Corticospinal excitability modulation by pairing peripheral nerve stimulation with cortical states of movement initiation

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Running title: Corticospinal plasticity by pairing nerve stimulation with movements

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Abbreviations: CSE, corticospinal excitability; EEG, electroencephalography; EMG, electromyography; TMS, transcranial magnetic stimulation; M1, motor cortex

Key points (133/150 w):

- We compare the effects on corticospinal excitability of repeatedly delivering peripheral nerve stimulation at three time points (-30ms, 0ms, +50ms) relative to muscle onset in a cue-guided task.
- Plastic changes in excitability are only observed when stimuli are delivered immediately before the time when muscles activate, while stimuli delivered at muscle onset or shortly later (0, +50 ms) have no effect.
- Plastic effects are abolished if there is ongoing volitional EMG activity in the muscles prior to onset of the phasic contraction.
- The plastic effects induced by timing peripheral stimulation relative to electromyographic markers of muscle activation are as effective as those that occur if stimulation is timed relative to electroencephalographic markers of motor cortical activation. We provide a simple alternative protocol to induce plasticity in people in whom EEG recording is difficult.

Abstract (245/250 words limit)

Plastic changes in corticospinal excitability (CSE) and motor function can be induced in a targeted and long-term manner if afferent volleys evoked by peripheral nerve stimulation are repeatedly associated with the peak of premovement brain activity assessed with electroencephalography (EEG). Here we ask whether other factors might also characterise this optimal brain state for plasticity induction. In healthy human volunteers (N=24) we find that the same reliable changes in CSE can be induced by timing peripheral afferent stimulation relative to the electromyography (EMG) onset rather than using the EEG peak. Specifically, we observed an increase in CSE when peripheral stimulation activated the cortex just before movement initiation. By contrast, there was no effect on CSE if the afferent input reached the cortex at the same time or after EMG onset, consistent with the idea that the temporal order of synaptic activation from afferent input and voluntary movement is important for production of plasticity. Finally, in 14 volunteers we found that background voluntary muscle activity prior to movement also abolished the effect on CSE. One possible explanation is that the intervention strengthens synapses that are inactive at rest, but change their activity in anticipation of movement, and that the intervention fails when the synapses are tonically active during background EMG activity. Overall, we demonstrate that, in individuals with voluntary control

of muscles targeted by our intervention, EMG signals are a suitable alternative to EEG to induce plasticity by coupling movement-related brain states with peripheral afferent input.

Introduction

Several reports have suggested that long-term changes in corticospinal excitability (CSE) can be produced by repeatedly coupling movement-related brain states and peripheral afferent stimulation (Mrachacz-Kersting *et al.*, 2012; Kraus *et al.*, 2015, 2018). It has been proposed that movement-related peripheral stimulation (MRPS) can achieve a more selective action on targeted neural structures and produce more functionally relevant behavioural effects (Mrachacz-Kersting *et al.*, 2012, 2019) when compared to standard plasticity protocols. However, there remain theoretical and practical issues associated with defining the optimal brain states targeted by MRPS interventions, which potentially limit their efficacy and application in research and clinical settings.

Previous research suggested that the optimal brain state for MRPS interventions occurs at the time of the peak negativity of the contingent negative variation (PN_{CNV}), a cortical marker of M1 activation that is present at around the onset time of cue-guided movements (Xu *et al.*, 2014; Mrachacz-Kersting *et al.*, 2019). There is no effect if the afferent input from peripheral stimulation is timed to arrive about 200 ms before or after $PN_{CNV.}$ However this still leaves a relatively broad range of timings to define the optimal brain state for MRPS considering the fast brain dynamics during the early phases of movement generation (Hannah *et al.*, 2018; Lara *et al.*, 2018). There are also practical difficulties with using the PN_{CNV} since it can be small and variable (within and across subjects), particularly in patients who may have brain lesions (Tecce, 1971; Fang *et al.*, 2007; Ibáñez *et al.*, 2014*b*; Mrachacz-Kersting *et al.*, 2016). The present work examined whether there are other ways to identify the optimal brain state for MRPS and define its important characteristics.

In visually-cued movement tasks that have been used in previous studies, the PN_{CNV} usually occurs just prior to electromyographic (EMG) onset (Jochumsen *et al.*, 2015; Martínez-Expósito *et al.*, 2017). We therefore explored whether it was possible to define the timing of MRPS relative to EMG onset rather than PN_{CNV}. In fact, EMG markers have been used previously in a study looking at the after-effects of repeatedly delivering transcranial magnetic stimulation (TMS) over M1 at different times relative to EMG onset (Thabit *et al.*, 2010). This showed that TMS given just before movement (50 ms before movement onset) increased M1 excitability, while other stimulus times either had no effect (TMS given 100ms before or 50ms after movement onsets) or decreased M1 excitability (TMS given 100ms after the onsets). These experiments show that EMG signals can be used to define the optimal time windows for stimulation in TMS-based movement-related stimulation interventions. However, it remains unclear if EMG-referenced triggers are equally effective in MRPS protocols that

use peripheral afferent stimulation. Additionally, there has been no direct comparison of the effectiveness of electroencephalography- (EEG) and EMG-defined triggers in MRPS interventions.

In the first experiment we used a cue-guided movement paradigm and measured the changes in CSE induced by repeatedly delivering peripheral nerve stimulation (PNS) at different times relative to the onset of EMG activity in the target muscle. Moreover, by concurrently recording EEG, we also compared CSE changes obtained when PNS was timed relative to EMG onset or to the PN_{CNV}. We found that there were clear after-effects on CSE when PNS was timed to occur 30 ms before average EMG onset, but there was no effect if PNS was timed to coincide with EMG onset, suggesting that the optimal brain state for MRPS interventions occurs prior to discharge of corticospinal neurons. A second experiment allowed us to probe the role of ongoing corticospinal activity by showing that the after-effect of MRPS was no longer present if there was tonic EMG activity in the targeted muscle prior to movement onset.

Materials and methods

Ethical approval

This study was approved by the University College London Ethics Committee (Ethics Application 10037/001) and warranted to be in accordance with the Declaration of Helsinki, except for registration in a database. All participants recruited gave written informed consent before experiments took place.

Participants

A total of 53 participants (23male; 30 females; aged 20-40 years, mean ± SD: 28 ± 6; right handed) were recruited for the experiments in this study. None of the participants had a history of neurological or psychiatric disorders or was under any drug treatment.

Recordings and stimulation

Throughout the experiments, participants were seated comfortably in an armchair. EMG activity was recorded from the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles of the right hand, using surface electrodes (19mm Whitesensor AMBU A/S, Denmark). EMG signals were amplified 1000×, bandpass filtered (20-2kHz; D360 amplifier, Digitimer, UK) and stored at 5 kHz sampling rate (CED Power 1401 acquisition board, Cambridge Electronic Design, UK).

TMS was used to assess changes in CSE. We used a Magstim 200 stimulator (Magstim, Carmarthenshire, UK) and a 70-mm figure-of-eight coil held tangentially on the top of the scalp over

M1 and with its handle forming a 45-degree angle with the sagittal plane of the brain. The motor hotspot was defined as the TMS coil location eliciting the largest and most stable motor evoked potentials (MEPs) in the right FDI muscle. MEPs were assessed to estimate CSE changes resulting from the tested interventions. At the beginning of each experiment, the resting motor threshold (RMT) of the relaxed FDI was obtained. The RMT was defined as the minimum TMS intensity that elicited MEPs with peak to peak amplitudes greater than 50 uV in at least 5 out of 10 consecutive pulses (Rothwell *et al.*, 1999; Rossini *et al.*, 2015). In all the interventions, TMS pulses were applied at 120% and 140% RMT to measure changes in MEPs.

During the interventions, PNS was delivered using a constant-current mono-phasic controller isolated stimulator (DS7A, Digitimer, UK). Surface electrodes were placed over the right ulnar nerve at the wrist, with the cathode proximal to avoid the risk of anodal block (Bertasi *et al.*, 2000). Stimulation intensity was set at the minimum level inducing small index finger twitches (Brown *et al.*, 2016). Pulse width was set to 0.2 ms.

In 20 participants taking part in experiment 1, EEG signals were recorded from nine scalp locations using Ag-AgCl scalp electrodes according to the International 10-10 system: F3, F4, C1, C2, P3, P4, Fz, Cz and Pz (WaveGuard 64-channel Cap and a TMSi Refa amplifier were used; Oldenzaal, Netherlands). The reference was set to the common mastoids voltage and the ground electrode was AFz. The recording was performed in DC mode, with 2048 Hz sampling rate.

Movement task

A cue-guided paradigm with predictable movement times was used to pair PNS with movement-related brain states in order to make our results comparable to previous similar studies (Mrachacz-Kersting *et al.*, 2012). Each trial of the paradigm consisted of four stages: rest, pre-movement, movement and feedback. Trials started with the presentation of the word “REST” on the screen for 2 s. Then, four circles at each of the four edges of a cross appeared and started moving towards the intersection point with a velocity inversely proportional to the distance to the centre of the cross (distance 4.5cm), (Fig.1A). Participants were instructed to press a keypad button with their right index finger at the time when the four circles overlapped at the centre of the cross (“GO” time). After this, and in a certain number of trials only (see below), the task performance (*i.e.*, the time interval between the “GO” time and the button press time) was displayed for a random period of time ranging between 1.5-2.5 s. This feedback was intended to motivate participants to perform movements in a consistent manner at around the “GO” time throughout the intervention phase. The relationship between button press times and feedback was based on preliminary tests with our set

up, showing that there is an average interval of 80-90 ms between EMG onsets and button press events. The messages “TOO SOON” and “TIMEOUT” were given when participants pressed the button before the “GO” time or more than 150 ms after it, respectively. The message “GOOD!” was presented when button presses were performed within the 50-100 ms interval after the “GO” time. Finally, “Ok” was shown in any other case.

Experimental Paradigm

The structure of the experimental sessions is illustrated in Fig. 1B. On average, each of them took about 70 min. Recording blocks (PRE, POST0 and POST15) indicate the times at which CSE was probed with TMS before and after the intervention. Thirty MEP for each intensity of stimulation (120% and 140% RMT) and block were collected. In every session, after practising the task (20-30 trials) and before the blocks, participants performed 30 additional trials of the movement task to estimate their average movement time based on the EMG (MT_{emg}). EMG onset times were defined as the time when the rectified and smoothed EMG (using a sliding window of 5 ms) exceeded five times the EMG at rest (*i.e.*, during the resting periods at the beginning of the trials).

Three sets of experiments were performed, each using different participants. In all experiments and sessions, participants underwent a task-related peripheral nerve stimulation (TRPNS) intervention. The common element in all interventions was that participants performed the movement task for 120 trials (two blocks of 60 trials each) and, in 90 % of the trials, PNS was delivered at specific points relative to movement initiation. In these trials, feedback about the button press times was not given to the participants to avoid compensatory delays in response times (Agarwal & Gottlieb, 1972; Ziemann *et al.*, 1997). The remaining 10% of trials were non-stimulation trials providing feedback of the button press times as described in the previous section.

Experiment 1: 24 participants (14 females) took part in three sessions, each involving a different type of intervention: $TRPNS_{-30}$, $TRPNS_0$ and $TRPNS_{+50}$. Different sessions were run on different days (separated by at least 2 days). The order of the intervention sessions was randomized in each participant. In $TRPNS_{-30}$, PNS was programmed to be delivered 30 ms before MT_{emg}. This level of anticipation of stimuli relative to MT_{emg} was used to ensure that, in most trials, stimuli were delivered in close proximity to movements, i.e. approximately right before the time when motor commands were transmitted through the corticospinal tract, but in the absence of voluntary muscle. In $TRPNS_0$ and $TRPNS_{+50}$ stimulation was delivered at the online estimated EMG onset time ($TRPNS_0$) or 50 ms after it ($TRPNS_{+50}$). Therefore, in these two cases, instead of using $MT_{emg,}$ EMG onsets were estimated online using the same approach as the one described above for MT_{emg}. In the three

intervention sessions, FDI and ADM were at rest during the intervals preceding the movements (Exp. 1 panel in Fig.1C).

Experiments 2 and 3: $TRPNS_{-30}$ in experiment 1 differs from $TRPNS_0$ and $TRPNS_{+50}$ in three factors: 1) PNS is triggered at fixed times relative to the visual cue (in $TRPNS_0$/$TRPNS_{+50}$ PNS is triggered based on the EMG); 2) in most trials, PNS is delivered when FDI and ADM are relaxed and 3) PNS is triggered during the movement initiation phase (before the motor command is sent through the corticospinal tract).

Experiment 2 was designed to test the possibility that differences in the outcomes obtained in $TRPNS_{-30}$ and $TRPNS_0$/$TRPNS_{+50}$ were due to factor 1. Fifteen participants (11 females) took part in a one-session intervention, $TRPNS_{+30}$, which was similar to $TRPNS_{-30}$ in that PNS was delivered at a constant time relative to the "GO" (unlike in $TRPNS_0$ and $TRPNS_{+50}$). However, this time PNS was given 30 ms after the MT_{emg} to ensure PNS was delivered during muscle contraction (Exp. 2 panel in Fig.1C).

Experiment 3 was designed to test the possibility that differences between $TRPNS_{-30}$ and $TRPNS_0$/$TRPNS_{+50}$ were due to factor 2 or 3. Fourteen participants (6 females) took part in a one-session intervention, $TRPNS_{-30ACTIVE}$, that was like $TRPNS_{-30}$ but, in this case, participants were instructed to produce mild contractions (~200 mV peak-to-peak) with the FDI muscle in the intervals preceding the "GO" time (Exp. 3 panel in Fig.1C).

Data Analysis

To assess CSE changes, only MEPs from PRE, POST0 and POST15 blocks with amplitudes higher than 50 uV peak-to-peak and with pre-stimulus (200 ms windows before the TMS) peak-to-peak activity below 50 uV were considered. Overall, 2.0 ± 3, 4.6 ± 5.0 and 2.4 ± 3.7 % of the recorded MEPs were discarded in experiments 1, 2 and 3 respectively. We then excluded participants showing average MEP amplitudes in block "PRE" (before the intervention took place) that were more than two standard deviations higher or lower than the group mean MEPs in each experiment. The reasoning behind this method was to avoid that our readout (MEP amplitude) would not be informative of plasticity induction due to floor or ceiling effects. One participant from experiment 1 was removed from the analysis using this criterion.

To know if timing of stimulation relative to the PN_{CNV} was an important factor determining the excitability changes induced in our intervention, we used the data acquired in experiment 1 to generate an additional "virtual" intervention ($TRPNS_{CNV}$) by selecting, for each participant, the

intervention in which PNS was timed to reach cortical areas at the closest possible time to PN_{CNV}. This intervention was intended to replicate the methodology used in previous studies suggesting a pivotal role of the PN_{CNV} in the induction of plastic effects (Mrachacz-Kersting *et al.*, 2012)). To do this, EEG from all three sessions in experiment 1 performed by each participant were analysed to estimate the average time of the peak of the CNV. The continuous EEG data were re-referenced to the average potential in F3, Fz, F4, P3, Pz, P4 and down-sampled to 128 Hz. Then, EEG signals were low pass filtered (< 5 Hz, 2nd-order Butterworth filter) and high pass filtered (> 0.5 Hz, 1st-order Butterworth filter) to remove high frequency components and DC drifts (Ibáñez *et al.*, 2014*a*; Olsen *et al.*, 2018). Data from each trial were time-locked to the end of the "GO" time and divided into 4-s epochs (from -3 s to +1 s with respect to the "GO" time). Epochs containing artefacts (due to eye blinks, muscle activity, etc.) were rejected following visual inspection. A grand average of the resulting epochs was computed for channels C1, Cz and C2. Then, the largest negative peak of the CNV waveform within a 100ms window around the "GO" time (50 ms on either side) was identified as the CNV peak. We then compared the amplitudes of the estimated peaks in C1, Cz and C2 and selected the electrode with the largest amplitude. The timing of the peak negativity of the CNV was finally estimated with respect to the "GO" cue. For each participant, the session in which the PNS had been delivered closest to the CNV peak time was selected to construct a new "virtual" session for the $TRPNS_{CNV}$ intervention (for this selection, we compensated for the ~20 ms conduction time that takes afferent volleys to reach cortical areas (Rushton *et al.*, 1981; Stefan *et al.*, 2000; Brown *et al.*, 2016)). For cases in which no CNV peaks were observed in the analysed 100 ms window, the CNV for that participant and session was considered absent. Participants not showing a CNV peak in at least two of the three recording sessions were not included in the analysis. EEGLAB 14.1.1 and MATLAB R2015b (The MathWorks, Natick, MA) were used to analyse EEG data (Delorme & Makeig, 2004).

Statistical analysis

To probe CSE changes in this study, we used TMS intensities based on RMT levels. This was decided to make results comparable with previous similar studies (Mrachacz-Kersting *et al.*, 2012; Kraus *et al.*, 2018) and it led to variable average MEP amplitudes across participants both for 120 % and 140 % RMT levels. To ensure that the MEP averages were normally distributed, for each intervention session, TMS intensity and muscle, z-scores were used to normalize all valid MEP amplitudes registered.

We used the Kolmogorov-Smirnov test to evaluate normality of the dependent variables in all the statistical tests run. Normality was confirmed for the normalized MEP amplitudes in all three

experiments (z-scores for skewness and kurtosis obtained from the sample residuals of the general linear models were below a critical value of 2). Normality was also confirmed for MT_{emg} ($P > 0.05$), but not for RMT and for the PNS intensities used in experiment 1 ($P < 0.05$).

For Experiment 1, we compared MT_{emg}, RMT, PNS intensities used across the 3 interventions tested. We used repeated measures ANOVA (rmANOVA) for MT_{emg} and Friedman ANOVA for RMT and PNS intensities. Changes in MEP amplitudes across interventions were assessed by means of a 4-way rmANOVA with factors INTENSITY (120%RMT, 140%RMT), INTERVENTION ($TRPNS_{-30}$, $TRPNS_{0}$, $TRPNS_{+50}$), MUSCLE (FDI, ADM), and TIME (PRE, POST0, POST15). Since z-scores were used for MEP normalization, the outcomes of the rmANOVA relative to main effects of factors INTENSITY, INTERVENTION and MUSCLE as well as of the interactions between these factors without including TIME were artificially made non-significant. For this reason, in order to rule out that the initial MEPs in the compared three intervention sessions were different, the absolute (non-normalized) MEPs from blocks PRE in $TRPNS_{-30}$, $TRPNS_{0}$ and $TRPNS_{+50}$ were compared using paired comparisons for each intensity and muscle. Since absolute MEP amplitudes were not normally distributed, Wilcoxon signed rank tests were used for this purpose.

MEP changes in experiments 2 and 3 and in the $TRPNS_{CNV}$ intervention were assessed using normalized MEPs and separate 3-way rmANOVAs with factors INTENSITY, MUSCLE and TIME. Finally, to compare changes induced by $TRPNS_{-30}$ and $TRPNS_{CNV}$ interventions, a paired t-test was used.

In all cases, effects were considered significant when $P<0.05$. Paired t-test comparisons with Bonferroni corrections were used for post-hoc paired comparisons of MEP amplitudes. Statistical tests were done using SPSS25 statistic software (IBM Corp., Armonk, N.Y., USA). Data are presented as mean±SD unless indicated otherwise.

Results

Experiment 1

Table 1 shows the MT_{emg}, RMT and PNS intensities used in $TRPNS_{-30}$, $TRPNS_{0}$ and $TRPNS_{+50}$. There were no significant differences in these variables across interventions ($P>0.1$ in all cases).

To assess whether PNS in the $TRPNS_{-30}$ condition was triggered right before EMG onset, we quantified the percentage of trials in which PNS preceded the onset of EMG resting EMG activity preceded PNS in each participant. PNS was delivered before the EMG onsets in 80±13% of the trials. On the contrary, in $TRPNS_{0}$ and $TRPNS_{+50}$, PNS was always triggered by the presence of EMG activity (stimuli were triggered based on online processing of the EMG).

MEP amplitudes in the FDI increased after $TRPNS_{-30}$ (in POST0 and POST15 average MEP amplitudes are higher than in PRE), but not following the application of $TRPNS_0$ or $TRPNS_{+50}$ (Figs. 2A-C and 3A-C). Average MEP amplitudes in the task-irrelevant ADM did not change after any of the interventions. The statistical tests confirmed this (see Table 2). First, there was a significant main effect of factor TIME ($F_{[2,44]}$=3.721; P=0.032; η^2=0.145). There was also a significant INTERVENTION × MUSCLE × TIME interaction ($F_{[4,88]}$=2.843; P=0.029; η^2=0.114). Post-hoc comparisons revealed significant differences for $TRPNS_{-30}$ in FDI MEP amplitudes between blocks PRE and POST0 (P=0.005) and PRE and POST15 (*P*=0.028). No significant differences were found in the other two interventions (P>0.3 for all paired comparisons between PRE and POST0/POST15 FDI MEPs). Fig. 3 shows individual changes in MEP amplitudes for blocks POST0 and POST15 (the average of both) relative to PRE, for the three compared interventions.

Comparisons between interventions of the absolute MEP amplitudes in the PRE blocks for the two muscles and two TMS intensities tested did not show any significant differences (P>0.2 in all comparisons; see Tables 1 and 4). This result confirms that all three sessions were started from comparable baseline levels of CSE.

Experiment 2

Table 1 summarizes the MT_{emg} estimated from the initial training trials in each $TRPNS_{+30}$ session, together with RMT estimates and PNS intensities. Figs. 2D and 3D show the MEP amplitudes at the different measurement times before and after applying $TRPNS_{+30}$. The rmANOVA did not show any significant main effects or interactions.

Experiment 3

Table 1 summarizes the MT_{emg} estimated from the initial training trials in each $TRPNS_{-30ACTIVE}$ session, together with RMT estimates and PNS intensities. Figs. 2E and 3E show the MEP amplitudes at the different measurement times before and after applying $TRPNS_{-30ACTIVE}$. No significant main effects or interactions were found.

Comparison of effects induced by $TRPNS_{-30}$ and $TRPNS_{CNV}$ interventions

The application of the criteria described in the methods section resulted in us having usable data from 11 participants to analyse the possible plasticity effects induced by the $TRPNS_{CNV}$ intervention (the discarded participants did not show a consistent PN_{CNV} around the "GO" time across the experimental sessions). Examples of the CNV patterns of the participants who presented a consistent peak are shown in Fig. 4. The figure shows that, although the average CNV peaks at the time of the "GO" cue, individual CNV patterns and PN_{CNV} times vary across subjects. The average

time of the PN_{CNV} across subjects was 1.36±30.73ms relative to the "GO" time. A 3-way rmANOVA revealed a significant main effect of factor TIME ($F_{[2,20]}$ =4.620; P=0.022; η^2=0.316) for the $TRPNS_{CNV}$ intervention. This result is in line with previous similar studies testing plastic M1 changes induced by pairing PNS with the PN_{CNV} (Mrachacz-Kersting *et al.*, 2012). Fig. 5 compares individual MEP changes with $TRPNS_{CNV}$ and $TRPNS_{-30}$ across participants. $TRPNS_{CNV}$ led to smaller MEP changes than $TRPNS_{-30}$ in 3 cases, larger in 2 cases and identical (same sessions considered) in the rest of the cases (Fig. 5). A t-test comparison did not reveal a significant difference between the two interventions for the considered sample (P=0.388).

Finally, we tested the correlation between the MEP changes induced by the $TRPNS_{CNV}$ intervention and the intervals (in each participant) between the estimated PN_{CNV} and the PNS time. This was done to assess whether the inter-individual variation in CSE changes was related to the accuracy with which the PNS was timed relative to the PN_{CNV}. CSE changes and the interval between the CNV peak and the PNS time were uncorrelated, irrespective of whether the signs of the PN_{CNV}-PNS intervals were considered (P=0.368) or absolute values were used instead (P=0.637). These results thus suggest that, at least over the range of time intervals explored here, the precise temporal proximity between the times of the PN_{CNV} and PNS does not predict the outcome of our $TRPNS_{CNV}$ intervention.

Discussion

Repeatedly pairing naturally-occurring brain states with precisely timed afferent input is suggested to be more selective and effective in promoting long-term changes in cortical function than interventions that exclusively use artificial stimulation methods such as TMS or transcranial electrical stimulation to elicit artificial brain-states (Liepert *et al.*, 1998; Mrachacz-Kersting *et al.*, 2012). Previous studies have found that one such brain state occurs during the movement initiation period of a visually-cued movement task, at the time of the PN_{CNV}, and that pairing this state with peripheral afferent input produces after-effects on CSE that last 30 min or more (Mrachacz-Kersting *et al.*, 2012, 2016, 2019). The present experiments define additional features of this state. First, we find that its timing can be reliably determined from the time of EMG onset in individuals with normal control of movement, and that this method is equivalent to employing PN_{CNV} in terms of the plasticity induction. Second, the state occurs immediately prior to the discharge of corticospinal neurones involved in the task. Finally, a critical feature of this brain state is that it occurs in the transition from rest to movement, but not from a state of tonic corticospinal activity to movement.

Using EEG vs EMG in movement-related stimulation interventions

The results of the first experiment here show that, if the peripheral nerve stimulus during the plasticity protocol was timed to arrive 30 ms before average EMG onset ($TRPNS_{-30}$), there was an increase in CSE that lasted for at least 15 min after the intervention. There was no effect if the stimulus was given at the onset of EMG activity nor 50 ms after it. By reanalysing the same data, we could also show that the magnitude of the $TRPNS_{-30}$ effect was the same as it would have been if we had timed the stimulus with respect to the PN_{CNV} ($TRPNS_{CNV}$). In fact, this is not so surprising since several previous studies show that, in cue-guided paradigms similar to the one used here, the PN_{CNV} occurs just before the EMG onset (Mrachacz-Kersting *et al.*, 2012; Jochumsen *et al.*, 2015; Martínez-Expósito *et al.*, 2017). In this sense, it has been previously suggested that the motor potential observed in the EEG in planned movements is associated with the point at which CSE starts to raise just before EMG onset (Chen *et al.*, 1998). From a physiological point of view, this suggests that, regardless of whether EMG- or EEG-derived markers of brain states are used, a critical element required to induce plasticity with MRPS interventions may be that afferent brain stimuli reach M1 circuits during movement initiation phase (*i.e.*, tens of ms before EMG onset), when CSE increases sharply (Starr *et al.*, 1988; Chen *et al.*, 1998; Chen & Hallett, 1999; Zaaroor *et al.*, 2003). Indeed, previous studies have proposed that transient periods of increased excitability during which brain responses are destabilized and undergo increased variability may predispose toward cortical remodelling (Kozyrev *et al.*, 2018). From a practical standpoint, the result means that in cases where the CNV is unclear or has a variable peak time, it is still possible to perform MRPS interventions by timing PNS relative to EMG onset. The EMG and CNV methods would therefore seem to complement one another. The former would work well in individuals capable of producing a voluntary muscle activity, whereas the CNV method would be necessary in patients unable to do so.

The results also show that timing peripheral stimuli relative to EMG onset fails to increase CSE if the peripheral afferent input arrives at the onset or during EMG activity ($TRPNS_0$ or $TRPNS_{+30}$). This would be consistent with the idea that the induction of plasticity by the present protocol employs a form of spike-timing dependent plasticity, in which the temporal order of synaptic activation from afferent input and voluntary movement is important for production of plasticity. The same phenomenon is observed with the method of paired associative stimulation (PAS), in which transcranial magnetic stimulation is paired with timed afferent input (Stefan *et al.*, 2000; Weise *et al.*, 2013). If afferent input arrives at sensorimotor cortex prior to the TMS pulse, then repeated pairing increases CSE, whereas the opposite happens if the order of stimulation is reversed. Thabit et al

(2010) employed similar reasoning in their experiments pairing TMS pulses with volitional movement (Thabit *et al.*, 2010).

The results observed here are similar to those of previous PNS-based MRPS interventions that used motor imagery rather than overt movement to induce plasticity (Mrachacz-Kersting *et al.*, 2012). During motor imagery there is no direct activation of corticospinal neurones, implying that the increase in CSE is not caused by an increase in the effectiveness of synaptic inputs to corticospinal neurones, since these are presumably not active during imagery, but at more "upstream" connections. One possible location is at synapses onto the "pre-corticospinal" interneurons. A likely possible mechanism is via changes in the synaptic efficacy of inputs to neurons in layer 2/3. These neurones have excitatory synaptic connections to corticospinal neurones and contribute to late descending I-waves following TMS (Lazzaro *et al.*, 2012; Weise *et al.*, 2013).

Lack of plasticity when there is ongoing EMG activity

There were no changes in CSE after interventions in which there was ongoing background EMG activity in the muscle prior to the phasic movement ($TRPNS_{-30ACTIVE}$). In fact, since overt movements generated from a pre-activated condition are expected to produce movement-related cortical potentials that are similar to those in normal movements initiated from a relaxed state (Terada *et al.*, 1995, 1999), the conclusion is likely to apply equally well in situations where PNS is timed in relation to the PN_{CNV}.

One possible interpretation for this observed difference between $TRPNS_{-30}$ and $TRPNS_{-30ACTIVE}$ is that mild contractions exerted in the latter case lead to sensory attenuation effects dampening the afferent volleys induced by PNS (Rushton *et al.*, 1981). However, sensory attenuation is known to start building up from 80-100 ms before voluntary movements begin (Cohen & Starr, 1987) which is earlier than the volleys used in the $TRPNS_{-30}$ protocol that produced the most significant effects. Thus, sensory attenuation seems unlikely to be an important contributing factor. An alternative possibility is that $TRPNS_{-30}$ strengthens synapses that are inactive at rest, and that the intervention fails when the synapses are tonically active during background EMG activity (Brown *et al.*, 2016). This could cancel out the expected LTP-like STDP effect induced by the intervention when muscles are activated during movement preparation stages.

Limitations

Previous work using stimulation of lower-limb nerves in stroke patients suggests that these interventions can have important clinical benefits (Mrachacz-Kersting *et al.*, 2016, 2019), and thus the results here may be of relevance to therapeutic interventions. Since our primary outcome

measure was CSE, further research will need to be done to evaluate the functional impact of our proposed MRPS intervention on movement. Previous studies characterizing the effects induced by PNS- and TMS-based associative interventions have shown that MEP changes are mostly explained by cortical changes, with spinally-mediated reflexes showing little to no changes (Stefan *et al.*, 2000; Thabit *et al.*, 2010; Mrachacz-Kersting *et al.*, 2012). We therefore assume that the changes in CSE here are also mostly cortically-driven, though we cannot rule out a potential subcortical contribution. Finally, since EEG and EMG signals can be severely altered in patients with neural damage affecting motor functions (Daly *et al.*, 2006; Fang *et al.*, 2007; Ibáñez *et al.*, 2014*a*), future research should also be dedicated towards further defining the advantages and limitations of using EMG and EEG signals to drive MRPS interventions in different populations of patients.

Conclusions

PNS paired with movement initiation states induces task-related long-term increases in CSE. For these effects to be induced, peripheral stimuli need to be given repeatedly while muscles are at rest and immediately before muscles activate to produce movements. We propose that the induced effects using PNS paired with motor processing may be associated with changes in synaptic efficacy affecting the activity of interneurons stimulating corticospinal cells.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

The experiments were performed in the laboratories of the Department of Clinical and Movement Neurosciences, University College London (UK).

Conception and design of the experiments: RH, JCR, JI; acquisition, analysis and interpretation of data: LF, LR, GX, JCR, JI; drafting the article or revising it critically for important intellectual content: LF, LR, RH, GX, JCR, JI.

All authors approved the final version for publication, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Table 1.- MT_{emg}, RMT, PNS intensities and average pre-intervention MEP amplitudes for each experimental session (mean ± SEM)

Intervention	MT_{emg} (ms)	RMT (%MSO)	PNS (mA)	PRE MEP 120%RMT (mV)		PRE MEP 140%RMT (mV)	
				FDI	ADM	FDI	ADM
$TRPNS_{-30}$	-5.9 ± 4.9	53.4 ± 1.9	10.9 ± 0.8	0.88 ± 0.13	0.42 ± 0.07	1.71 ± 0.22	1.05 ± 0.14
$TRPNS_{0}$	-6.5 ± 4.8	53.8 ± 1.8	10.7 ± 0.7	0.91 ± 0.12	0.51 ± 0.09	1.65 ± 0.21	1.16 ± 0.18
$TRPNS_{+50}$	-0.4 ± 5.4	52.6 ± 1.9	11.6 ± 0.8	0.86 ± 0.12	0.45 ± 0.07	1.63 ± 0.19	0.99 ± 0.12
$TRPNS_{+30}$	-12.6 ± 7.3	45.6 ± 1.9	10.1 ± 0.8	0.94 ± 0.10	0.48 ± 0.10	1.92 ± 0.25	1.39 ± 0.24
$TRPNS_{-30ACTIVE}$	0.3 ± 7.6	48.5 ± 2.7	9.4 ± 0.6	0.82 ± 0.12	0.50 ± 0.09	1.54 ± 0.22	1.16 ± 0.19

Table 2.- Results of the rmANOVAs evaluating the effects of the interventions tested in experiments 1, 2 and 3 on the normalized MEP amplitudes. Significant main effects and interactions are indicated using bold fonts.

		$F_{[DF,error]}$	P	η2
Experiment 1	TIME	$3.721_{[2,44]}$	**0.032**	0.145
	INTENSITY×TIME	$0.359_{[2.44]}$	0.700	0.016
	INTERVENTION×TIME	$1.870_{[4,88]}$	0.123	0.078
	MUSCLE×TIME	$0.103_{[2,44]}$	0.902	0.005
	INTENSITY×INTERV.×TIME	$0.699_{[4,88]}$	0.595	0.031
	INTENSITY×MUSCLE×TIME	$0.245_{[2.44]}$	0.784	0.011
	INTERVENTION×MUSCLE×TIME	$2.843_{[4,88]}$	**0.029**	0.144
	INTENS.×INTERV.×MUSCLE×TIME	$0.568_{[4.88]}$	0.687	0.025
Experiment 2	TIME	$2.401_{[2,28]}$	0.109	0.146
	INTENSITY×TIME	$1.082_{[2,28]}$	0.353	0.072
	MUSCLE×TIME	$0.334_{[2,28]}$	0.719	0.023
	INTENSITY×MUSCLE×TIME	$1.591_{[2,28]}$	0.222	0.102
Experiment 3	TIME	$0.096_{[2,26]}$	0.909	0.007
	INTENSITY×TIME	$0.804_{[2,26]}$	0.458	0.058
	MUSCLE×TIME	$0.939_{[2,26]}$	0.404	0.067
	INTENSITY×MUSCLE×TIME	$0.158_{[2,26]}$	0.855	0.012

Table 3.- P values of paired comparisons between MEP amplitudes measured in each intervention and muscle at times PRE, POST and POST15 for TMS intensities of 120% of the estimated RMT. Bonferroni corrections are applied to the P values.

Intervention	Muscle	Time	
		PRE vs POST0	PRE vs POST15
$TRPNS_{-30}$	FDI	**0.005**	**0.028**
	ADM	0.633	0.059
$TRPNS_{0}$	FDI	1.000	0.747
	ADM	1.000	0.953
$TRPNS_{+50}$	FDI	1.000	1.000
	ADM	1.000	1.000
$TRPNS_{+30}$	FDI	0.347	1.000
	ADM	1.000	0.845
$TRPNS_{-30ACTIVE}$	FDI	1.000	1.000
	ADM	1.000	1.000

Table 4.- P values from the paired comparisons (Wilcoxon signed rank tests) between MEP amplitudes measured in the PRE blocks in each intervention for each muscle and intensity. These control comparisons served to reject the possibility that MEPs collected in each session were significantly different.

	$TRPNS_{-30}$ vs $TRPNS_{0}$	$TRPNS_{-30}$ vs $TRPNS_{+50}$	$TRPNS_{0}$ vs $TRPNS_{+50}$
FDI 120%RMT	0.447	0.927	0.738
ADM 120%RMT	0.346	0.605	0.715
FDI 140%RMT	0.879	0.879	0.831
ADM 140%RMT	0.316	0.346	0.287

Fig. 1.- Summary of the interventions tested. A) Schematic representation of the movement task performed by participants. B) Structure of a session consisting of 120 pairs of movements and PNS. CSE was measured using single-pulse TMS before, immediately and 15 min after the end of the interventions (left). C) Examples of the FDI EMG traces in each of the interventions tested in experiment 1 (left plots), and in experiments 2 and 3 (right plots).

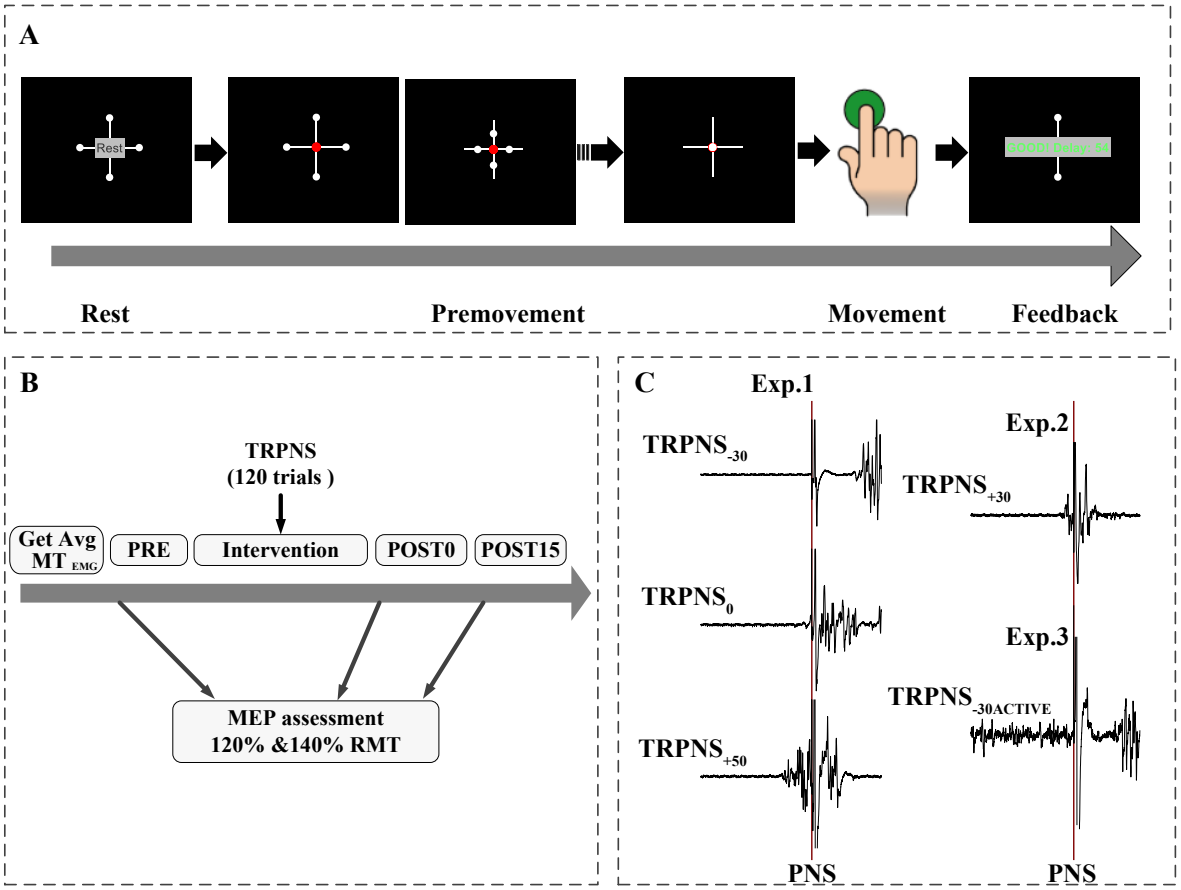

Fig. 2. MEP amplitudes before (PRE) and after (POST0 and POST15) the interventions (results for TMS intensities of 120% RMT). Top row: interventions in experiment 1 - $TRPNS_{-30}$ (A), $TRPNS_{0}$ (B), $TRPNS_{+50}$ (C); Bottom row: $TRPNS_{+30}$ (experiment 2; D) and $TRPNS_{-30ACTIVE}$ (experiment 3; E). (*$P < 0.05$, ** $P < 0.01$).

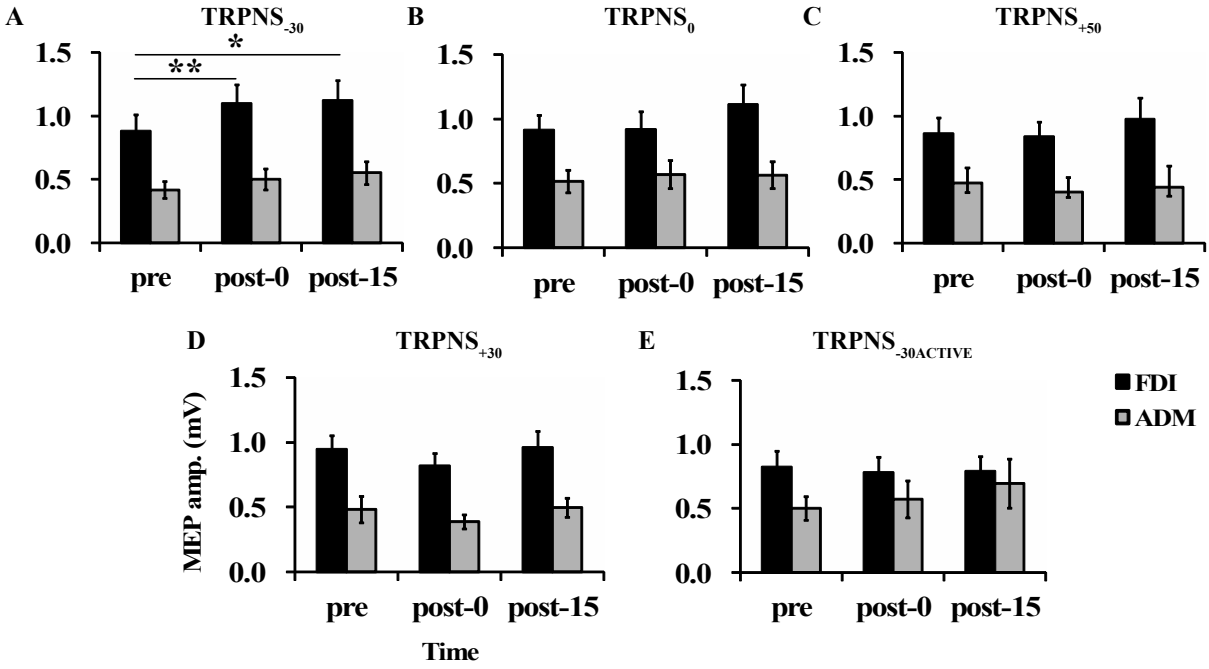

Fig. 3. Normalized MEP amplitudes before (PRE) and after (POST0 and POST15) the interventions (results for TMS intensities of 120% RMT). Top row: interventions in experiment 1 - $TRPNS_{-30}$ (A), $TRPNS_{0}$ (B), $TRPNS_{+50}$ (C); Bottom row: $TRPNS_{+30}$ (experiment 2; D) and $TRPNS_{-30ACTIVE}$ (experiment 3; E). (*$P < 0.05$, ** $P < 0.01$).

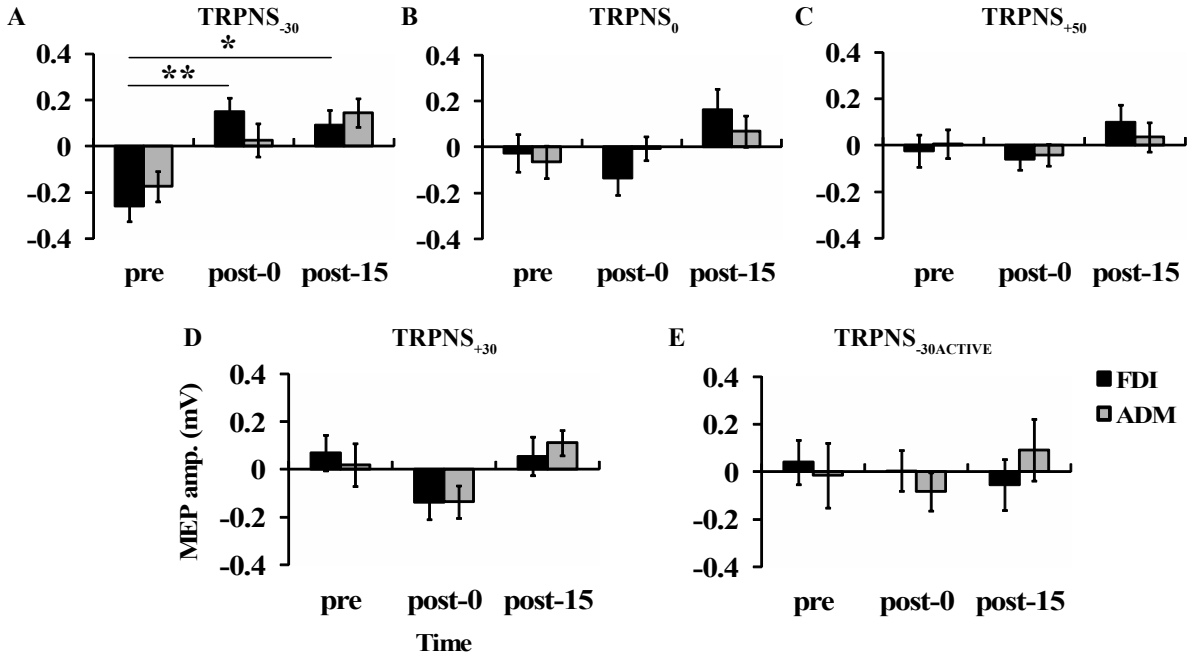

Fig. 4. Individual MEP changes in all three interventions tested in Experiment 1 (TRPNS-30, TRPNS0 and $TRPNS_{+50}$). Points represent individual average MEP amplitudes for blocks POST0 and POST15 relative to PRE for 120% RMT TMS intensities.

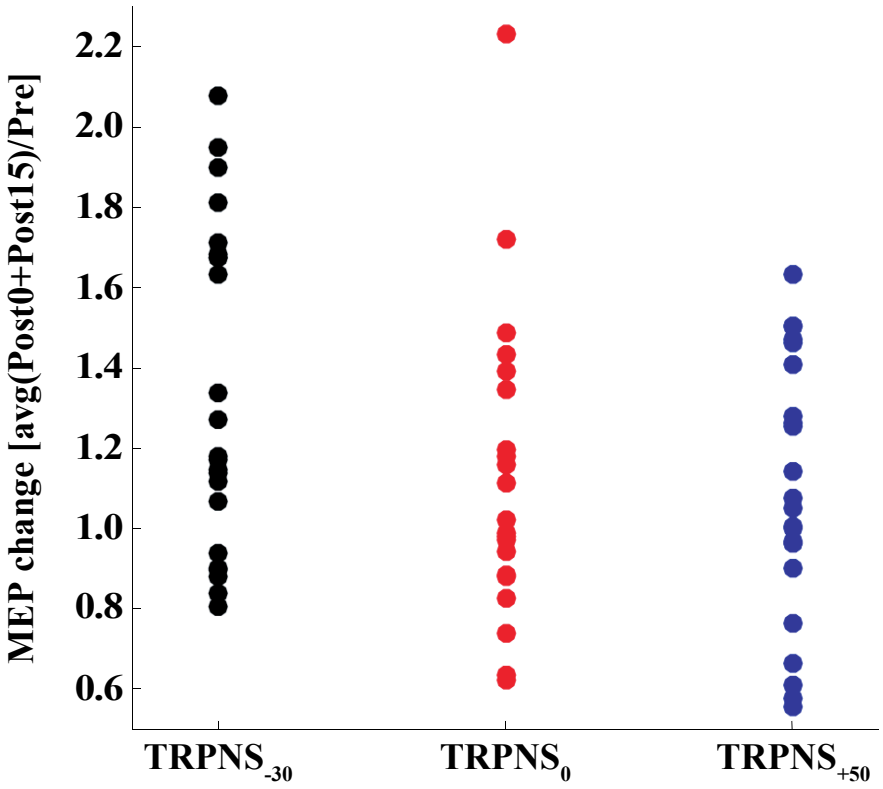

Fig. 5. Individual CNV patterns of all individuals (dashed lines) used to generate the $TRPNS_{CNV}$ intervention. The average CNV is plotted with a solid line. Although on average a clear CNV pattern peaking at the time to move is observed, individual CNV patterns and PN_{CNV} times vary across subjects.

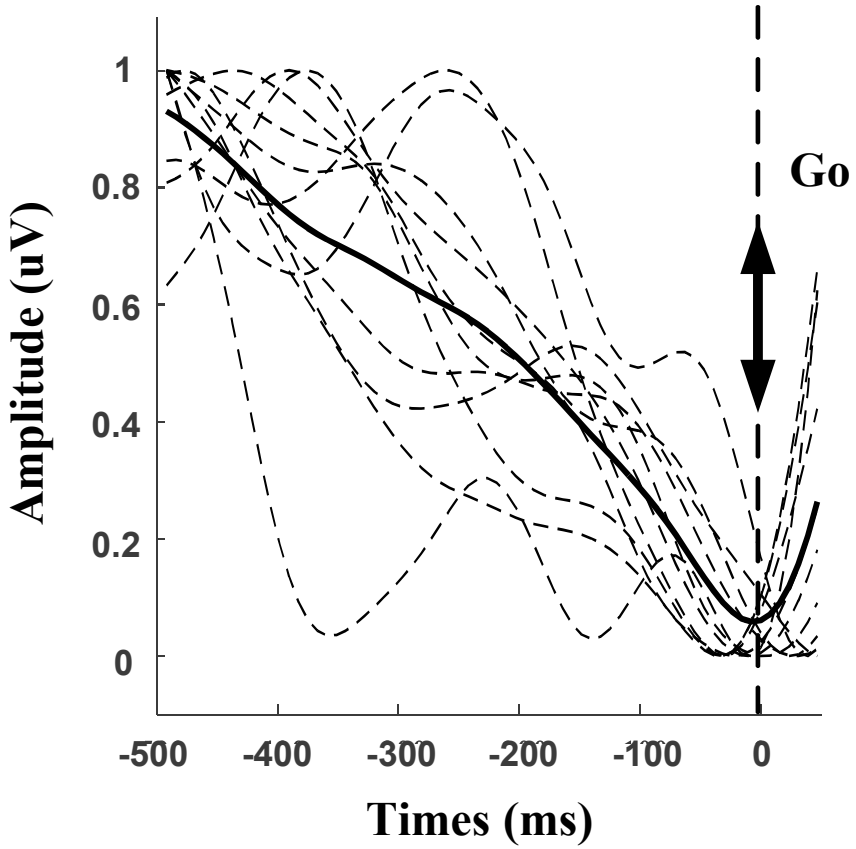

Fig. 6. CSE changes induced by the $TRPNS_{CNV}$ and $TRPNS_{-30}$ interventions for 120% RMT TMS intensities (left panel). Changes in MEP amplitudes with $TRPNS_{CNV}$ as a function of the interval between the PN_{CNV} peak and the PNS time (right panel).