vecchio et al., 2018). The present experiment provides evidence that the capsaicin patch induced moderate pain over a 24-hour period, however, secondary hyperalgesia was not identified. This absence of secondary hyperalgesia may be due to a need for stronger nociceptor activation, as e.g., non-responders to punctate stimulation after intradermal capsaicin application has been reported (Mouraux and Iannetti, 2009), but combining heat and topical capsaicin produce significant punctate secondary hyperalgesia (Meeker et al., 2019). The heat-capsaicin experimental pain model has additionally shown acceptable reliability and stability when painful cold or hot stimuli were applied (Petersen and Rowbotham, 1999). Future studies should therefore investigate the reliability of punctate hyperalgesia and self-reported pain during prolonged exposure to topical capsaicin. Previous studies have demonstrated how capsaicin-induced pain and hyperalgesia are critically temperature-dependent and that mild cooling of the skin provides instant relief from ongoing pain (Koltzenburg et al., 1992; Grönroos and Pertovaara, 1993; Knolle et al., 2013). If the skin is cooled before application of a capsaicin patch, the pain can be virtually abolished (Knolle et al., 2013), which may indicate that ice-induced pain relief is achieved through peripheral receptor activation (Koltzenburg et al., 1992). At the functional receptor level, whole-cell patch clamp studies have demonstrated that cooling inhibits capsaicin-induced currents in human embryonic kidney (HEK) 293 cells expressing the TRPV1 receptor (Chung and Wang, 2011). This is likely due to a decrease in the open probability of the capsaicin bound TRPV1, and recent cryo-electron microscopy data suggest that even with saturating levels of capsaicin, TRPV1 decreases its open probability below 50% before reaching 0° with a structure shape similar to the apo state (Yang et al., 2018). An alternative explanation that cannot be ruled out is that the ice-induced pain relief was achieved by activation of other TRP receptors such as the TRP melastatin-8 (Proudfoot et al., 2006), albeit its role in pain alleviation is still controversial (Brederson et al., 2013). Future research is encouraged to investigate if prevention of capsaicin-induced pain (by pre-cooling the skin) still produces an impaired homeostatic response. This would support the notion that sustained nociception, and not pain intensity, is the primary driver for cortically impaired

homeostatic control, as application of ice after capsaicin does not affect the H-reflex (Grönroos and Pertovaara, 1993).

Corticomotor excitability changes during pain for 24 hrs

Similar to previous studies, the capsaicin patch reduced corticomotor excitability 30 minutes post patch application. Farina et al. (2001) investigated the interaction between capsaicin pain and corticomotor excitability by applying low concentration capsaicin on the skin adjacent to the FDI and flexor carpi radialis (FCR) in 17 healthy participants. They found reduced MEP amplitudes of abductor pollicis brevis (APB), abductor digiti minimi (ADM), and extensor carpi radialis (ECR) muscles only when capsaicin was applied over the FDI and FCR muscles and corticomotor excitability was measured from the same muscles (Farina et al., 2001). Moreover, capsaicin may also affect intracortical inhibition. For instance, Fierro et al. (2010) applied capsaicin on the dorsal surface of the right hand of seven healthy participants and found significantly reduced MEP amplitudes and decreased SICI (Fierro et al., 2010). Taken together, these findings suggest that capsaicin may affect corticomotor excitability through inhibitory gamma-aminobutyric acid A receptor activity (Di Lazzaro et al., 2006).

Homeostatic plasticity changes during pain for 24 hrs

The homeostatic plasticity induction protocol of seven minutes of cathodal tDCS followed by three minutes of no stimulation and another block of five minutes of cathodal tDCS successfully induced a homeostatic response as observed in the placebo patch condition. These findings are consistent with previous studies using similar homeostatic plasticity induction protocols (Thapa and Schabrun, 2018; Wittkopf et al., 2021b). Specifically, a recent systematic review analysing 55 experiments indicated that two blocks of inhibitory stimulation with an interval of no stimulation between blocks only effectively induced a homeostatic response when the duration of the interval of no stimulation was 10 minutes or less (Wittkopf et al., 2021a). In the present study, a homeostatic response was effectively induced 30 minutes post placebo patch application. Interestingly, a second subsequent homeostatic plasticity induction resulted in a homeostatic response, indicating that homeostatic plasticity can be induced in sequence. This was further corroborated by the induction of a third homeostatic response (HP3), 24 hrs post patch application. This is in line

with previous reliability studies that have demonstrated the protocol's reliability over one, two, seven, and 14 days (Thapa and Schabrun, 2018; Wittkopf et al., 2021b).

Disrupted homeostatic plasticity has been identified in patients with chronic pain where migraineurs (Antal et al., 2008; Cosentino et al., 2014) and chronic low back pain patients present with impaired homeostatic plasticity responses (Thapa et al., 2018). Moreover, in a recent mechanistic study, Thapa et al. (2021), reported that prolonged nerve-growth factor-induced pain (21 days), impaired homeostatic plasticity in a time-dependent fashion, where the maximum impairment was seen four days after injection, with a slow return-to-normal after 14 days. The current findings are in line with this, in that sustained nociception disrupted the homeostatic response. Our group (De Martino et al., 2018; Larsen et al., 2018, 2019) and others (Farina et al., 2001; Schabrun and Hodges, 2012; Schabrun et al., 2013) have demonstrated that experimental musculoskeletal pain models provoke alterations in both corticomotor and somatosensory cortex excitability. As homeostatic responses can also be elicited in the somatosensory cortex (Bliem et al., 2008; Jones et al., 2016), future research is encouraged to approach homeostatic plasticity as a global regulation of cortical excitability and investigate its disruption during sustained nociception.

It is important to highlight that the current study setup uses an inhibitory tDCS paradigm on an already inhibited system, i.e., the capsaicin-induced nociception decreased MEPs 30 min after patch application. This reduction was compensated for by increasing the stimulation intensity to match MEPs to those at baseline. Nonetheless, when participants received the capsaicin patch, the HP1 response yielded a further overall decrease in corticomotor excitability when compared to pre-HP induction (in contrast to the expected increased corticomotor excitability). One possible explanation is that the attempt to induce a homeostatic response using inhibitory tDCS stimulations further exacerbated the inhibition. To investigate this, corticomotor excitability should be measured 30 mins post patch, and again after the first block of inhibitory tDCS, as this would yield information on the priming effect of capsaicin pain on a subsequent inhibitory tDCS stimulation. We are unable to definitively assess whether priming stimulation did alter cortical excitability, as we did not assess changes in MEP amplitudes directly following the priming block of stimulation, to not disrupt any ongoing plasticity. Nevertheless, this should have little impact on our findings as homeostatic plasticity does not depend on changes in synaptic efficacy following priming (Abraham, 2008), and several experiments in animals and humans have reported

homeostatic plasticity without excitability changes following the priming block (Hamada et al., 2009; Murakami et al., 2012).

A limitation of the present experiment is that the investigator was not blinded to the condition (i.e., capsaicin or placebo patch) nor to the groups. This may pose a limitation to the homeostatic response parameter, but since extreme care was taken to standardize TMS delivery, and that the homeostatic plasticity induction method has shown excellent test-retest reliability (Thapa and Schabrun, 2018; Wittkopf et al., 2021b), this is unlikely to have impacted the current findings. The present findings are limited to homeostatic plasticity induced and assessed at the primary motor cortex of healthy participants using a tDCS protocol. Further research is needed to determine the effect of capsaicin-induced pain on homeostatic plasticity in other brain regions, such as the primary somatosensory cortex. As capsaicin demonstrably reduced the MEPs, one possibility for the lack of homeostatic response for the capsaicin condition is that the priming cathodal tDCS block induced a homeostatic response, i.e., facilitated MEPs between the priming and test tDCS blocks in HP1. We find this possibility unlikely as the subsequent blocks (i.e., HP2 and HP3) would then be expected to induce similar effects on the MEPs, as the capsaicin patch was applied for 24 hrs, and nociception was sustained. It could be speculated that the current results mirror those reported by Farina et al. (2001), where dorsum hand capsaicin reduced corticomotor excitability 20 to 30 mins after application, but recovered to near-baseline after 40 mins. This may imply that the ongoing nociception sustained a disruption of the homeostatic plasticity responses, induced by tDCS. Nonetheless, future studies are needed to investigate the potential priming effect of capsaicin (or other experimental acute pain models) on cathodal tDCS and if such combination can elicit a homeostatic response. This would need further consideration on the interval between pain application and cathodal tDCS stimulation, as to ensure a homeostatic response can be captured (Karabanov et al., 2015)."

In conclusion, this study provides evidence that capsaicin-induced pain can temporally disrupt homeostatic response up to 24 hrs post patch application and that the homeostatic response is not restored by ice-induced pain relief.

AUTHOR CONTRIBUTIONS (CRediT)

PGW: Conceptualization, Methodology, Data collection, Data Curation, Formal analysis, Writing – original draft/review & editing, Visualization **DBL:** Methodology, Formal analysis,

Data Curation, Writing – original draft/review & editing, Visualization **LG:** Data collection (partly), Writing – review & editing **TGN:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision

ACKNOWLEDGMENTS

Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). Declarations of interest: None.

REFERENCES

Abbott LF, Nelson SB (2000) Synaptic plasticity: taming the beast. Nat Neurosci 3:1178–1183.

Abraham WC (2008) Metaplasticity: Tuning synapses and networks for plasticity. Nat Rev Neurosci 9:387–399.

Antal A, Lang N, Boros K, Nitsche M, Siebner HR, Paulus W (2008) Homeostatic metaplasticity of the motor cortex is altered during headache-free intervals in migraine with aura. Cereb Cortex 18:2701–2705.

Arendt-Nielsen L, Andersen OK (2005) Capsaicin in human experimental pain models of skin, muscle and visceral sensitization. Turn up Heat Pain TRPV1 Recept Pain Inflamm:117–144.

Babes A, Amuzescu B, Krause U, Scholz A, Flonta ML, Reid G (2002) Cooling inhibits capsaicin-induced currents in cultured rat dorsal root ganglion neurones. Neurosci Lett 317:131–134.

Bliem B, Müller-Dahlhaus JFM, Dinse HR, Ziemann U (2008) Homeostatic metaplasticity in the human somatosensory cortex. J Cogn Neurosci 20:1517–1528.

Boudreau S, Romaniello A, Wang K, Svensson P, Sessle BJ, Arendt-Nielsen L (2007) The effects of intra-oral pain on motor cortex neuroplasticity associated with short-term novel tongue-protrusion training in humans. Pain 132:169–178 Available at: http://www.ncbi.nlm.nih.gov/pubmed/17870237.

Brederson JD, Kym PR, Szallasi A (2013) Targeting TRP channels for pain relief. Eur J Pharmacol 716:61–76 Available at: http://dx.doi.org/10.1016/j.ejphar.2013.03.003.

Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D (2006) Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. Eur J Pain 10:287–333.

Chipchase L, Schabrun S, Cohen L, Hodges P, Ridding M, Rothwell J, Taylor J, Ziemann U

(2012) A checklist for assessing the methodological quality of studies using transcranial magnetic stimulation to study the motor system: An international consensus study. Clin Neurophysiol 123:1698–1704 Available at: http://dx.doi.org/10.1016/j.clinph.2012.05.003.

Chung MK, Wang S (2011) Cold suppresses agonist-induced activation of TRPV1. J Dent Res 90:1098–1102.

Cooper LN, Bear MF (2012) The BCM theory of synapse modification at 30: Interaction of theory with experiment. Nat Rev Neurosci 13:798–810.

Cosentino G, Brighina F, Talamanca S, Paladino P, Vigneri S, Baschi R, Indovino S, Maccora S, Alfonsi E, Fierro B (2014) Reduced Threshold for Inhibitory Homeostatic Responses in Migraine Motor Cortex? A tDCS/TMS Study. Headache J Head Face Pain 54:663–674 Available at: https://onlinelibrary.wiley.com/doi/10.1111/head.12249.

De Martino E, Zandalasini M, Schabrun S, Petrini L, Graven-Nielsen T (2018) Experimental Muscle Hyperalgesia Modulates Sensorimotor Cortical Excitability, Which is Partially Altered by Unaccustomed Exercise. Pain 00:1 Available at: http://insights.ovid.com/crossref?an=00006396-900000000-98889.

Di Lazzaro V, Pilato F, Dileone M, Ranieri F, Ricci V, Profice P, Bria P, Tonali PA, Ziemann U (2006) GABA A receptor subtype specific enhancement of inhibition in human motor cortex. J Physiol 575:721–726 Available at: https://onlinelibrary.wiley.com/doi/10.1113/jphysiol.2006.114694.

Farina S, Valeriani M, Rosso T, Aglioti S, Tamburin S, Fiaschi A, Tinazzi M (2001) Transient inhibition of the human motor cortex by capsaicin-induced pain. A study with transcranial magnetic stimulation. Neurosci Lett 314:97–101 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11698155.

Fierro B, De Tommaso M, Giglia F, Giglia G, Palermo A, Brighina F (2010) Repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex (DLPFC) during capsaicin-induced pain: modulatory effects on motor cortex excitability. Exp Brain Res 203:31–38 Available at: http://link.springer.com/10.1007/s00221-010-2206-6.

Fricke K, Seeber AA, Thirugnanasambandam N, Paulus W, Nitsche MA, Rothwell JC (2011) Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol 105:1141–1149.

Grandl J, Kim SE, Uzzell V, Bursulaya B, Petrus M, Bandell M, Patapoutian A (2010) Temperature-induced opening of TRPV1 ion channel is stabilized by the pore domain. Nat Neurosci 13:708–714.

Grönroos M, Pertovaara A (1993) Capsaicin-induced central facilitation of a nociceptive flexion reflex in humans. Neurosci Lett 159:215–218 Available at: https://linkinghub.elsevier.com/retrieve/pii/030439409390837B.

Hamada M, Hanajima R, Terao Y, Okabe S, Nakatani-Enomoto S, Furubayashi T, Matsumoto H, Shirota Y, Ohminami S, Ugawa Y (2009) Primary motor cortical metaplasticity induced by priming over the supplementary motor area. J Physiol 587:4845–4862.

Hassanzahraee M, Zoghi M, Jaberzadeh S (2018) How different priming stimulations affect the corticospinal excitability induced by noninvasive brain stimulation techniques: A systematic review and meta-analysis. Rev Neurosci 29:883–899.

Henrich F, Magerl W, Klein T, Greffrath W, Treede RD (2015) Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. Brain 138:2505–2520.

Jones CB, Lulic T, Bailey AZ, Mackenzie TN, Mi YQ, Tommerdahl M, Nelson AJ (2016) Metaplasticity in human primary somatosensory cortex: effects on physiology and tactile perception. J Neurophysiol 115:2681–2691 Available at: https://www.physiology.org/doi/10.1152/jn.00630.2015.

Karabanov A, Ziemann U, Hamada M, George MS, Classen J, Massimini M, Rothwell J, Roman H (2015) Consensus paper: Probing homeostatic plasticity of human cortex with non-invasive transcranial brain stimulation. Brain Stimul 8:442–454 Available at: http://dx.doi.org/10.1016/j.brs.2015.01.404.

Knolle E, Zadrazil M, Kovacs GG, Medwed S, Scharbert G, Schemper M (2013) Comparison of cooling and EMLA to reduce the burning pain during capsaicin 8% patch application: A randomized, double-blind, placebo-controlled study. Pain 154:2729–2736 Available at: http://dx.doi.org/10.1016/j.pain.2013.08.001.

Koltzenburg M, Lundberg LER, Torebjörk EH (1992) Dynamic and static components of mechanical hyperalgesia in human hairy skin. Pain 51:207–219 Available at: https://journals.lww.com/00006396-199211000-00010.

Kregel J, Meeus M, Malfliet A, Dolphens M, Danneels L, Nijs J, Cagnie B (2015) Structural and functional brain abnormalities in chronic low back pain: A systematic review. Semin

Arthritis Rheum 45:229–237.

Larsen DB, Graven-Nielsen T, Boudreau SA (2019) Pain-Induced Reduction in Corticomotor Excitability Is Counteracted by Combined Action-Observation and Motor Imagery. J Pain Available at: https://linkinghub.elsevier.com/retrieve/pii/S1526590019300549.

Larsen DB, Graven-nielsen T, Hirata RP, Boudreau SA (2018) Differential Corticomotor Excitability Responses to Hypertonic Saline-Induced Muscle Pain in Forearm and Hand Muscles. Neural Plast 2018:1–9.

Lo Vecchio S, Andersen HH, Arendt-Nielsen L (2018) The time course of brief and prolonged topical 8% capsaicin-induced desensitization in healthy volunteers evaluated by quantitative sensory testing and vasomotor imaging. Exp Brain Res 236:2231–2244 Available at: http://dx.doi.org/10.1007/s00221-018-5299-y.

Malmberg AB, Mizisin AP, Calcutt NA, Von Stein T, Robbins WR, Bley KR (2004) Reduced heat sensitivity and epidermal nerve fiber immunostaining following single applications of a high-concentration capsaicin patch. Pain 111:360–367.

Massé-Alarie H, Beaulieu LD, Preuss R, Schneider C (2016) Corticomotor control of lumbar multifidus muscles is impaired in chronic low back pain: concurrent evidence from ultrasound imaging and double-pulse transcranial magnetic stimulation. Exp Brain Res 234:1033–1045.

Meeker TJ, Keaser ML, Khan SA, Gullapalli RP, Seminowicz DA, Greenspan JD (2019) Non-invasive motor cortex neuromodulation reduces secondary hyperalgesia and enhances activation of the descending pain modulatory network. Front Neurosci 13:1–18.

Mouraux A, Iannetti GD (2009) Nociceptive laser-evoked brain potentials do not reflect nociceptive-specific neural activity. J Neurophysiol 101:3258–3269.

Murakami T, Müller-Dahlhaus F, Lu MK, Ziemann U (2012) Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. J Physiol 590:5765–5781.

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113 Available at: http://www.ncbi.nlm.nih.gov/pubmed/5146491.

Petersen KL, Rowbotham MC (1999) A new human experimental pain model: The heat/capsaicin sensitization model. Neuroreport 10:1511–1516.

Pozo K, Goda Y (2010) Unraveling Mechanisms of Homeostatic Synaptic Plasticity. Neuron

66:337–351 Available at: https://linkinghub.elsevier.com/retrieve/pii/S0896627310002990.

Proudfoot CJ, Garry EM, Cottrell DF, Rosie R, Anderson H, Robertson DC, Fleetwood-Walker SM, Mitchell R (2006) Analgesia Mediated by the TRPM8 Cold Receptor in Chronic Neuropathic Pain. Curr Biol 16:1591–1605.

Quartarone A, Morgante F, Sant'Angelo A, Rizzo V, Bagnato S, Terranova C, Siebner HR, Berardelli A, Girlanda P (2008) Abnormal plasticity of sensorimotor circuits extends beyond the affected body part in focal dystonia. J Neurol Neurosurg Psychiatry 79:985–990.

Quartarone A, Pisani A (2011) Abnormal plasticity in dystonia: Disruption of synaptic homeostasis. Neurobiol Dis 42:162–170 Available at: http://dx.doi.org/10.1016/j.nbd.2010.12.011.

Rolke R, Magerl W, Campbell KA, Schalber C, Caspari S, Birklein F, Treede RD (2006) Quantitative sensory testing: A comprehensive protocol for clinical trials. Eur J Pain 10:77–88.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2011) Screening questionnaire before TMS: an update. Clin Neurophysiol 122:1686 Available at: http://dx.doi.org/10.1016/j.clinph.2010.12.037.

Rossini PM et al. (2015) Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 126:1071–1107 Available at: http://linkinghub.elsevier.com/retrieve/pii/S1388245715000711.

Schabrun SM, Hodges PW (2012) Muscle pain differentially modulates short interval intracortical inhibition and intracortical facilitation in primary motor cortex. J Pain 13:187–194 Available at: http://dx.doi.org/10.1016/j.jpain.2011.10.013.

Schabrun SM, Jones E, Kloster J, Hodges PW (2013) Temporal association between changes in primary sensory cortex and corticomotor output during muscle pain. Neuroscience 235:159–164 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23357117.

Thapa T, Graven-Nielsen T, Chipchase LS, Schabrun SM (2018) Disruption of cortical synaptic homeostasis in individuals with chronic low back pain. Clin Neurophysiol 129:1090–1096 Available at: https://doi.org/10.1016/j.clinph.2018.01.060.

Thapa T, Graven-Nielsen T, Schabrun SM (2021) Aberrant plasticity in musculoskeletal pain: a failure of homeostatic control? Exp Brain Res 239:1317–1326 Available at: https://doi.org/10.1007/s00221-021-06062-3.

Thapa T, Schabrun SM (2018) Test-Retest Reliability of Homeostatic Plasticity in the Human Primary Motor Cortex. Neural Plast 2018:1–9 Available at: https://www.hindawi.com/journals/np/2018/6207508/.

Wittkopf PG, Larsen DB, Graven-Nielsen T (2021a) Protocols for inducing homeostatic plasticity reflected in the corticospinal excitability in healthy human participants: A systematic review and meta-analysis. Eur J Neurosci 54:5444–5461.

Wittkopf PG, Larsen DB, Gregoret L, Graven-Nielsen T (2021b) Prolonged corticomotor homeostatic plasticity – Effects of different protocols and their reliability. Brain Stimul 14:327–329.

Yang F, Xiao X, Lee BH, Vu S, Yang W, Yarov-Yarovoy V, Zheng J (2018) The conformational wave in capsaicin activation of transient receptor potential vanilloid 1 ion channel. Nat Commun 9 Available at: http://dx.doi.org/10.1038/s41467-018-05339-6.

FIGURE LEGENDS

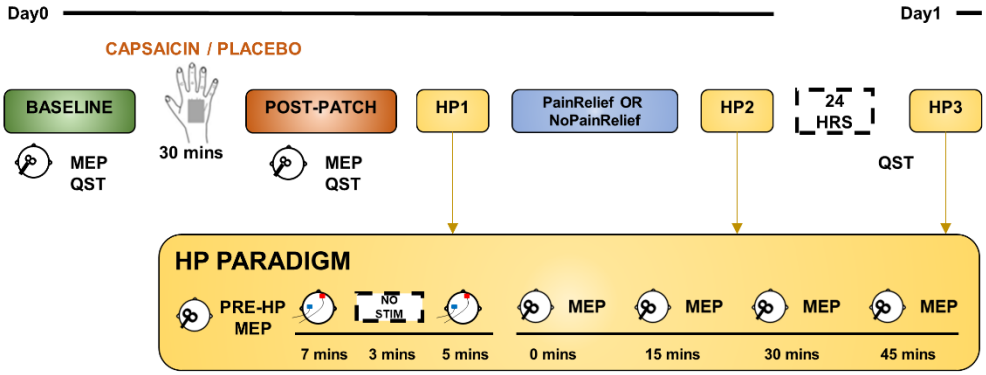

Figure 1. Timeline of one experimental session. After baseline assessments of motor evoked potentials (MEPs), a placebo or capsaicin patch was applied on the right-hand dorsum. After 30 minutes, the impact of patch application was assessed through two different approaches (1) applying a transcranial magnetic stimulation (TMS) intensity equal to baseline, and (2) modifying the TMS intensity to match the amplitude of baseline MEP. To characterize the impact of pain and pain relief on homeo

static plasticity (HP), first and second induction of HP took place (HP1 and HP2), respectively, separated by an application of ice over the patch (capsaicin and placebo). After 24 hours, a third induction of HP was conducted (HP3) to characterise the effect of 24-hour prolonged pain on HP.

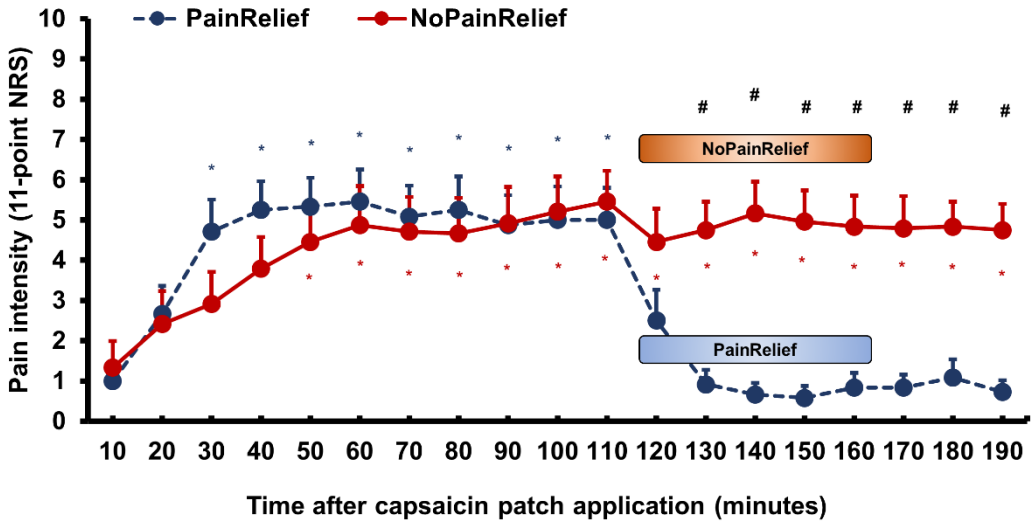

Figure 2. Pain intensity after capsaicin patch application and ice-induced pain relief. Mean and standard error of the mean of pain intensity measured by numerical rating scale (NRS) scores every 10 minutes post-patch application.

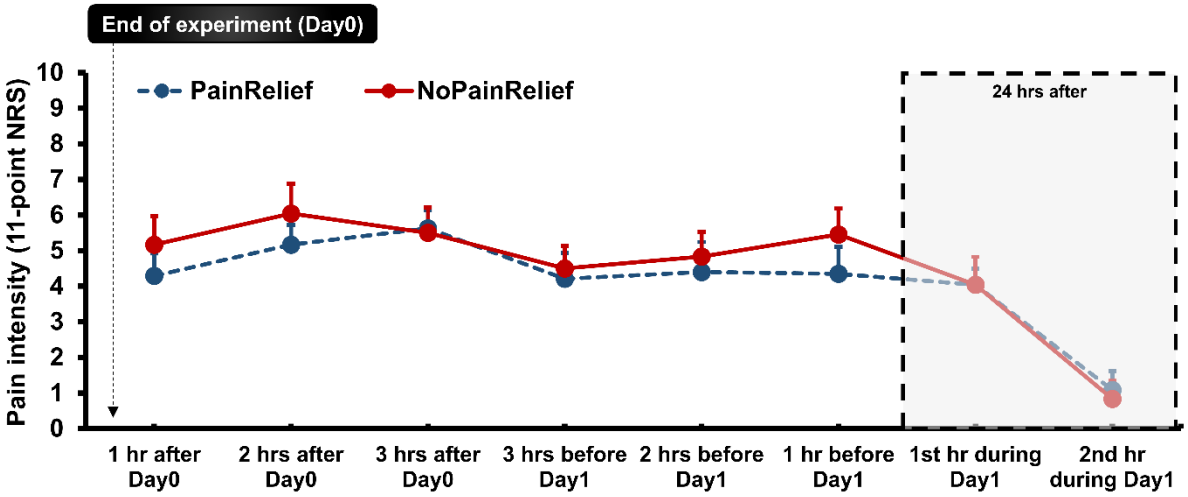

Figure 3. Pain intensity over the two days of testing. Mean and standard error of the mean of pain intensity measured by numerical rating scale (NRS) scores after Day0, and before and during Day1. The capsaicin patch was removed at the end of experiment on Day1.

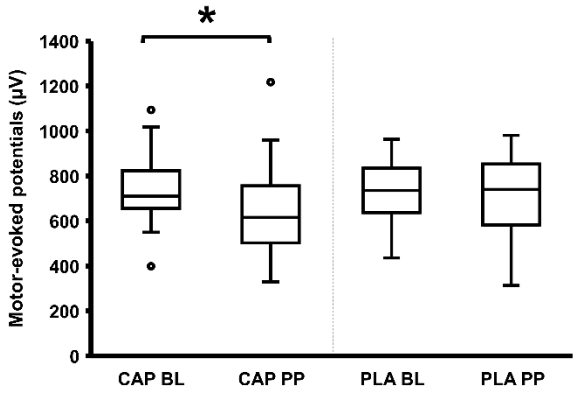

Figure 4. Baseline cortical excitability assessed by motor evoked potentials (MEPs) after capsaicin and placebo patch application, and before homeostatic plasticity (HP) induction. Median MEP amplitudes (μV) and interquartile range (25th and 75th quartile) represented by the box and minimum and maximum values represented by the whiskers. Outliers are shown as dots. The capsaicin patch significantly reduced the MEP amplitude (*, $p<0.005$). CAP BL and CAP PP: Capsaicin baseline and post patch, respectively (pain condition); PLA BL and PLA PP: Placebo baseline and post patch, respectively (control condition).

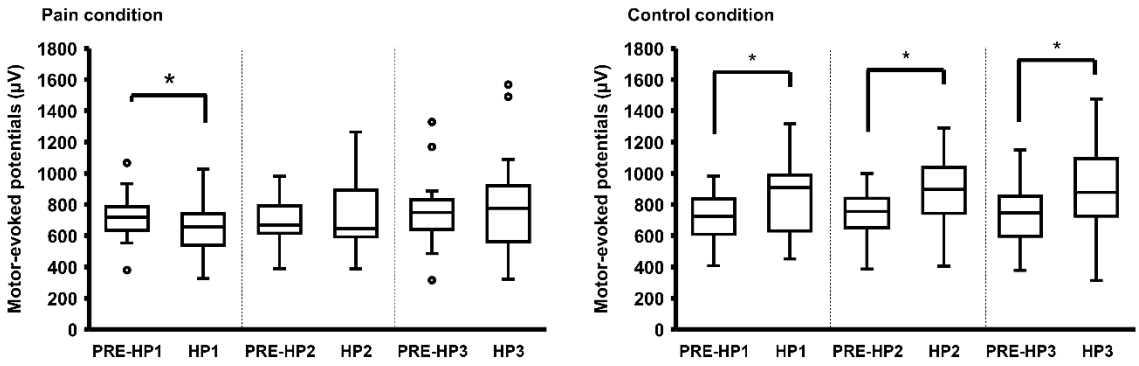

Figure 5. Corticomotor excitability changes after homeostatic plastic (HP) induction.
Median MEP amplitudes (µV) and interquartile range (25th and 75th quartile) represented by the box and minimum and maximum values represented by the whiskers. Outliers are shown as dots. The HP1, HP2, and HP3 MEPs reflect pooled responses at 0-, 15- and 30-min post HP induction. Compared with pre-HP, MEPs were significantly increased in all three HP blocks in the control condition ($p<0.005$). A significant decrease in MEPs was found for HP1 in the pain condition ($p=0.013$).