Supplementary Results and Methods

Exploring the role of the left DLPFC in fatigue during unresisted rhythmic movements

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Running Head: DLPFC involvement in repetitive movement fatigue

Supplementary Results

In the case of FT patterns, neither the application of tSMS nor increasing cognitive demands with Stroop modified the movement features described below. Some variables reflected the classic consequences of fatigue in FT.

Maximal FT frequency along 30 s

The drop in maximal frequency within the 30-s repetitions was approximately 13%, comparing median scores of 0–5 s (PRE) vs. 25–30 s (POST) periods. This significant effect ($F_{1,18}$=267.6, $p<0.001_{TIME}$; **Supp. Fig. 1A)** was not differentially expressed across sessions. **Supp. Fig. 1A.1 inset** presents results split by stimulation mode as a representative example of the same behavior across stimulation modes. This was also the case for some other variables, but we did not split the results to increase simplicity.

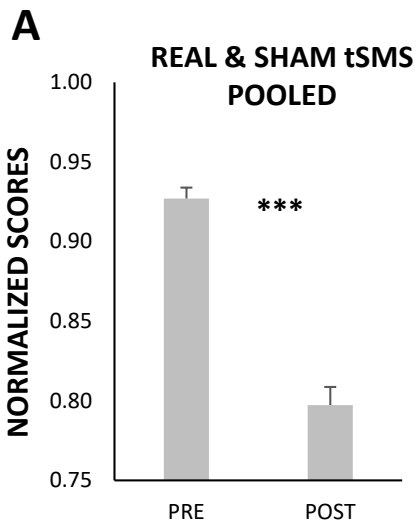
ROM amplitude during maximal FT

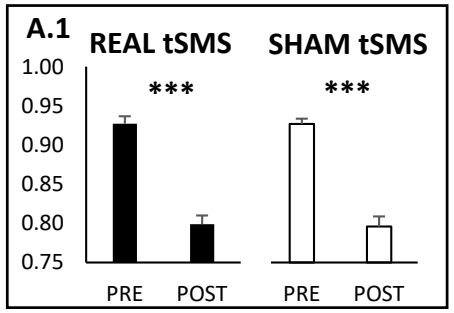
ROM amplitudes were reduced (~4.5%) across the 30 s ($F_{1,18}$=12.8, $p=0.002_{TIME}$; **Supp. Fig. 2A**). Within each set, 2nd repetitions were always executed with smaller ROM amplitudes than the first repetitions ($F_{1,18}$=63.3, $p<0.001_{REP}$; **Supp. Fig. 2B**), although the differences were reduced with set progression ($F_{2,36}$=18.1, $p<0.001_{SET \times REP}$). The effect was due to the ROM amplitude reduction during the first repetitions (**Supp. Fig. 2C**). Overall, this shows that fatigue within repetitions and over the course of the protocol is expressed as a reduction in ROM amplitude.

ROM amplitude during self-selected tapping rates

Changes in ROM along self-selected FT did not differ between real and sham tSMS sessions (**Supp. Fig. 3A** shows the results of pooling stimulation modes). Self-selected FT ROM increased across sets ($F_{2,36}$=3.6, $p=0.038_{SET}$), but the change was small in magnitude, and none of the pair-wise comparisons reached statistical significance.

FREQUENCY AT MAXIMAL FT

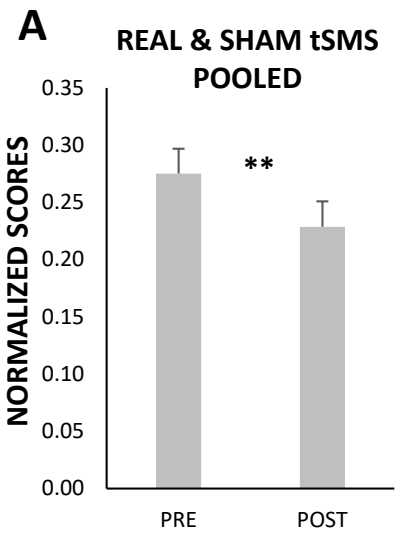


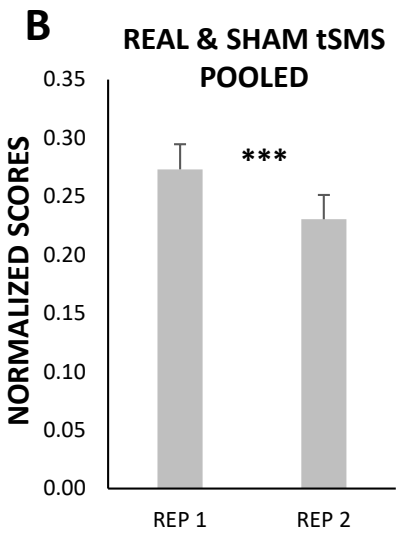


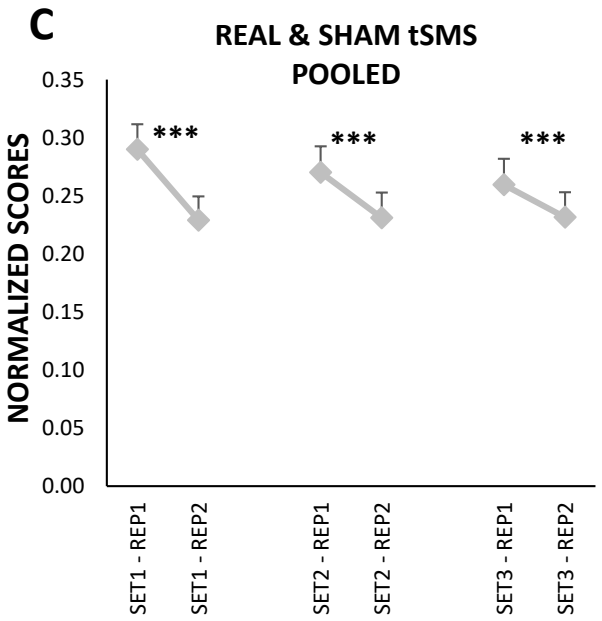
SUPPLEMENTARY FIGURE 1

Supplementary Figure 1. A) Significant reduction in maximal FT frequency from PRE (0–5 s) to POST (25–30 s) within repetitions. Gray bars present responses pooled across real and sham tSMS sessions (**A.1 inset**).

ROM AMPLITUDE AT MAXIMAL FT



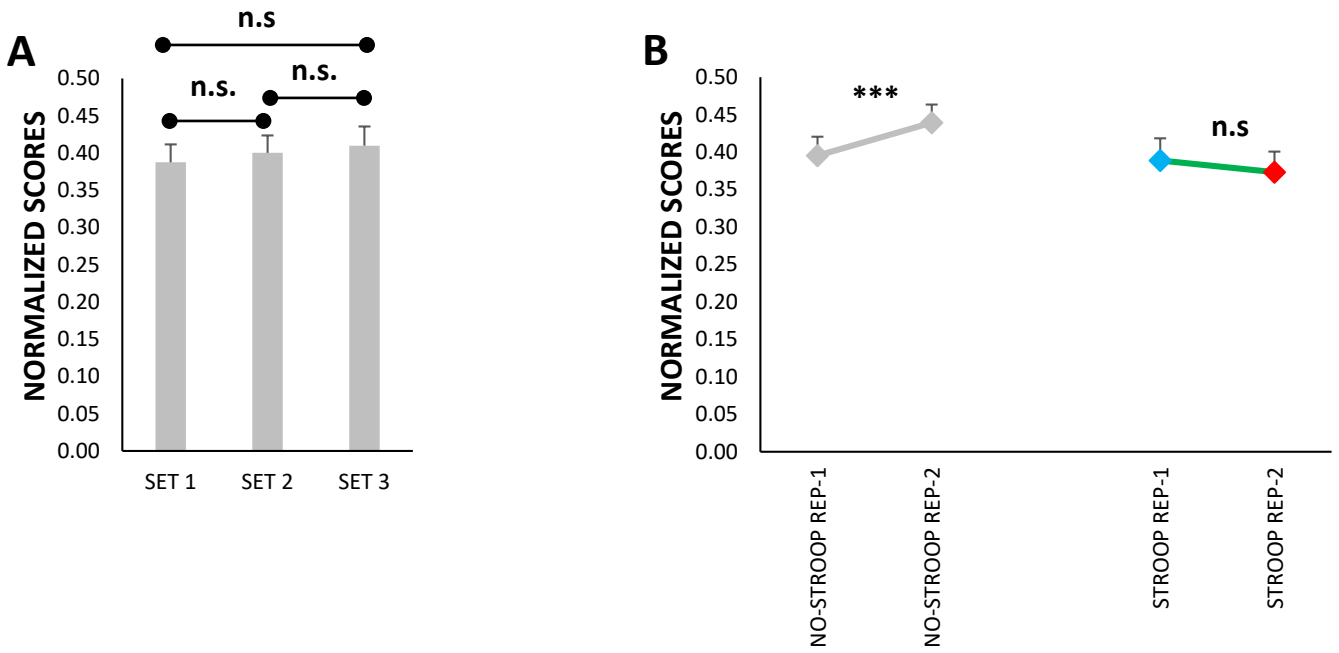




SUPPLEMENTARY FIGURE 2

Supplementary Figure 2. ROM amplitudes during maximal FT decreased within and between repetitions (**A** and **B**, respectively). Set progression (**C**) reduced amplitudes, especially in first repetitions. **p<0.01; ***p<0.001.

ROM AMPLITUDE AT SELF-SELECTED FT



SUPPLEMENTARY FIGURE 3

Supplementary Figure 3. A) ROM amplitude at self-selected FT remained stable across trials. However, while fatigued (2nd repetition) **(B)** ROM increased for no-Stroop trials, not for those with Stroop.

However, the ROM change from 1st to 2nd repetitions differed between Stroop and no-Stroop FT ($F_{1,18}$=33.8, $p<0.001_{CL \times REP}$). It increased from 1st to 2nd repetitions without concurrent Stroop execution (post hoc p<0.001) but remained stable if repetitions were performed with Stroop (post hoc p=0.288); see **Supp. Fig. 3B.**

CV of the ROM amplitude at self-selected FT

CV_{ROM} differed across stimulation sessions (in sets and repetitions) $F_{2,36}=4.8_{\varepsilon=0.7,}$ $p=0.030_{STIM \times CL \times SET \times REP}$. In the two sessions, variability was approximately 4% higher during Stroop execution (real tSMS session $F_{1,18}$=33.0, $p<0.001_{CL}$; sham tSMS session $F_{1,18}$=26.4, $p<0.001_{CL}$ **Supp. Fig. 4A)**.

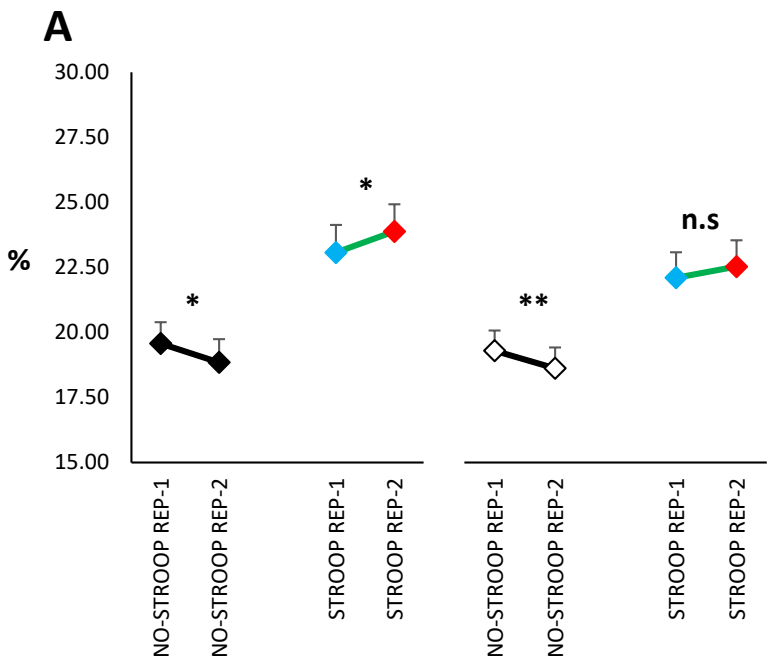
CV_{ROM} changed with fatigue (1st vs. 2nd repetitions) in a different way for Stroop and no-Stroop FT, both in real and sham tSMS sessions ($F_{1,18}$=21.7, $p<0.001_{CL \times REP}$ and $F_{1,18}$=31.0, $p<0.001_{CL \times REP}$, respectively). CV_{ROM} reduced with fatigue in no-Stroop trials. For Stroop trials, CV_{ROM} increased in real tSMS but did not change in sham tSMS (see **Supp. Fig. 4A** for post hoc comparisons). Only with real tSMS did changes across Stroop repetitions vary with sets ($F_{2,36}=5.0_{\varepsilon=0.7}$, $p=0.025_{CL \times SET \times REP}$). Post hoc comparisons were omitted for clarity in the **A.1 inset of Supp. Fig. 4**. Changes in variability from the 1st to 2nd repetitions were small, whereas changes due to increasing cognitive load during task execution were larger.

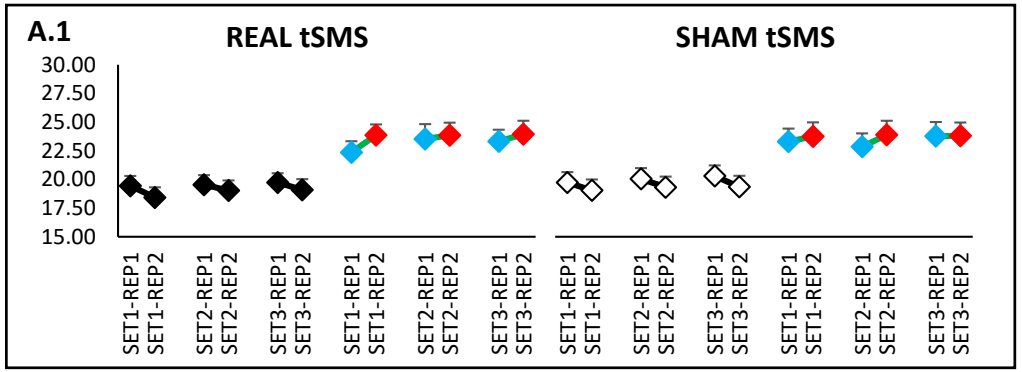
Stroop scores

At the end of the entire FT protocol (at rest), Stroop scores were higher than at the beginning of the session (before the execution of any motor task) ($F_{1,18}$=15.3, $p<0.001_{TIME}$; **Supp. Fig. 5A** suggests learning).

However, even in the absence of fatigue, scores were significantly reduced by the mere execution of self-selected FT. This was observed when comparing scores at rest (before any FT action) vs. scores obtained during the first time the Stroop and FT were executed together

ROM AMPLITUDE CV AT SELF-SELECTED FT

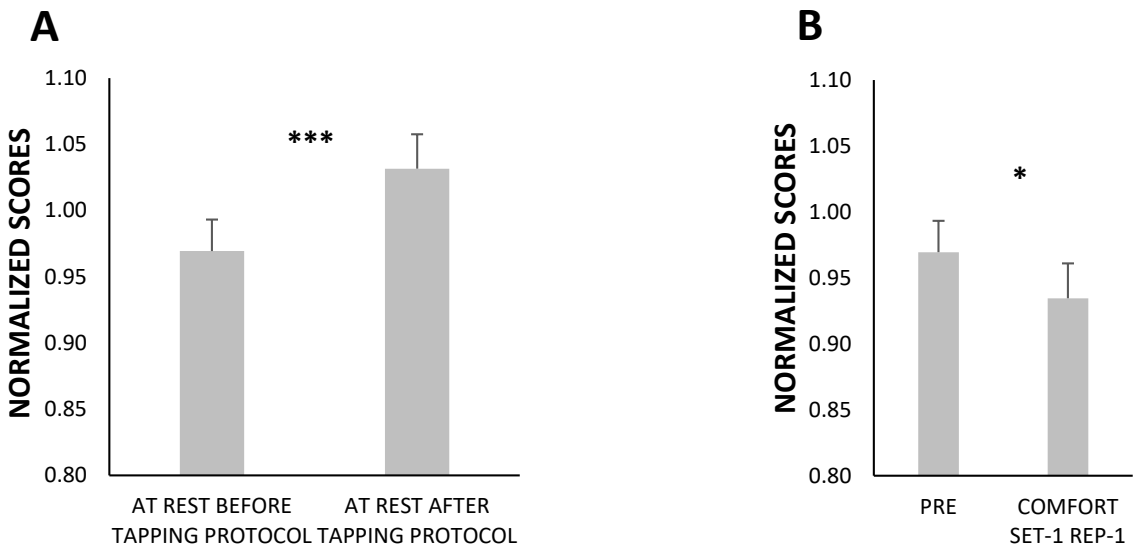




SUPPLEMENTARY FIGURE 4

Supplementary Figure 4. A) CV_{ROM} at self-selected FT increased while performing Stroop (vs. no-Stroop) in both sessions ($p<0.001_{CL}$ the two sessions; asterisk omitted). Additionally, in both sessions, CV_{ROM} was reduced in the presence of fatigue (2nd repetition) for no-Stroop FT. For Stroop FT, CV_{ROM} increase was significant in the presence of fatigue for real tSMS, an effect not observed for sham tSMS. **A.1 inset** displays behavior split by sets (asterisks omitted). *p<0.05; **p<0.01.

STROOP SCORES



SUPPLEMENTARY FIGURE 5

Supplementary Figure 5. A) Stroop scores at rest increased from the beginning to the end of the session. **B)** Stroop scores were reduced by concurrent execution of the first repetition of self-selected FT compared to scores obtained at motor-rest immediately before FT. *p<0.05; **p<0.01; ***p<0.001.

($F_{1,18}$=5.6, p=0.029$_{TIME}$; **Supp. Fig. 5B**). Stroop scores without FT were always higher than those with FT.

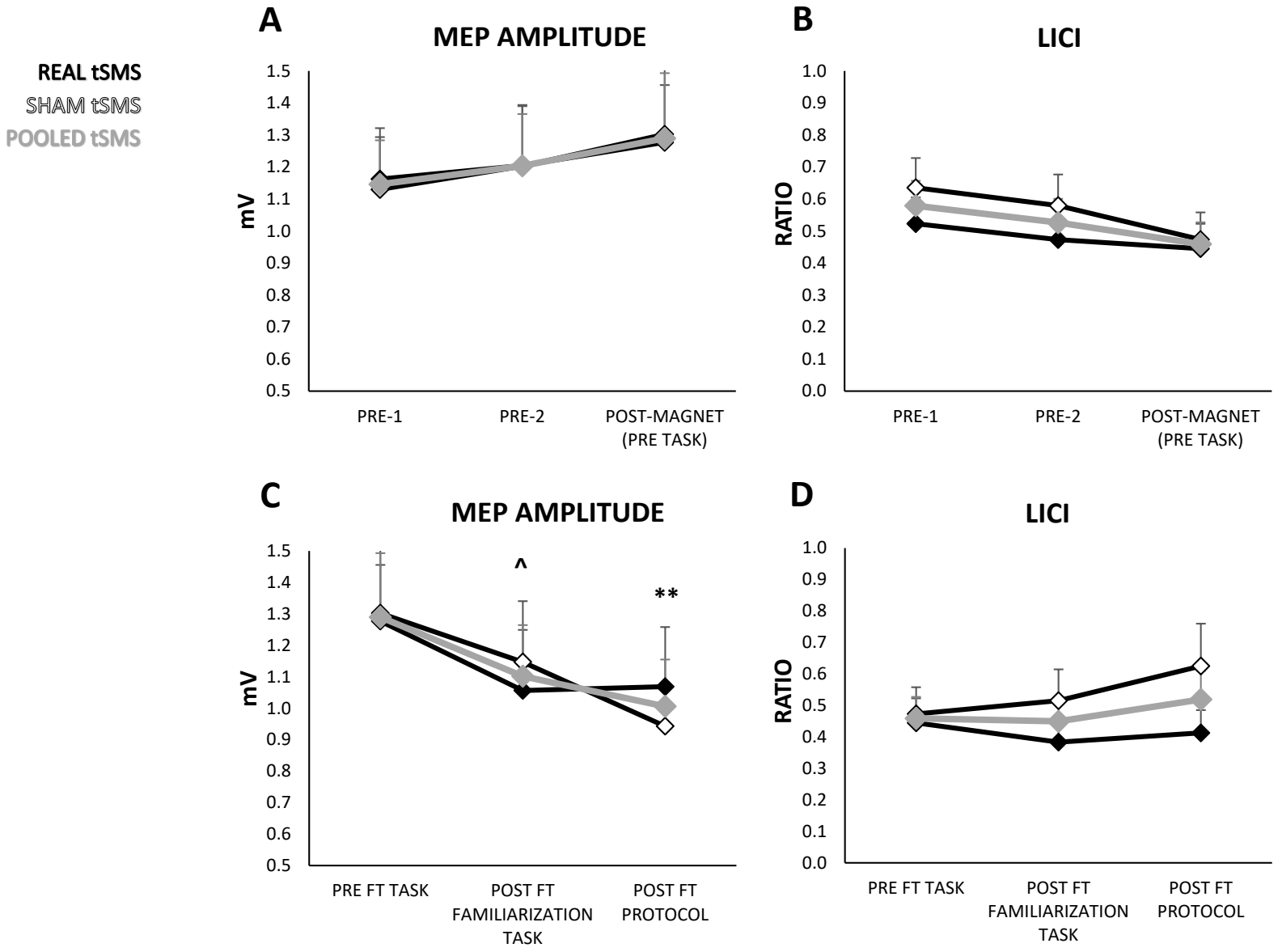
Modulation of cortico-spinal and cortico-cortical excitability by tSMS and FT

At the start of the sessions and before any actions were performed by participants, applying tSMS over the DLPFC for 15 minutes did not change motor evoked potentials (MEPs, i.e., M1 cortico-spinal excitability; $F_{2,36}$=1.0$_{\varepsilon=0.7}$, p=0.4$_{TIME}$; **Supp. Fig. 6A**) or M1 long intracortical inhibition (LICI) ($F_{2,36}$=3.2$_{\varepsilon=0.7}$, p=0.07$_{TIME}$; **Supp. Fig. 6B**). The remaining main effects and interactions were not significant.

MEP amplitudes decreased after motor execution compared with scores acquired immediately before the execution of FT ($F_{2,36}$=4.1, p=0.025$_{TIME}$). A significant reduction was evident after the familiarization FT round and progressed further at the end of the entire protocol (see **Supp. Fig. 6C** for post hoc comparisons). These responses did not differ between the real and sham tSMS ($F_{1,18}$≈0.0, p=1.0$_{STIM}$ and $F_{2,36}$=0.4, p=0.6$_{STIM \times TIME}$).

LICI (tested with matched amplitude MEPs, see Supplementary Methods) remained unchanged across sessions ($F_{2,36}$=0.8$_{\varepsilon=0.6}$, p=0.4$_{TIME}$; **Supp. Fig. 6D)** as did compound muscle action potential (CMAP) amplitudes ($F_{2,36}$=1.0$_{\varepsilon=0.7}$, p=0.4$_{TIME}$; remaining main effects and interaction were also non-significant). Background EMG activity at the time of stimulation remained unchanged across the different testing times and modalities (main effects and interactions were non-significant).

M1 CORTICO-CORTICAL AND CORTICOSPINAL EXCITABILITY



SUPPLEMENTARY FIGURE 6

Supplementary Figure 6. Excitability responses before any participant's action **(A, B)** did not change with 15' magnet application over the left DLPFC. When tested at rest after FT, MEP **(C)** but not LICI **(D)** reduced (similarly for real and sham tSMS sessions). *p<0.05; **p<0.01; ***p<0.001. ^p<0.05 (without Bonferroni correction).

Supplementary Methods

Session structure

Subjects wore a helmet three times every session, twice at rest, and the last time during several sets of FT (**Supp. Fig. 7** shows the session test sequence):

- Before the first tSMS application, we tested initial levels of perceived fatigue using the VAS (Hewlett, Dures, & Almeida, 2011) and determined the maximal active ROM of the index finger in a flexo-extension plane. We also tested baseline M1-cortico-motor excitability (CMAP, MEP, and LICI, see below).
- Next, an initial 15 min tSMS period was performed with subjects at rest, which was sufficient to produce changes in cortical excitability previously (Arias et al., 2017; Antonio Oliviero et al., 2011).
- At the end of this initial 15 min of tSMS, the helmet was removed, and cortico-motor excitability was immediately re-tested to understand the putative effects of left DLPFC-tSMS on M1 excitability, thus avoiding any interference produced by the execution of motor or cognitive tasks.
- After this procedure, subjects executed 30 s of Stroop without FT (as described below).
- Next, the helmet was worn again, and tSMS was applied for another 10 min; this was done to refresh the stimulation effects, given they are short-lasting (Antonio Oliviero et al., 2011).
- Then, the helmet was removed, and an initial set FT was executed to familiarize participants with task execution (data discarded from the analyses). Immediately after this familiarization FT set, and in this order, we tested cortico-motor excitability and fatigue perception with the VAS. Evaluation of VAS and evoked potentials aimed to understand the putative effects of preliminary familiarization tasks on the tested variables.

EVALUATION PROTOCOL ALONG SESSIONS

tSMS- HELMET – OFF	tSMS- HELMET – ON 15 min.	tSMS- HELMET – OFF 5 min.	tSMS- HELMET – ON 10 min.	tSMS- HELMET – OFF 10 min.	tSMS- HELMET – ON 30 min.	tSMS- HELMET – OFF 10 min.
• VAS – BASELINE • MAXIMAL ROM TESTING • PRE – CMAP • PRE – 1 – MEP-LICI • PRE – 2 – MEP-LICI	• PARTICIPANTS AT REST	• POST-MAGNET – MEP-LICI • STROOP (NO-FT TASK)	• PARTICIPANTS AT REST	• FT FAMILIARIZATION TRIAL NO-STROOP • POST FT-FAMILIARIZATION CMAP • POST FT-FAMILIARIZATION MEP-LICI • VAS (PRE MAIN FT-PROTOCOL)	• MAIN FT – PROTOCOL (FIGURE 1 MAIN DOCUMENT)	• POST – MAIN FT PROTOCOL CMAP • POST – MAIN FT PROTOCOL MEP-LICI • POST – MAIN FT PROTOCOL STROOP (NO-FT TASK)

VAS: Visual Analogue Scale, Fatigue Perception
ROM: Range of Movement Amplitude
CMAP: Compound muscle action potential
MEP: Motor evoked potentials
LICI: M1 long intra-cortical inhibition

SUPPLEMENTARY FIGURE 7

Supplementary Figure 7. Representation of the sequence of tests in a whole session. Elements including concurrent tSMS (either real or sham) display over a black background.

- Next, the helmet was worn again, and tSMS was applied during the main FT protocol, which lasted for 30 min. In total, the tSMS application lasted 55 min per session.
- Immediately after the main FT protocol, the helmet was removed, and we re-tested cortico-motor excitability at rest, followed by a final Stroop test without FT.

Evaluation of cortico-motor excitability

For this purpose, we recorded EMG activity on the first dorsal interosseous (FDI) muscle with surface electrodes and evoked potentials from M1 with single and double-pulse TMS. EMG activity was amplified (×250; bandpass filter, 3–3000 Hz; D360, Digitimer Ltd.) and sent to the CED1401 unit.

Initially, CMAP was acquired by supramaximal stimulation on Erb's point (Digitimer DS7AH; pulse width, 100 µs). This was tested to determine the baseline efficiency of neuromuscular transmission (subsequently, it was re-tested after muscle fatiguing activity). Notably, putative CMAP changes after muscle activity would influence the interpretation of MEP acquired with TMS.

Next, monophasic TMS paired pulses over M1 were delivered with a MagPro X100/MagOption and a figure-of-eight coil to test cortico-spinal excitability (1^{st} MEP amplitude) and LICI (i.e., 2^{nd} MEP/1^{st} MEP amplitude ratio). LICI is a good marker for cortical inhibition (thought to be a GABAb receptor-mediated inhibition) and GABAb receptor interneurons have been shown to increase their excitability during fatiguing FT (Arias et al., 2015; Madrid et al., 2018; Madrid et al., 2016). In contrast, cortico-spinal excitability depression has been reported for several minutes after fatiguing motor tasks (Samii, Wassermann, & Hallett, 1997).

For MEP acquisition, the coil was tangentially positioned over the FDI muscle "*hot-spot*" (Arias et al., 2015; Madinabeitia-Mancebo, Madrid, Jácome, Cudeiro, & Arias, 2020; Madrid et al., 2018) with the handle facing backward at 45° from the midline and current direction flowed in

the coil to induce currents in a postero-anterior direction in the brain. We adjusted the TMS intensity to produce (first) MEPs of approximately 1 mV in amplitude in the resting muscle. The second pulse of the pair (at the same intensity) was delayed by 120–200 ms. The inter-stimulus interval (ISI) was individually determined at the beginning of the first session to obtain a second MEP, which was inhibited to approximately 50% of the first MEP amplitude, and the same ISI was used in the second session. We delivered 20 paired pulses (one pair every 4.5–5.5 s) for each evaluation time point.

Two blocks (PRE-1 and PRE-2 time points) of 20 TMS pulse pairs were used to assess the stability of MEP responses before tSMS. Another 40 TMS pulse pairs were delivered after removing the helmet to test putative changes in MEP and LICI produced by left DLPFC stimulation (Cao et al., 2018). Twenty of the pulses had the same intensity as used in PRE-testing (i.e., *fixed intensity*), and another 20 TMS intensities scaled to have 1st MEP amplitudes equivalent to those obtained before tSMS (i.e., *matched amplitude)*. This last mode was tested to evaluate LICI across testing times with similar amplitudes of the 1st MEPs (important since LICI= 2nd MEP amplitude / 1st MEP amplitude). The fixed intensity and matched amplitude block orders were counterbalanced across participants. CMAP, MEP, and LICI testing described above were performed two more times during the protocol: once after the first execution of the familiarization FT set and again at the end of the main FT protocol (see **Supp. Fig. 7** for testing sequence details). For matched amplitude testing, we considered the putative changes in CMAP amplitude to adjust the TMS intensity to the desired 1st MEP amplitude.

For MEP, LICI, and CMAP computations, customized MATLAB programs defined amplitudes from peak-to-peak. We also considered the root mean square of the 50-ms interval prior to TMS delivery to assess the level of background muscle activity during MEP testing. The individual values introduced into the analyses were the median scores of the 20 events recorded for every testing time point.

Statistical analyses

Statistical analyses of corticomotor excitability were performed with ANOVA. ANOVA had the following factors: STIM (real and sham tSMS) and TIME (i.e., testing time points).

- For testing aftereffects of left DLPFC-tSMS on excitability at rest before any motor action, factor TIME had three levels: PRE-1, PRE-2, and PostMagnet.
- For testing aftereffects of left DLPFC-tSMS on excitability changes produced by FT execution, factor "TIME" included another three levels: PRE-task, POST-FT familiarization task, and POST-FT whole fatigue protocol.