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Homeostatic metaplasticity induced by the combination of two inhibitory brain stimulation techniques: Continuous theta burst and transcranial static magnetic stimulation

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ABSTRACT

Aftereffects of non-invasive brain stimulation techniques may be brain state-dependent. Either continuous theta-burst stimulation (cTBS) as transcranial static magnetic field stimulation (tSMS) reduce cortical excitability.

Our objective was to explore the aftereffects of tSMS on a M1 previously stimulated with cTBS.

The interaction effect of two inhibitory protocols on cortical excitability was tested on healthy volunteers (n = 20), in two different sessions. A first application cTBS was followed by real-tSMS in one session, or sham-tSMS in the other session.

When intracortical inhibition was tested with paired-pulse transcranial magnetic stimulation, LICI (ie., long intracortical inhibition) increased, although the unconditioned motor-evoked potential (MEP) remained stable. These effects were observed in the whole sample of participants regardless of the type of static magnetic field stimulation (real or sham) applied after cTBS.

Subsequently, we defined a group of good-responders to cTBS (n = 9) on whom the unconditioned MEP amplitude reduced after cTBS and found that application of real-tSMS (subsequent to cTBS) increased the unconditioned MEP. This MEP increase was not found when sham-tSMS followed cTBS. The interaction of tSMS with cTBS seems not to take place at inhibitory cortical interneurons tested by LICI, since LICI was not differently affected after real and sham tSMS.

Our results indicate the existence of a process of homeostatic plasticity when tSMS is applied after cTBS. This work suggests that tSMS aftereffects arise at the synaptic level and supports further investigation into tSMS as a useful tool to restore pathological conditions with altered cortical excitability.

Introduction

Understanding the effects of non-invasive brain stimulation (NIBS) techniques on corticospinal excitability (CSE) is essential from a basic and clinical point of view. In this sense, it is typical to group the most common NIBS techniques into those that produce an increase in excitability, such as high frequency repetitive transcranial magnetic stimulation (rTMS), intermittent theta burst stimulation (iTBS), or anodal transcranial direct current stimulation (a-tDCS), and others with opposite effects, such as low frequency rTMS, continuous TBS, or cathodal tDCS (Rossini et al., 2015; Antal et al., 2017). However nowadays, it seems evident that this classification is too simplistic, and it becomes clear that NIBS techniques aftereffects vary depending on various factors, including stimulation intensity and duration or the age of the participants. For instance, application of c-tDCS might result in increased cortical excitability, and it seems to change along the lifespan (Moliadze et al., 2018).

The same is applicable to new NIBS techniques that, currently, are not so well known. For instance, transcranial static magnetic field stimulation (tSMS) has been shown to reduce cortical excitability either in human or animal models (Oliviero et al., 2011; Aguila et al., 2016), being able to affect excitability at remote but functionally connected regions with the stimulated spot (Takamatsu et al., 2021; Caballero-Insaurriaga et al., 2023), as well as modifying human behaviour (Nojima et al., 2019; Pineda-Pardo et al., 2019; Vila-Villar et al., 2022). Interestingly, while 10 min of tSMS on the M1 reduces cortical

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excitability in humans (Oliviero et al., 2011; Paulus, 2011) by increasing short intracortical inhibition (SICI) and reducing short intracortical facilitation (SICF), the effect is reversed when the stimulation period is augmented to 30 min (SICI is reduced and SICF increases) (Dileone et al., 2018). The net effect on CSE, however, remains the same in both cases: CSE is reduced (Dileone et al., 2018). Notably, despite the various exposure times of tSMS investigated over the primary motor cortex and documented in the literature, there is a consistent observation: motor evoked potentials (MEP) were never facilitated in the reported studies (Oliviero et al., 2011; Paulus, 2011; Silbert et al., 2013; Oliviero et al., 2014; Nojima et al., 2015; Kirimoto et al., 2016; Arias et al., 2017; Dileone et al., 2018).

On the other hand, the results of NIBS techniques vary from subject to subject considerably (Muller-Dahlhaus et al., 2008; Wiethoff et al., 2014; Guerra et al., 2020), and the source of such variability is not well understood, which represents a limitation for their potential application in a clinical routine (Hinder et al., 2014). For instance, nearly 50 % of subjects undertaking an inhibitory protocol (like cTBS) display a paradoxical response, and CSE is increased (Heidegger et al., 2017). A factor to take into account when considering the effects of NIBS techniques is that they might depend on brain state (Talelli et al., 2007; Zrenner et al., 2018). For example, it has been shown that the effect of rTMS on cortico-spinal excitability depends on the different phases of the endogenous μ-rhythm (Zrenner et al., 2018). Furthermore, the effect of a given NIBS techniques may change as a function of what has happened before. In other words, it depends on the history of brain activity. The grounds of this phenomenon have their roots back in the early eighties with the formulation of a theoretical framework that allows an adjustment of the threshold for synaptic modification according to the previous history of postsynaptic neuronal activity (Bienenstock et al., 1982). This mechanism has been shown to influence the induction of LTP and hence to regulate synaptic plasticity (Huang et al., 1992).

Hence, metaplasticity, defined as the activity-dependent modulation of synaptic plasticity (Cantone et al., 2021), involves the alteration of plasticity induction concerning its duration, direction, and magnitude, contingent upon the preceding activity of the same postsynaptic neuron or neuronal network (Abraham and Bear, 1996; Abraham, 2008; Müller-Dahlhaus and Ziemann, 2015). The implications of metaplasticity extend significantly within the context of NIBS techniques.

The application of different protocols can lead to varying outcomes, influenced by both prior interventions (Abraham and Bear, 1996; Abraham, 2008) and recent activity in the stimulated area. Metaplasticity involves homeostatic mechanisms, where thresholds for increasing neuronal excitability rise with high previous activity or reduce with low previous activity (Bienenstock et al., 1982) and non-homeostatic plasticity (which permits the threshold for neuronal excitability to be lowered when the neuronal activity is high (Ziemann and Siebner, 2008), or to be raised when neuronal activity is low). These phenomena are likely at play in NIBS priming, such as preconditioning the motor cortex with tDCS, influencing the direction of after-effects evoked with rTMS (Lang et al., 2004; Siebner et al., 2004).

This paper extends our investigation by examining the combined effects of two inhibitory techniques, specifically tSMS in conjunction with cTBS. We anticipate that following the reduction of M1 excitability with cTBS, tSMS will counteract this effect, possibly through homeostatic mechanisms (Turrigiano and Nelson, 2004), leading to an increase in cortico-spinal excitability beyond baseline levels.

Experimental procedures

The protocols described herein adhere to the Declaration of Helsinki. Participants were informed about the nature of the work and signed consent forms for their participation. The protocol was part of a project approved by our local Ethics Committee.

Participants

Twenty healthy participants were enrolled (age range 18–43 years; 10 women). None had taken drugs in the week prior to the two testing sessions. Subjects were screened for incompatibility with brain stimulation protocols.

Protocol

Participants made two visits to our lab, with the objective of assessing the combined impact of two inhibitory NIBS techniques on both cortico-cortical and cortico-spinal excitability. A minimum inter-session interval of ten days was maintained. In each session, we initially assessed corticospinal and cortical excitability using paired-pulse TMS on M1. Subsequently, real cTBS was administered in both visits, and cortical excitability was immediately re-evaluated post-cTBS, providing insight into the initial individual response to cTBS. Following this, tSMS was applied to M1 in one visit (real) and sham in the other, with the order counterbalanced among participants. Excitability was re-tested at the conclusion of tSMS over several minutes (see Fig. 1).

Testing cortico-spinal and intracortical excitability

Excitability was tested using the *long intracortical inhibition* (LICI) protocol. Supra motor threshold monophasic paired-pulses of the same intensity were delivered over the right M1 by means of a MagPro X100 stimulator with the MagOption. The coil (MC-B70, figure of eight) was oriented to induce currents in a postero-anterior (PA) direction in the brain, and its position was marked with a soft-pen over the scalp. TMS intensity was set to induce unconditioned MEP amplitudes ≈ 0.5 mV in the relaxed first dorsal interosseous (FDI) muscle. In order to avoid floor-effects, the ISI between paired pulses was adjusted so that the amplitude of conditioned MEP was approximately half that of the unconditioned MEP, allowing the expression of putative increased/decreased inhibition induced by TBS/tSMS.

Table 1 shows the individuals' ISIs used in the first visit. At all testing time-points after cTBS and tSMS, we delivered 30 TMS paired-pulses. Fifteen pulses with the same intensity as *pre*-evaluation *–fixed intensity blocks-* and 15 pulses scaling the TMS intensity to have unconditioned MEP amplitudes ≈ to that obtained *pre* evaluation (i.e., *matched amplitude blocks*). The order of the *post* −testing blocks (*fixed intensity* and *matched amplitude*) was counterbalanced across subjects. Subjects #1, 3..19 were assigned to *fixed intensity* − *matched amplitude*, while subjects #2, 4,…20 and on were assigned to *matched amplitude* − *fixed intensity*, either in the first and second sessions (Fig. 1).

In the 2nd session, we proceeded similarly. The ISI was the same as in the 1st session, and the intensity of the TMS at the *pre*-evaluation was adjusted to have unconditioned MEP amplitudes similar to those of the 1st visit.

For recording the MEP, we used a Digitimer D360 amplifier (gain 1000, bandpass filtered 3–3000 Hz) that was connected to a CED1401 mk-II system (controlled with Signal 4 software). The CED unit sampled the EMG at 10 kHz and controlled the timing of the TMS. We tested excitability by delivering paired-pulses every 4.5–5.5 s.

Application of cTBS

To deliver cTBS, we used a refrigerated figure of eight coil (Cool-B65) positioned over the FDI hot-spot previously determined. Bi-phasic waveform pulses inducing currents with an AP-PA direction in the brain were used (Talelli et al., 2007; Zafar et al., 2008; Heidegger et al., 2017). Pulse intensity was set at 80 % of the active motor threshold determined on the FDI muscle with the same coil. The cTBS protocol lasted 40 s and consisted of bursts of 3 TMS pulses delivered at 50 Hz, with bursts being repeated every 200 ms (at 5 Hz) for a total of 600 pulses (Huang et al., 2005; Zafar et al., 2008). Subjects remained always at rest (Ataoglu et al., 2017).

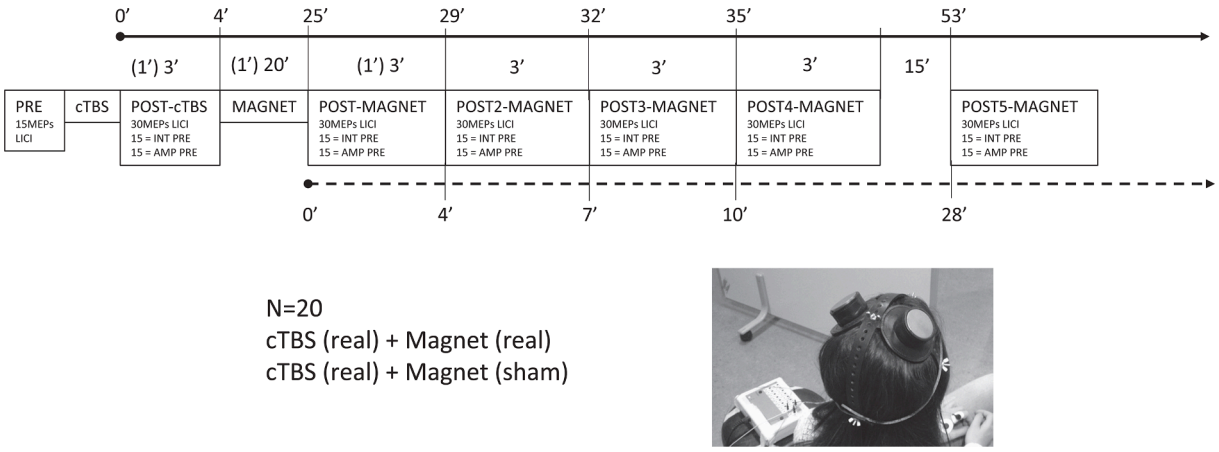

Fig. 1. Time-line for testing protocol and stimulation. Time-line in solid grey arrow are the lags from the end of cTBS. Time-line in dashed grey arrow are lag-times from the end of tSMS. Times in black font are the duration of every testing-time point, with transition time in brackets. The inset shows the application of the magnet to a participant. A magnetic cylinder (real magnet) is placed on M1 of one hemisphere and another identical cylinder without magnetic properties was placed on the opposite hemisphere to balance the holding harness.

Table 1
Individual ISIs.

Participant #	Good Responder to cTBS	ISI (ms)
S1	NO	150
S2	NO	150
S3	YES	150
S4	YES	170
S5	NO	150
S6	NO	110
S7	YES	120
S8	NO	160
S9	YES	180
S10	NO	150
S11	YES	140
S12	YES	160
S13	NO	150
S14	YES	180
S15	NO	150
S16	NO	140
S17	NO	170
S18	YES	150
S19	YES	150
S20	NO	210

Application of tSMS

For tSMS, we used a cylindrical nickel-plated (Ni–Cu–Ni) NdFeB magnet of 45 mm diameter and 30 mm of thickness (Model S-45-30-N, Supermagnete, Gottmadingen, Germany) (Oliviero et al., 2011) secured to the subject's head by means of a helmet (Neurek Ltd) as shown in Fig. 1. The maximum amount of magnetic energy stored in this magnet was 45 MGOe (megagauss-oersteds), with a nominal strength of 765 N (78 kg) and a magnetic field of 0.5 T. With the subject at rest, the magnet was placed over the skull on the representational field of the FDI muscle in the right hemisphere, previously identified with TMS (Arias et al., 2017). This form of stimulation, as indicated by previous studies, has consistently demonstrated a reduction in MEP amplitude (Oliviero et al., 2011; Paulus, 2011; Silbert et al., 2013; Nojima et al., 2015; Arias et al., 2017; Dileone et al., 2018), without any reported instances of MEP increase following the intervention. Stimulation lasted 20 min. For sham stimulation, a non-magnetic replica of the magnet (same size and weight) made in stainless steel was used. North and south pole exposition (producing the same effect on excitability (Oliviero et al., 2011) were counterbalanced across subjects.

Data processing

We analysed the effect of tSMS on the excitability of M1 previously conditioned by cTBS. For each subject, the median of the 15 MEP amplitudes was calculated at the different testing time-points. Because it is known that the responses to NIBS techniques vary from subject to subject, an intra-subject normalization was executed before data analyses. For this intra-subject normalization, and for each subject, the average value of the unconditioned MEP at the *pre*-evaluation across the *real* and *sham* sessions was calculated. This value divided all the MEP amplitudes of the same subject at all testing time-points in the two sessions. Previously, and using un-normalized data, we checked whether the unconditioned MEP amplitudes at the *pre*-evaluation differed across the two testing days.

We proceeded similarly with the root-mean square (*rms)* EMG amplitude extracted from the 50 ms time-window prior to the TMS pulse. This step was performed to evaluate the level of EMG background activity just prior to TMS pulses. We only calculated the *rms* amplitude for the unconditioned MEP since the *rms* amplitude just prior the conditioned MEP may be biased by the presence of the response to the first unconditioned TMS pulse.

Long intra-cortical inhibition (LICI) was defined as the amplitude of the conditioned MEP divided by the amplitude of the unconditioned MEP, such that the smaller the ratio, the greater the inhibition. LICI was calculated for each of the different time-points considered and was obtained from 15 TMS paired-pulses (for *fixed TMS intensity* blocks and *MEP matched amplitude* blocks) and the median was introduced in the analyses.

Statistical analyses

The assumption of normality of the distributions for the different variables was checked using the Kolmogorov-Smirnov test for one sample. Next, we evaluated if the amplitudes of the unconditioned MEP were different in the two visits with a paired-Student *t*-test on the non-normalized data.

To analyse the effects of brain stimulation (normalized data), a repeated-measures ANOVA was employed. Factors were STIM with two levels (1. *real cTBS+real magnet;* and *2. real cTBS+sham-magnet*) and TIME (*pre, post-cTBS, post-magnet, post2-magnet,…, post5-magnet*). Independent ANOVAs were executed for the *fixed-intensity* blocks and the *matched-amplitude* blocks. The same procedure was applied for the *rms* amplitude prior the unconditioned MEP and for the LICI. The Greenhouse-Geisser correction was applied in case of a sphericity

violation. Values shown in graphs and text are the mean and standard error (SE). Significance was considered if $p < 0.05$ and partial eta square (η_p^2) computed to estimate effect sizes. Bonferroni correction was applied for pair-wise follow-up comparisons after the ANOVA. In the case of p-values above 0.05, the observed power (o.p) was reported to inform about type-II error protection.

After the first analysis including all subjects (n = 20), we split the participants into two groups, good-responders to cTBS (n = 9) and bad-responders to cTBS (n = 11). This step was performed since the objective of the study was to understand the effect of tSMS on M1 excitability previously conditioned with cTBS, and it has been previously reported that the effect of cTBS may vary from subject to subject (Heidegger et al., 2017). We defined good-responders to cTBS as subjects that showed a decrease in the amplitude of the unconditioned MEP at the first and second testing right after the application of the *sham-magnet* (in the real-cTBS+sham-tSMS session). This criterion was chosen because these two time-points cover the duration of the inhibitory effect of the magnet reported in the literature (Oliviero et al., 2011; Paulus, 2011), and it was therefore optimal for the objective of this work: to test the interaction effects of these two inhibitory NIBS techniques.

Results

Table 2 shows the response at PRE for the two visits. The amplitude of the unconditioned MEP did not differ across days considering the 20 participants or after splitting the group into Good-Responders and Bad-Responders to cTBS. This lack of difference was also the case for the level of EMG background activity. On the other hand, LICI was significantly larger at PRE the day of cTBS+$MAGNET_{REAL}$ compared to the day of cTBS+$MAGNET_{SHAM}$ in the analyses with 20 participants and also in the sub-group of Bad-Responders to cTBS, but not in Good-Responders to cTBS.

The intensity of cTBS was set at 80 % of AMT for the two visits. For the cTBS+realMagnet visit, the AMT (in % from stimulator output) was 40.0 (SEM 1.6). For the cTBS+shamMagnet visit, it was 39.6 (SEM 1.5). They were not significantly different from each other ($t_{19} = 0.3p = 0.8$).

Table 2
PRE scores in the cTBS+$_{real}$Magnet and cTBS+$_{sham}$Magnet sessions.

Variable	cTBS+$_{real}$Magnet	cTBS+$_{sham}$Magnet	p-value (Student t-Test)
WHOLE SAMPLE			
MEP AMPLITUDE (mV)	0.654 (SEM 0.058)	0.629 (SEM 0.082)	$t_{19} = 0.403p = 0.691$
EMG background (mV)	0.027 (SEM 0.001)	0.027 (SEM 0.001)	$t_{19} = 0.472p = 0.642$
LICI (0–1; the lower, the more inhibition)	0.335 (SEM 0.064)	0.493 (SEM 0.079)	$t_{19} = 2.804p = 0.011$
GOOD RESPONDERS TO CTBS (N=9)			
MEP AMPLITUDE (mV)	0.789 (SEM 0.099)	0.822 (SEM 0.147)	$t_8 = 0.276p = 0.790$
EMG background (mV)	0.027 (SEM 0.001)	0.026 (SEM 0.001)	$t_8 = 0.535p = 0.608$
LICI (0–1; the lower, the more inhibition)	0.264 (SEM 0.102)	0.339 (SEM 0.111)	$t_8 = 1.15p = 0.283$
BAD RESPONDERS TO CTBS (N=11)			
MEP AMPLITUDE (mV)	0.544 (SEM 0.050)	0.472 (SEM 0.060)	$t_{10} = 1.336p = 0.211$
EMG background (mV)	0.028 (SEM 0.001)	0.028 (SEM 0.001)	$t_{10} = 0.098p = 0.924$
LICI (0–1; the lower, the more inhibition)	0.393 (SEM 0.082)	0.619 (SEM 0.101)	$t_{10} = 2.67p = 0.024$

Interaction of cTBS with tSMF

In the whole sample of participants (n = 20), the two intervention protocols had no effect on the amplitude of the unconditioned MEP at the different testing time-points (Fig. 2; $F_{6,114} = 1.8p = 0.141_{TIME}$ o.p = 0.5, and $F_{6,114} = 0.7p = 0.564_{TIME\ x\ STIM}$ o.p = 0.2).

Despite this apparent null effect of the stimulation protocols on excitability, the evaluation of the LICI revealed a more complex scenario since the LICI ratio (conditioned/unconditioned MEP) decreased (therefore inhibition increased) and then recovered along the testing time-points $F_{6,114} = 2.5p = 0.027_{TIME}$ $\eta_p^2 = 0.12$. Remarkably, this pattern was observed for the two stimulation protocols ($F_{6,114} = 1.1_{\varepsilon=0.5}$ $p = 0.349_{TIME\ x\ STIM}$ o.p = 0.3) suggesting that it was a cTBS aftereffect with no influence of tSMS. The LICI ratio decrement (greater inhibition) was ≈15 % in size at the time point of maximal effect, approximately 30 min after cTBS, regardless of whether it was followed by real or sham tSMS (Fig. 3).

Thus far, LICI was measured in the *fixed intensity blocks* since the MEP amplitude had not changed over time. However, a similar pattern of response appeared for *matched amplitude blocks* ($F_{6,114} = 5.3_{\varepsilon=0.5}$ $p = 0.004_{TIME}$ $\eta_p^2 = 0.22$ and $F_{6,114} = 1.8_{\varepsilon=0.5}$ $p = 0.143_{TIME\ x\ STIM}$ o.p = 0.5). Therefore, the absent effect on MEP might actually be driven by a "good compensation at network level" to the effect of cTBS on the $GABA_B$ M1 interneurons tested with LICI.

Finally, the EMG background activity was also modified significantly during the protocol ($F_{6,114} = 39.9$ $p < 0.001_{TIME}$ $\eta_p^2 = 0.67$) in a linear fashion ($F_{1,19} = 127.2$ $p < 0.001$ $\eta_p^2 = 0.88$, as clearly shown in Fig. 4). The effect was ≈8 % when comparing the two time-points with larger differences, and the application of the magnet (*real* or *sham*) did not modify this response ($F_{6,114} = 0.2_{\varepsilon=0.5}p = 0.863_{TIME\ x\ STIM}$ o.p = 0.1).

Interaction of cTBS with tSMF in the Good-Responders to cTBS

To shed further light on the main objective of this study, to evaluate the interaction of two inhibitory NIBS techniques, the sample of participants was split into two groups, namely, *good* or *bad responders* to cTBS (based on the results of the sessions with sham-tSMS; see methods for criteria). In these two groups, unconditioned MEP amplitudes in the fixed intensity blocks of the cTBS+$_{sham}$SMS session differed across testing times ($F_{6,108} = 2.4$ $p = 0.032_{TIME\ x\ GROUP}$ $\eta_p^2 = 0.12$ Fig. 5).

In good-responders to cTBS, we observed a significant change in the MEP amplitude over time ($F_{6,48} = 4.2$ $p = 0.002_{TIME}$ $\eta_p^2 = 0.35$), which was different if cTBS was followed by a real or sham magnet stimulation ($F_{6,48} = 2.4$ $p = 0.041_{TIME\ x\ STIM}$ $\eta_p^2 = 0.23$), as shown in Fig. 6a.

In the case of cTBS+$_{SHAM}$Magnet, the MEP amplitude was reduced immediately after cTBS, and the effect remained for more than 30 min until post3-MAG ($p < 0.001$, pair-wise comparisons with Bonferroni correction). Remarkably, at the 2nd and 3rd testing times after Magnet application, the MEP was significantly larger for cTBS+$_{REAL}$Magnet compared to cTBS+$_{SHAM}$Magnet ($p = 0.022$ and $p = 0.007$, respectively), and at these two points, MEP responses following cTBS+$_{REAL}$Magnet exceeded their baseline values, although the statistical significance of this increase was not observed. Therefore, in those subjects with good response to cTBS, the application of the Magnet after cTBS increased the size of MEP. The value of LICI in the good-responders to cTBS (in *matched amplitude* blocks) was not different when cTBS was followed by $_{REAL}$Magnet or $_{SHAM}$Magnet, ($F_{6,48} = 1.6$ $p = 0.180_{TIME\ x\ STIM}$ o.p = 0.5). In both cases, LICI ratio decreased (greater inhibition) ($F_{6,48} = 2.3$ $p = 0.054$ $_{TIME}$ $\eta_p^2 = 0.2$) and started to recover the last testing time-point (Fig. 7). Therefore, the interaction between the Magnet and cTBS seems not to occur at the population of interneurons responsible for LICI.

As in the whole sample, the effect of the stimulation protocols on the EMG background activity at the moment of MEP testing was also present in the Good-Responders to cTBS ($F_{6,48} = 22.9$ $p < 0.001_{TIME}$ $\eta_p^2 = 0.7$), regardless of the type of Magnet stimulation (real or sham), and the EMG

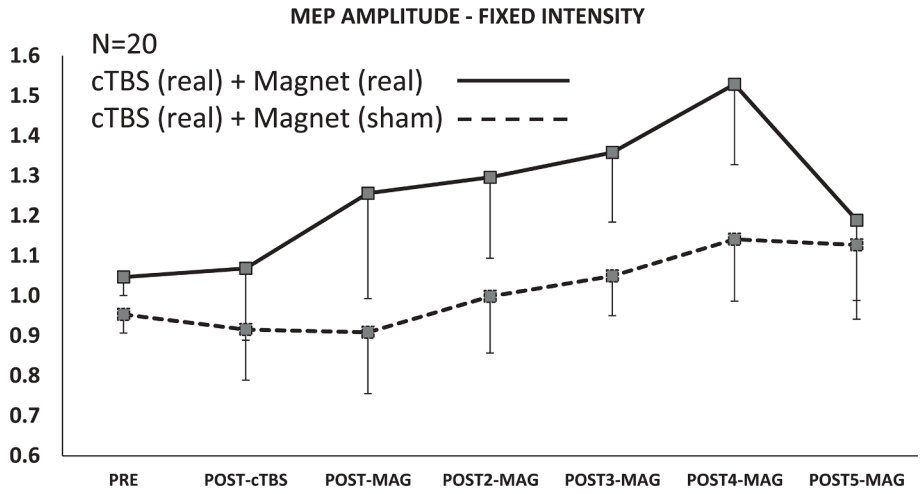

Fig. 2. Effects of the protocols on cortico-spinal excitability (amplitude of the unconditioned MEP) with fixed TMS intensity at all testing times. Values are means and SE in the whole sample of participants (n = 20). In all graphs, Y-axis represents normalized units (see Methods and Table 2).

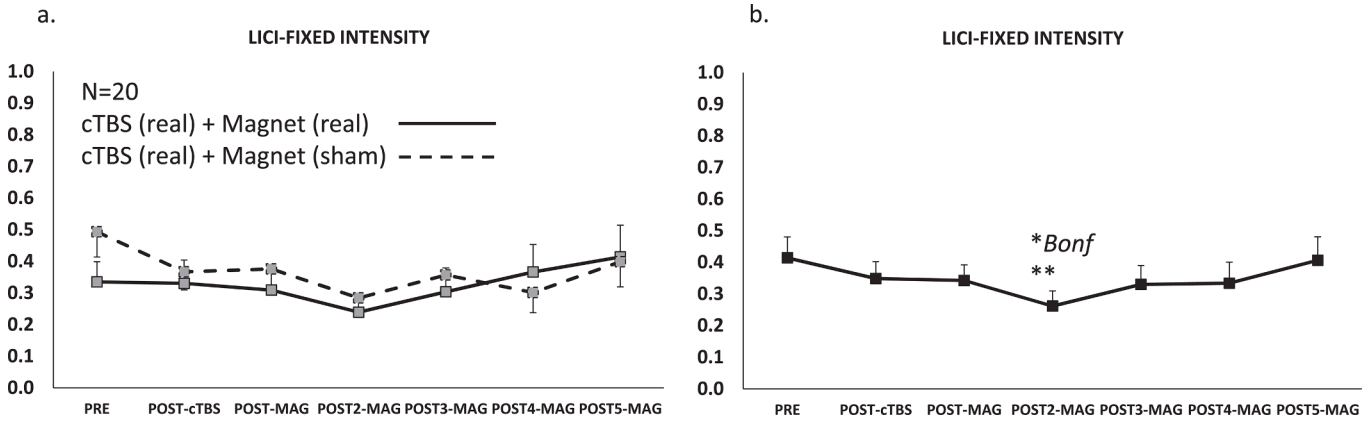

Fig. 3. Effects of the protocols on LICI with fixed TMS intensity at all testing times. Section a) shows the behaviour in the two sessions since their behaviour was not different and section b) shows the responses pooling across sessions. Scores are means and SE in the whole sample of participants (n = 20). *p < 0.05; **p < 0.01.

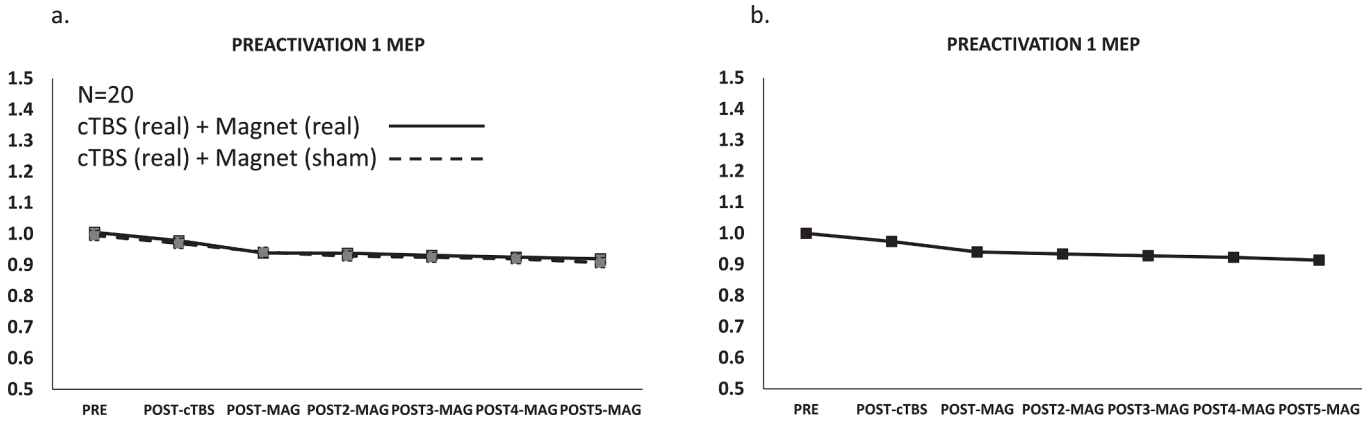

Fig. 4. Effects of the protocols on background EMG activity at the time of TMS testing at all testing times in the fixed intensity blocks. Scores are means and SE in the whole sample of participants (n = 20). The plot at the left (a) shows behaviours over two sessions, and the plot at the right (b) shows behaviours that are pooled because they did not differ.

background was reduced.

Bad-Responders to cTBS did not show the effects on the MEP amplitude observed in Good-Responders, but they did show a similar effect on the EMG background and LICI (see Table 3 and Fig. 6b).

Discussion

The objective of this study was to explore possible metaplastic effects induced by two different inhibitory NIBS techniques applied to the motor cortex and to suggest some possible underlying mechanisms

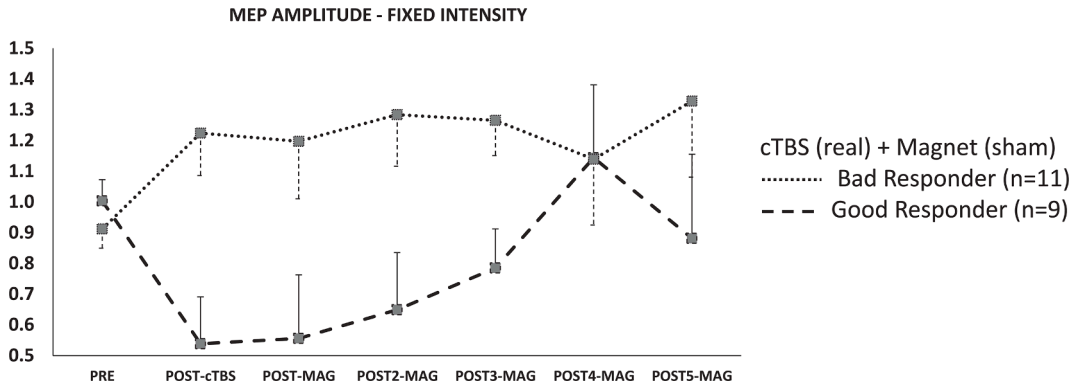

Fig. 5. Cortico-spinal excitability responses (unconditioned MEP amplitudes) with fixed intensity TMS across all testing times for the cTBS+$_{SHAM}$Magnet visit. Dashed and dotted lines illustrate the behaviour of Good and Bad Responders to cTBS, respectively. Scores are means and SE. Pair-wise comparisons are not reported since this plot aims to represent the different responses of the two sub-groups along the testing time-points.

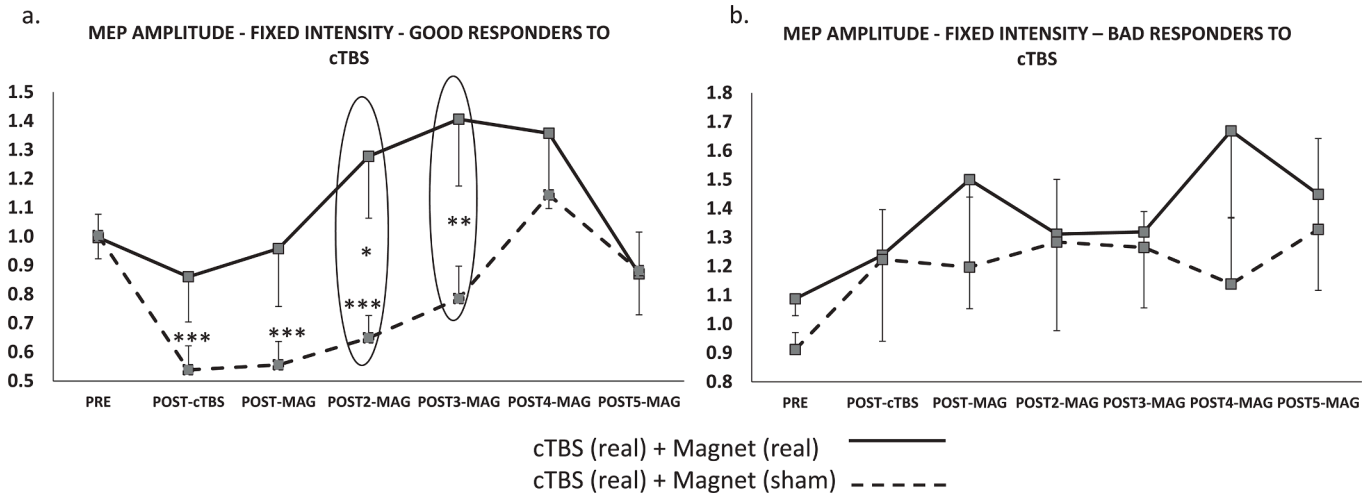

Fig. 6. a) Good-responders to cTBS. Cortico-spinal excitability responses (unconditioned MEP amplitudes) with fixed intensity TMS along all testing times when cTBS was followed by $_{REAL}$Magnet (solid line) or $_{SHAM}$Magnet (dashed line). b) Results for bad-responders to cTBS. Scores are means and SE. *p < 0.05; **p < 0.01; ***p < 0.001.

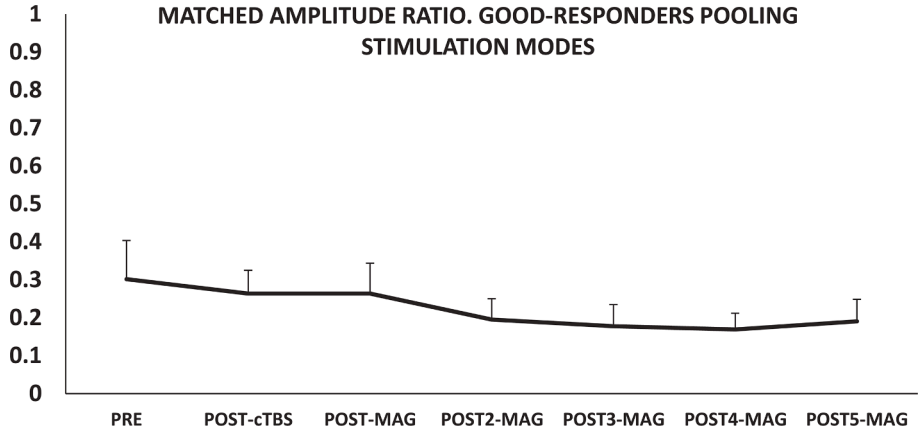

Fig. 7. LICI responses to cTBS in Good Responders. The black line represents both visits (cTBS+$_{REAL}$Magnet and cTBS+$_{SHAM}$Magnet) pooled since their responses did not change significantly from each other.

(Fischer et al., 1997; Edwards et al., 2008; Müller-Dahlhaus and Ziemann, 2015). While metaplasticity has been extensively examined at the cellular level for excitatory neurotransmission, it is also observed for inhibitory synapses (Fischer et al., 1997; Edwards et al., 2008; Müller-Dahlhaus and Ziemann, 2015). In our study, we employed cTBS to decrease M1 excitability as a priming technique, with tSMS as the subsequent test technique. To validate our hypothesis that homeostatic responses would emerge after priming M1 with cTBS and applying tSMS −the latter known for consistently reducing MEP amplitudes without reported MEP increases (Oliviero et al., 2011; Paulus, 2011; Silbert

Table 3
ANOVA Main Effects and Interactions for Bad-Responders to cTBS.

Variable	TIME	TIME X STIM
MEP AMPLITUDE (mV)	$F_{6,60} = 0.984$ p = 0.444 o. p = 0.4	$F_{6,60} = 0.617$ p = 0.716 o.p = 0.2
EMG background (mV)	$F_{6,60} = 18.367$ p < 0.001 $\eta_p^2 = 0.6$	$F_{6,60} = 0.708$p = 0.645 o.p = 0.2
LICI MATCHED AMPLITUDE	$F_{6,60} = 3.510_{\varepsilon=0.3}$ p = 0.049 $\eta_p^2 = 0.3$	$F_{6,60} = 1.563_{\varepsilon=0.5}$ p = 0.222 o.p = 0.4

et al., 2013; Oliviero et al., 2014; Nojima et al., 2015; Kirimoto et al., 2016; Arias et al., 2017; Dileone et al., 2018)- we initially assessed participants' responses to cTBS alone (cTBS+sham tSMS). We pursued this approach due to indications that up to 50 % of participants undergoing cTBS may experience increased excitability (Heidegger et al., 2017). In contrast, numerous studies have consistently demonstrated tSMS's inhibitory modulation of motor cortex excitability (Oliviero et al., 2011; Paulus, 2011; Silbert et al., 2013; Oliviero et al., 2014; Nojima et al., 2015; Kirimoto et al., 2016; Arias et al., 2017; Dileone et al., 2018) when tested with conventional TMS protocols (Davila-Pérez et al., 2019). In our assessment of responses to cTBS (+sham tSMS) across the entire participant sample (n = 20), we observed an overall lack of modulation in MEPs. However, the situation proved intricate as approximately half of the participants exhibited the expected positive response to cTBS, with MEPs decreasing. The remaining participants, labelled as Bad-Responders, demonstrated an unexpected response. Despite this variability, the overall sample, likely influenced by the Good-Responders, showed an increase in GABAb inhibition (LICI) after cTBS, which commenced recovery by the end of the testing protocol.

It is remarkable that for Good-Responders to cTBS, on which MEP was reduced by ≈40 % right after cTBS (and remained below baseline levels for approximately 35 min in the sham tSMS session), the subsequent application of real tSMS on M1 interacted in such a way that corticospinal excitability changed significantly, and inhibition was cancelled. This observation supports the notion of a homeostatic effect, indicative of homeostatic metaplasticity.

Conversely, in the Bad-Responders group to cTBS, where MEP remained unchanged in the cTBS+sham tSMS condition, the application of real tSMS after cTBS did not elicit any further changes in excitability. These effects are unlikely driven by changes in the level of pre-activation of the motor system during MEPs testing, as the pre-activation change was small (≈8 % for the largest difference) and showed a linear reduction throughout the testing protocol. Moreover, it is known that a reduction in EMG background at the time of MEP testing does not result in an increase in MEP amplitude (Mazzocchio et al., 1994). Our results in the Good-Responders group can be understood within the framework of homeostatic plasticity. In essence, this suggests a mechanism aimed at regulating Long-Term Potentiation (LTP) and Long-Term Depression (LDP) to uphold synaptic activity within physiological ranges. As it was shown in the early 90's in slice preparations, the same pattern of tetanic stimulation can induce either LTD or LTP depending on the level of depolarization of the postsynaptic neuron (Artola et al., 1990). This finding suggests that the state of the brain at a given time is a key element to induce and direct synaptic plasticity. In the human brain, a particular rTMS protocol with a null effect on cortical excitability may induce increased excitability of M1 if applied after an inhibitory technique (cathodal tDCS) on the same motor cortex, the latter serving as priming protocol; the effect is reversed and cortical excitability is reduced if the priming technique is excitatory (anodal tDCS) (Lang et al., 2004). As mentioned, this sort of effect has been shown even if the priming technique has no observable effect on cortical excitability on its own (Lang et al., 2004; Siebner et al., 2004). However, similar findings can be observed when the priming technique produces changes in excitability. In fact, the larger the reduction in CSE produced by a priming cathodal tDCS, the larger the reversal effect of 1H-rTMS, and the reverse occurs if the priming technique is excitatory (Siebner et al., 2004).

In the case of Bad-Responders to cTBS, for which MEPs remained unchanged along the whole testing session during the cTBS+sham tSMS, the application of real tSMS produced no effects on the previously conditioned M1 (with cTBS). At least two possible explanations may account for this response. First, it might be possible that in this subsample of participants either homeostatic as non-homeostatic responses (Bienenstock et al., 1982; Ziemann and Siebner, 2008) might have expressed with a different time-window between the priming (TBS) and the testing technique (tSMS). While this is a plausible scenario, it seems that the time windows for interactions (across all directions and magnitudes of change) are notably extensive (Hassanzahraee et al., 2018). Another potential explanation is that individuals identified as Bad-Responders to cTBS may also demonstrate poor responsiveness to other NIBS techniques modalities, such as tSMS. In this subgroup of Bad Responders, not only did MEPs remain unchanged in both sessions, but LICI also exhibited no alterations, suggesting that this subgroup might simply exhibit lower responsiveness to various NIBS techniques interventions.

This observation could also imply that the mechanisms of action of cTBS and tSMS might overlap (hence, non-responders to cTBS do not respond to the subsequent application of tSMS). It is possible that individuals who are bad responders to one NIBS technique may also be non-responders to other NIBS techniques. This argument finds support in the idea that brain plasticity induced by NIBS techniques could be influenced by different BDNF gene phenotypes (Cheeran et al., 2008; Bath et al., 2012; Mastroeni et al., 2013; Ni et al., 2014). However, this assumption may lose strength when examining results from different NIBS techniques applied in pathological conditions. For example, there are reports indicating positive responses (mean pain ratings in responders) in 32.6 % for rTMS and 29.6 % for tDCS, but half of these responders are sensitive to only one technique (André-Obadia et al., 2023).

In addition to the above mentioned mechanisms, *meta*-analyses conducted by Hassanzahraee et al. (2018) have suggested that the reversal effect of the priming technique on test technique critically depends on the time-window between these interventions. There appears to be an optimal time-window for inducing a reversal effect (opposite to the conventionally reported effect) (i.e., homeostatic plasticity). In our protocol, the lag between the end of cTBS and the initiation of tSMS was aproximately 3 min for all subjects. It seems unlikely that the lack of effects of tSMS on the group of Bad-Responders to cTBS is solely attributed to an inappropriate time-window between cTBS and tSMS application. However, it remains unclear whether time-window constraints might also exhibit inter-participants variability. On the other hand, we observed reversal of tSMS effects after cTBS exclusively on Good-Responders to cTBS, not in Bad-Responders. This suggests a potential dependency of the facilitatory effects of tSMS on the preceding brain activity/state. In individuals where cTBS successfully reduced M1 excitability, subsequent tSMS induced homeostatic reactions. Conversely, in Bad-Responders where cTBS failed to reach the threshold for MEP reduction, tSMS did not reverse the effect or potentiate the prior impact of cTBS. It is plausible that these subjects are less inclined to exhibit plastic responses after NIBS techniques, akin to Val66Met gene carriers compared to Val66Val phenotypes (Cheeran et al., 2008; Bath et al., 2012; Mastroeni et al., 2013).

Which pool of neurons is involved in the observed homeostatic responses? We do not have a definitive answer to that question, but we can provide some clues. In the group of Good-Responders to cTBS, LICI was identically affected regardless of the type of tSMS (real or sham). This result suggest that the mechanism of action of tSMS and the subsequent homeostatic response originates from interneurons not recruited by LICI protocols, at least under the conditions explored in our work (tSMS after cTBS).

Study limitations

Although our sample size included 20 participants, only about 50 % of them were "good-responders" to cTBS. While this proportion of good responders to cTBS is in agreement with literature (Heidegger et al., 2017), sub-grouping has an impact of statistical power. To account for this limitation, we have reported the observed power in those cases were significance was not reached to inform about type-II error protection. In the case of Good-Responders to cTBS (despite the small sample size), the subsequent application of real tSMS (cTBS+realSMS) after cTBS resulted in significant modulation excitability compared to cTBS+shamSMS, which therefore does not question statistical power. Anyway, further studies with larger samples should confirm our observation.

In conclusion, in our hands, tSMS is likely modifying excitability at the synaptic level and may induce homeostatic changes in cortical excitability. The notion that tSMS is able to reduce cortical inhibition supports the possibility of investigating the use of the technique when aiming to restore excitability in pathological conditions with altered cortical inhibition.

CRediT authorship contribution statement

Pablo Arias: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Lucía Adán-Arcay:** Investigation, Methodology, Project administration. **Elena Madinabeitia-Mancebo:** Investigation, Methodology. **Javier Cudeiro:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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