Sessions of prolonged continuous theta burst stimulation or high-frequency 10 Hz stimulation to left dorsolateral prefrontal cortex for three days decreased pain sensitivity by modulation of the efficacy of conditioned pain modulation

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Running title: rTMS to left DLPFC modulates CPM efficacy

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ABSTRACT

10Hz repetitive transcranial magnetic stimulation (10Hz-rTMS) to the left dorsolateral prefrontal cortex (L-DLPFC) produces analgesia, probably by activating the pain modulation system. A newer rTMS paradigm, called theta burst stimulation (TBS), has been developed. Unlike 10Hz-rTMS, prolonged continuous TBS (pcTBS) mimics endogenous theta rhythms, which can improve induction of synaptic long-term potentiation. Therefore, this study investigated whether pcTBS to the L-DLPFC reduced pain sensitivity more efficiently compared with 10Hz-rTMS, the analgesic effects lasted beyond the stimulation period, and the reduced pain sensitivity was associated with increased efficacy of conditioned pain modulation (CPM) and/or intra-cortical excitability. Sixteen subjects participated in a randomized cross-over study with pcTBS and 10Hz-rTMS. Pain thresholds to heat (HPT), cold (CPT), pressure (PPT), intra-cortical excitability assessment, and CPM with mechanical and heat supra-pain threshold test stimuli and the cold pressor test as conditioning were collected before (Baseline), 3 (Day3) and 4 days (Day4) after 3-day session of rTMS. HPTs and PPTs increased with 10Hz-rTMS and pcTBS at Day3 and Day4 compared with Baseline (P=0.007). Based on pooled data from pcTBS and 10Hz-rTMS, the increased PPTs correlated with increased efficacy of CPM at Day3 (P=0.008), while no correlations were found at Day4 or with the intra-cortical excitability.

Perspective: Preliminary results of this comparative study did not show stronger pain sensitivity reduction by pcTBS compared with 10Hz-rTMS to the L-DPFC. Both protocols maintained increased pain thresholds up to 24-hours after the last session, which were partially associated with modulation of CPM efficacy but not with the intra-cortical excitability changes.

Trial registration number: NCT03733015

Key words: pain; repetitive transcranial magnetic stimulation; dorsolateral prefrontal cortex; conditioned pain modulation, intracortical excitability; diffuse noxious inhibitory control

INTRODUCTION

Non-invasive brain stimulation has received a lot of attention as a potential pain therapy [23,34]. For instance, 10Hz repetitive transcranial magnetic stimulation (rTMS) applied to the left dorsolateral prefrontal cortex (L-DLPFC) decreased pain sensitivity in healthy subjects [13,62] and reduced experimental [56,61,62], post-operative [9,10,14], and chronic pain [12,45,57]. Although several studies demonstrated pain relief effects of daily 10Hz L-DLPFC rTMS sessions in chronic [12,45,57] and in experimental pain [56], no studies have proven whether the decreased pain sensitivity in healthy subjects could be maintained for several hours by repetitive sessions of rTMS, which may have a clinical relevance if one considers rTMS to be potentially useful before painful procedures.

Although still unclear, the mechanism underlying 10Hz rTMS-induced analgesia may be mediated by the activation of descending pain control systems [61,62] or the neuro-modulatory effects on the intra-cortical excitability [21]. For instance, a recent study demonstrated an anatomical circuitry from the periaquaductal grey and the nucleus cuneiformis to the L-DLPFC [29], and 10Hz L-DLPFC rTMS increased the L-DLPFC activity and attenuated the brainstem and medulla responses to painful stimuli [61], indicating that L-DLPFC rTMS may drive top down analgesia by modulating the descending nociceptive control pathways [61]. An alternative explanation can be the modulation on the intra-cortical excitability. Indeed, short intra-cortical inhibition (SICI) was reduced in chronic pain [36,40] and in healthy subjects during experimental pain [21,52,53]. Using 5Hz L-DLPFC rTMS during experimental pain, SICI normalization and pain reduction have been shown [21], indicating a possible intra-cortical modulatory action of L-DLPFC rTMS.

A newer paradigm of rTMS, called theta burst stimulation (TBS), has been recently developed [25,28,47], which is much shorter than “classical” 10Hz rTMS and appear to have stronger and more reproducible clinical and neuromodulatory effects [28,42,60]. The application

of theta burst patterns of stimulation to induce synaptic long-term potentiation comes from the burst discharge at theta rhythms (ranges from 4 to 7 Hz) described in hippocampus of animals during exploratory behavior [60]. In humans, continuous (cTBS) with 600 pulses have been demonstrated to induce long-term depression [33,60]. However, when cTBS is prolonged to 1200 pulses (pcTBS), the cortical effect becomes facilitatory [24], similar to what it has been described following intermittent TBS (iTBS). Although pcTBS and iTBS are both facilitatory paradigms, a stronger analgesic effect have been recently reported by pcTBS compared with the iTBS and 10 Hz rTMS to the primary motor cortex (M1)[42]. In addition, in patients with treatment-resistant depression, a similar antidepressant effect between 10Hz and iTBS to the LDPFC has been recently demonstrated [7]. To date, no studies have compared whether L-DLPFC pcTBS would reduce pain sensitivity more efficiently compared with the "classical" 10Hz-LDPFC rTMS. Therefore, this study aimed to compare the analgesic effects of 3-day consecutive sessions of two rTMS protocols and investigated whether 1) L-DLPFC pcTBS produced stronger reduction in pain sensitivity compared with 10Hz L-DLPFC rTMS, 2) the effects lasted beyond the last day of stimulation, and 3) the increase of pain thresholds were associated with changes in the descending pain control system and/or in the intra-cortical excitability.

METHODS

Participants

Based on a previous study showing the strongest analgesic effect of pcTBS compared with 10Hz rTMS to the primary motor cortex (M1) on 13 healthy subjects [42], 16 participants were recruited in this preliminary randomized cross-over study at Hospital das Clínicas (University of São Paulo, Brazil), between September and November 2018. The study was approved by the local Ethics Committee (54271916.8.0000.0068), registered at ClinicalTrials.gov (NCT03733015), and

performed in accordance with the Helsinki Declaration. Written informed consent was obtained prior to study commencement. All participants were naïve to transcranial magnetic stimulation (TMS), and without any history of chronic pain or neuropsychiatric disorders. At the day of the recruitment, participants completed the following questionnaires: 1) Pain Catastrophizing Scale (PCS)[59], 2) Beck Depression Inventory (BDI-II)[5], 3) Positive and Negative Affective Schedule (PANAS)[63], and 4) insomnia questionnaire [43]. Also, participants filled out a screening questionnaire to screen for potential contraindications for rTMS [50].

Study design

Participants were randomly assigned to two intervention sequences of four experimental sessions on consecutive days (from Day1 to Day4; Fig. 1). A researcher prepared the concealed allocation schedule by computer randomization of these 2 intervention sequences to a consecutive number series without any involvement on the data collection. Both participants and the investigator involved in the data collection were blind to intervention sequences. Sequence 1 consisted of active pcTBS + sham 10Hz rTMS (7/16 participants) and Sequence 2 of sham pcTBS + active 10Hz rTMS (9/16 participants). Participants received the opposite protocol with an interval of 16 ± 4 days (min 11 days, max 26 days).

Each experimental session at Day1 and Day3 began with cortical excitability assessment by TMS. After this, mechanical and thermal pain thresholds (PTs) were collected in a randomized order. Subsequently, mechanical and heat supra-pain threshold stimulations as test-stimuli (TS) were evaluated in a randomized order. Finally, the cold pressor test was used as conditioning stimulus, and mechanical and heat supra-pain threshold test stimuli, and mechanical pain thresholds were repeated in the randomized order as used before the conditioning stimulus. At Day4, PTs, TS and conditioned TS were repeated.

Pain sensitivity

Pressure (PPT), cold (CPT), and heat pain thresholds (HPT) were recorded. The PPT was measured using a handheld pressure algometer (1-cm^2 probe, Algometer type II, SBMEDIC Electronics, Solna, Sweden), applying pressure at a rate of 30 kPa/s perpendicular to the skin. The pain sensitivity to thermal stimuli was recorded using a Medoc (TSA II Neurosensory Analyzer, Ramat Yishai, Israel), using a standard thermode of 30x30 mm [2]. The PPT, CPT and HPT were defined as the point where the stimulus perception changed to a perception of pain. The participants were instructed to press a button as soon as the stimulation became painful. The interval between each measure was 30 s and the mean of three successive measures were used for the analyses.

Four different sites were assessed for the PPT: 1) Right extensor carpi radialis brevis (ECRB), 2) left ECRB, 3) right tibialis anterior (TA), and 4) left TA muscles [37]. The recordings were made at the right and left thenar eminences for the CPT, and over the right mid-thigh for HPT [2]. The mean value of PPT and CPT across different sites was used for the statistical analysis.

Conditioned pain modulation

For the conditioned pain modulation (CPM), supra-pain threshold TS were collected before and immediately after the cold pressor test. Participants rated the pain intensity using a visual analogue scale (VAS, 'no pain' = 0 to 'worst imaginable pain' = 10 cm). The heat supra-pain threshold TS (HTS) was delivered for 5 s at 3° C above the HPT over the right mid-thigh. The pain intensity was scored on the VAS immediately after the HTS. The mechanical supra-pain threshold TS (MTS) was applied over the right TA muscle. The MTS intensity was estimated by applying increasing pressure (30 kPa/s) until the participants pressed a button as soon as the stimulation reached 5 cm on the VAS; subsequently this intensity was used as the MTS with a fast increase to the target intensity, applied for 5 s and rated on the VAS.

According to CPM recommendations [64], participants immersed the left hand in a bucket of water and ice at 4° C for up to 60 s (cold pressor test). Immediately after they withdrew their hand, HTS and MTS were reassessed over the right thigh and TA muscle, respectively. In addition, three measures of PPT over the right TA were repeated. CPM was calculated as a relative difference to the TS before conditioning (e.g. HTS during conditioning minus HTS before conditioning). The mean value of VAS reduction across HTS and MTS was used for the statistical analysis.

Cortical excitability

Participants were comfortably seated and instructed to maintain their hand completely relaxed. Magnetic stimulation was applied (MagPro X100, MagVenture A/S, Farum, Denmark) with a circular coil (MCF-125) to the left M1. Motor evoked potentials (MEPs) were recorded using surface disposable recording electrodes (Kendall™ Electrode, Danlee Medical Products, New York, USA) located on the right first dorsal interosseous muscle-. MEP signals were filtered at 5 Hz - 1 kHz and sampled at 1 kHz (Neuro-MEP-Micro, 2-channel Ultraportable EMG, Ivanovo, Russia). The optimal cortical site (hotspot) was determined as the coil position that provoked a maximal peak-to-peak MEP for a given stimulation intensity. 7 measures were collected at the hotspot: Resting motor threshold (rMT), motor-evoked potentials (MEPs) at 120%, MEPs at 140%, short intra-cortical inhibition (SICI) at 2 and 4 ms, and intra-cortical facilitation (ICF) at 10 and 15 ms [19].

The rMT was defined as the lowest intensity eliciting a MEPs of at least 50 μV in 50% of trials [51]. MEPs were recorded at 120% (MEP120) and 140% (MEP140) of the rMT at rest to evaluate the corticomotor excitability [51], and the stimulus–response gain (ratio of the amplitudes: MEP140/MEP120) was extracted [19]. Paired pulses were delivered randomly at 2, 4, 10 and 15 ms inter-stimulus interval, with the intensity of the first stimulus set at 80% of the rMT and the intensity of the second stimulus at 120% of the rMT [19,21,36,40]. For each measurement, the

results of 4 trials were averaged, and the changes in test MEP induced by conditioned stimuli (paired pulses) were expressed as a percentage of the unconditioned MEP amplitude at 120% [18,19,40]. The mean percentage inhibition with inter-stimulus interval 2 ms and 4 ms and facilitation with the inter-stimulus interval 10 ms and 15 ms were used for the statistical analysis [42].

Repetitive transcranial magnetic stimulation

rTMS was applied (MagPro X100, MagVenture A/S, Farum, Denmark) with a double coil (MCF-B65 Butterfly Coil), with the main phase of the induced current in the anterior-posterior direction [19,40,42]. The coil was fixed to an arm, positioned over the L-DLPFC, according to the BeamF3 algorithm [4,41], and stimulation intensity was set at 90% of the rMT of the FDI muscle.

pcTBS consisted of 3 pulses at 50 Hz repeated 400 times with inter-stimulus intervals of 200 ms [25,42]. The total pulses of pcTBS were 1200 delivered in 1 min and 44 s. The 10Hz rTMS consisted of 30 trains of 10 s with an interval of 20 s between trains [9,11]. Each train included 100 pulses and the total number of pulses was 3000 given in 15 min.

Sham stimulation was performed with a sham coil of identical size, color and shape, emitting the same sound of the active coil [19,40,42]. In each stimulation session, participants received two sequential rTMS applications, one active and one sham stimulation (either sham-10Hz rTMS or sham-pcTBS). Both types of stimulation were delivered sequentially one immediately after the other, so that participants received a total of 16m 44s (15 min + 1 min 44 s) of stimulation in each stimulation session. This design was chosen because both stimulation methods have durations that are too different and this could affect blinding if sham stimulation was not added to equalize the total amount of stimulation duration.

Because the rTMS procedure is known to be slightly painful [8], pain ratings of the procedure was acquired at the end of the study, using a numerical rating scale (NRS) for pain intensity, where

0 was no pain and 10 was most intense pain imaginable. Besides, blinding was assessed at the end of the study, by asking the participants whether they could guess the correct sequence of rTMS administered.

Statistics

All data are presented as mean and standard deviations (SD). Statistical significance was set at $P < 0.05$. All data were assessed for normality using visual inspection.

The effects of the two interventions on pain sensitivity, neurophysiological and CPM measures were assessed by 3-way mixed-model analysis of variance (ANOVA) with *Days* (Day1, Day3, and Day4) and *Interventions* (pcTBS and 10Hz rTMS) as within subject factors and *Sequence* (Sequence-1 and Sequence-2) as a between subject factor. In case of significant differences, post-hoc analyses were performed using Bonferroni to correct for multiple comparisons.

To investigate whether the cold pressor test produced a CPM effect and the paired-pulses produced an SICI and ICF, a 3-way mixed-model ANOVA with *Days* (Day1, Day3 and Day4), *Condition* (unconditioned and conditioned stimulus), and *Interventions* (pcTBS and 10Hz rMT) as within subject factors and *Sequence* (Sequence-1 and Sequence-2) as between subjects were performed on TS and PPT, as well as single pulses MEPs and paired-pulses MEP (SICI and ICF).

Association between changes in pain thresholds (HPT, CPT and PPT), the intracortical excitability (SICI and ICF) and CPM (supra-pain threshold TS and PPTs) were explored as the differences at Day3 and Day4 (only CPM), relative to Day1 using Pearson correlations. Whether changes in pain thresholds were stable from Day3 to Day4 were investigated as the differences from Day1 to Day3 and Day4, respectively, which were correlated using Pearson correlation. To compensate for multiple correlations, the P-value was Bonferroni corrected.

RESULTS

The morphology and questionnaires are shown in Table 1 and they were within the normal ranges [5,43,59,63]. All participants performed all sessions and no data were missing.

Pain sensitivity

A main effect of Days was found for the PPT (Table 2; $F_{2,28}$ = 24.69; P < 0.001), CPT ($F_{2,28}$ = 5.86, P = 0.008), and HPT ($F_{2,23.9}$ = 7.54, P = 0.007). Post-hoc testing demonstrated increased PPTs and HPTs at Day3 (increased by 39.1±46.1 kPa and 1.3±1.6 °C; P < 0.016) and at Day4 (71.7±41.7 kPa and 1.5±2.2 °C; P < 0.039) compared with Day1. A tendency towards decreased CPT was found at Day3 (-1.5±2.1 °C; P = 0.059) and at Day4 (-1.9±2.6 °C; P = 0.060) compared with Day1. No significant main effects or interactions of Intervention and Sequence were found for the PPT, HPT, and CPT, indicating that both protocols produced similar decrease in pain sensitivity.

Conditioned pain modulation

Three-ways mixed-model ANOVA did not show any statistical changes in perceived pain intensity for HTS and MTS across Day, Intervention or Sequence. Similarly, the conditioned painful stimuli were not significantly modified by the two interventions (Table 3). The conditioning stimulation led to a decrease in pain VAS scores of 1.0±1.7 cm for the HTS ($F_{1,14}$ = 17.96; P < 0.001), 1.6±1.8 cm for the MTS, and an increase of 98.8±110.4 kPa for the PPT ($F_{1,14}$ = 46.87; P < 0.001) across all days. No statistical changes across Day, Intervention or Sequence were found (Table 4).

Cortical excitability

All raw data are reported in Table 5 and there were no significant ANOVA factors or interactions across Day, Intervention or Sequence. The paired pulses led to a modulation of the single pulse MEP ($F_{2,28}$ = 37.73; P < 0.001). Indeed, the average 2 ms and 4 ms inter-stimulus interval (SICI)

produced an MEP inhibition of 57.9±22.6% (P < 0.001) compared with MEP at 120% rMT. In contrast, average 10 ms and 15 ms inter-stimulus interval (ICF) produced an MEP increase of 48.3±73.4% (P = 0.008) compared with the single pulse MEP at 120%. A Day*Intervention interaction was found in the SICI at 2 ms interval ($F_{1,14}$ = 6.65, P = 0.022), but post-hoc testing did not show any statistical difference (all P > 0.15).

Associations between pain sensitivity, intracortical excitability and conditioned pain modulation

Since no group effect was found for the two rTMS protocols the data were pooled for the correlations. No significant association were revealed between the relative differences (Day1 to Day3) of HPT, CPT and PPT with SICI, ICF (Pearson r > -0.287; P = 1).

A significant association between the differences (Day1 to Day3) of PPT with supra-pain thresholds CPM (average of MTS and HTS CPM; Pearson r = -0.578; P = 0.008; Fig. 2), but not with PPT CPM (Pearson r = -0.215; P = 0.713). No statistical correlations were found between CPT or HPT with supra-pain thresholds CPM (average of MTS and HTS CPM) or PPT CPM (Pearson r < -0.349; P > 0.585). No statistical changes were found between the differences (Day1 to Day4) of PPT, CPT or HPT with supra-pain thresholds CPM or PPTs CPM (Pearson r < -0.438; P > 0.121).

Maintained reduced pain sensitivity at Day4

A correlation between the difference relative to Day1 at Day3 and at Day4 were found for the PPT (Pearson r = 0.64, P < 0.001), CPT (Pearson r = 0.62, P < 0.001), and HPT (Pearson r = 0.75, P < 0.001), indicating the analgesic effect was steadily maintained up to 24 hours (Fig. 3).

Adverse effects and blinding

No adverse effects occurred in the study. The mean pain VAS during the stimulations was 0.8±1.3 cm. Only one volunteer was able to identify the correct sequence of the stimulation administered.

DISCUSSION

The present study assessed, for the first time, the temporal profile and nature of the reduced pain sensitivity of two different patterns of multiple sessions of rTMS to the same cortical target in healthy subjects. Opposite to the first hypothesis, the 10Hz-rTMS and the pcTBS to the L-DLPFC produced a similar increase of the pain thresholds. Besides, the pain threshold modulation induced by rTMS lasted up to 24 hours after the last stimulation. Finally, a correlation between the changes in PPT and CPM was found at Day3, indicating that both pain modulations could be a consequence of the short-lasting effect of repeated magnetic stimulation to the L-DLPFC.

Temporal profile of increased pain thresholds

Recently, L-DLPFC iTBS has been successfully tested in major depression [7] and approved by the Food and Drug Administration for patients with medication-resistant depression. In pain research, no studies have investigated the effect of L-DLPFC pcTBS on pain thresholds or during experimental and chronic pain. The results of the present study indicate that L-DLPFC pcTBS did not show any stronger analgesic effect compared with 10Hz L-DLPFC rTMS. Previously, Moisset et al. reported a CPT decrease of ~3°C after pcTBS to the M1 [42], while in the current study, L-DLPFC pcTBS and L-DLPFC 10Hz-rTMS showed a CPT decrease of ~1.5°C. Besides, a CPT decrease from 2 to 3°C has been reported in response to 10Hz-rTMS to right DLPFC [18,19] and M1 [18,19,42,44], indicating a less effective effect of L-DLPFC stimulation compared with M1 [18,19,42,44], and slightly lower effect compared with the right DLPCF [18,19]. However, since the HPT and PPT have not evaluated in these previous studies, it is impossible to know whether this stronger analgesic effect is specific to CPT or generalized to all pain sensation. Indeed, Nahmias et al. reported that neither 10Hz-rTMS to M1 or right DLPFC modified the HPT [44], while Taylor et al. showed a HPT increase of 1 to 2°C after 10Hz L-DLPFC rTMS [62] as the present study. Globally, these studies

indicate that CPT and HPT are differently affected by rTMS to M1, right or left DLPFC [44,61,62], probably because diverse brain regions or mechanisms are involved in different types of pain [3].

Experimental [56] and chronic pain studies [32,40,48] showed that the peak of analgesic effect induced by rTMS required few days after the beginning of the treatment (3-5 days), and can last few days after the last rTMS sessions (from 3 days to 2 weeks) [32,40,48,57], suggesting a cumulative analgesic effect of rTMS. In healthy subjects, previous studies reported that a single session of 10Hz L-DLPFC rTMS increased the HPT of around 1-2°C up to 1 hour [13,62]. The results of the present study expanded on this knowledge by demonstrating that the increase of pain thresholds lasted at least up to 24 hours after the last session. Importantly, a similar effect on pain thresholds after both stimulations was found, although the different number of pulses. However, previous studies showed that increasing or reducing the number of TBS pulses does not extend the excitatory effects and might produce an opposite effect [25,28]. Future studies are needed to evaluate whether 1) repeated L-DLPFC rTMS sessions produce an increase of pain thresholds longer than 24 hours, 2) repeated daily sessions of pcTBS before a clinical painful procedure can reduce the pharmacological-controlled analgesia in the following days [14] and may prevent the development of chronic pain following acute injury or surgery. In fact, high pain intensity in the early stage of acute pain appears to be one of the strongest predictors of chronic pain development [20,31]. Therefore, intervention like left DLPFC rTMS, able to increase pain thresholds with minimal side effects, may have the potential future clinical application of reducing pain sensitivity. Finally, the main practical advantage of TBS is the shorter stimulation time (below 2 minutes) and fewer number of pulses (1200) compared with the 'classical' 10Hz-rTMS (from 1500-4000 pulses in 15-20min) [42,62].

Descending pain modulation system

Neuroimaging studies reported that 10Hz L-DLPFC rTMS induced local cortical activity changes, but also in distant brain regions, such as the medulla and the brainstem [17,58,61]. Naloxone pretreatment abolished the medulla and the brainstem response induced by 10Hz L-DLPFC rTMS, as well as the analgesic effect, suggesting that L-DLPFC stimulation drive a top-down opioidergic analgesia thought the diffuse inhibitory pain system [61]. To test this hypothesis, CPM was systematically measured before and after 3-day sessions L-DLPFC rTMS. The results of the current study did not show any facilitation of the CPM, however a correlation between the increase in PPT and the increase in CPM was found, suggesting that both adaptations could be a consequence of a common driving factor. Similar to the current study, previous studies investigated whether a single session of 10Hz-rTMS and pcTBS to M1 and right DLPCF were able to modulate the CPM, but no correlation between the changes in CPT and the changes in CPM was found [42,44]. A possible explanation of the different findings may be the cortical target. Indeed, M1 and right DLPFC 10Hz-rTMS may induce analgesic effect by means of different brain mechanisms [6,55]. However, based on PPT measures, previous studies showed that transcranial direct current stimulation to M1 potentiated CPM in healthy subjects [22,49], indicating that M1 stimulation may modulate the pain descending modulatory systems. It is interesting to note that only the PPTs changes from Day1 were associated with CPM in the current study, while the thermal pain thresholds changes did not correlate with CPM as shown in previous studies [42,44]. This may suggest that descending modulation could act differently on diverse pain stimulations [44]. An alternative explanation may be the number of rTMS sessions. Indeed, when multiple sessions of rTMS are delivered, cumulative neuroplastic and therapeutic effects have been demonstrated [1,26–28], indicating long-lasting and more robust effects induced by multiple daily sessions of rTMS compared with a single session. Finally, no correlation was found between PPTs and CPM at Day 4, suggesting a short-lasting effect of the neuromodulation of the descending pain modulation system.

Intra-cortical excitability

As previous studies applying 10Hz-rTMS to right DLPFC [19] and M1 [42], SICI was not influenced by the present two L-DLPFC rTMS protocols. Reduced SICI have been described in several chronic pain condition, such as neuropathic [35,36,39,54] and musculoskeletal pain [15,38]. Besides, when prolonged experimental pain was applied in healthy subjects, reduced SICI has been also demonstrated [21,52]. Chronic pain studies demonstrated that several sessions of 10Hz-rTMS to M1 produced pain relief [32,40,48] and normalized the SICI [35,36,40]. In line with these clinical findings, applying topical capsaicin in healthy subjects, 5Hz L-DLPFC rTMS reduced the pain intensity and normalized the SICI [21]. Yet, in the chronic [36,40] and experimental pain studies [21], the changes observed after the rTMS treatment showed a normalization of the reduced SICI, suggesting that rTMS modulation of SICI may depend upon the presence of baseline continuous pain and subsequent altered cortical excitability to occur. Indeed, others have also reported lack of effect of rTMS on cortical excitability parameters, despite significant analgesic effects on pain thresholds [19,42,44].

Limitations

There are some notable limitations to the current study. First, a sham group has not been included in this study, since the aims were to investigate the pain sensitivity difference between two active protocols and the association between the analgesic effect and the intracortical excitability or CPM, rather than the efficacy of rTMS versus sham rTMS in healthy subjects. Several previous studies showed that 10Hz L-DLPFC rTMS produced analgesic effects in healthy subjects and in patients compared with a sham stimulation [9,12,14,45,57,61,62]. Importantly, the changes in HPT in the current study are similar to those described in previous active 10Hz L-DLPFC rTMS groups [13,62], but the effect on CPT are lower compared with M1 and right DLPCF stimulations, indicating an unlikely placebo effect.

Several complementary mechanisms associated with pain relief by rTMS have not been investigated in the current study. In fact, 10Hz L-DLPFC rTMS provokes secondary changes in several brain areas, such as orbitofrontal cortex, the insula and the anterior cingulate cortex [17,58]. All these areas are implicated, for instance, in reward, emotion, sympathetic and parasympathetic activity and, consequently, in the regulation of pain perception. Further specific studies are needed to determine these changes in adjacent cortical areas.

A third limitation in the current study is the TBS protocol selected. Recent studies have reported excellent effects with 30Hz (rather than 50Hz) bursts repeated at 10 Hz (rather than 5Hz) [30,46].

Finally, the study has not been performed in patients where additional factors play a crucial role in pain sensitivity, such as stress, anxiety, and medical expectations [16].

Conclusions

Preliminary results of this comparative study showed that the increase of the pain thresholds after 3-day sessions of pcTBS and 10Hz-rTMS to the L-DLPFC were similar for both protocols, lasted at least up to 24 hours after the last rTMS session, and were correlated with modulation of the CPM efficacy at Day3. Thus, the less extensive pcTBS protocol may be attractive for future studies clarifying its clinical potential.

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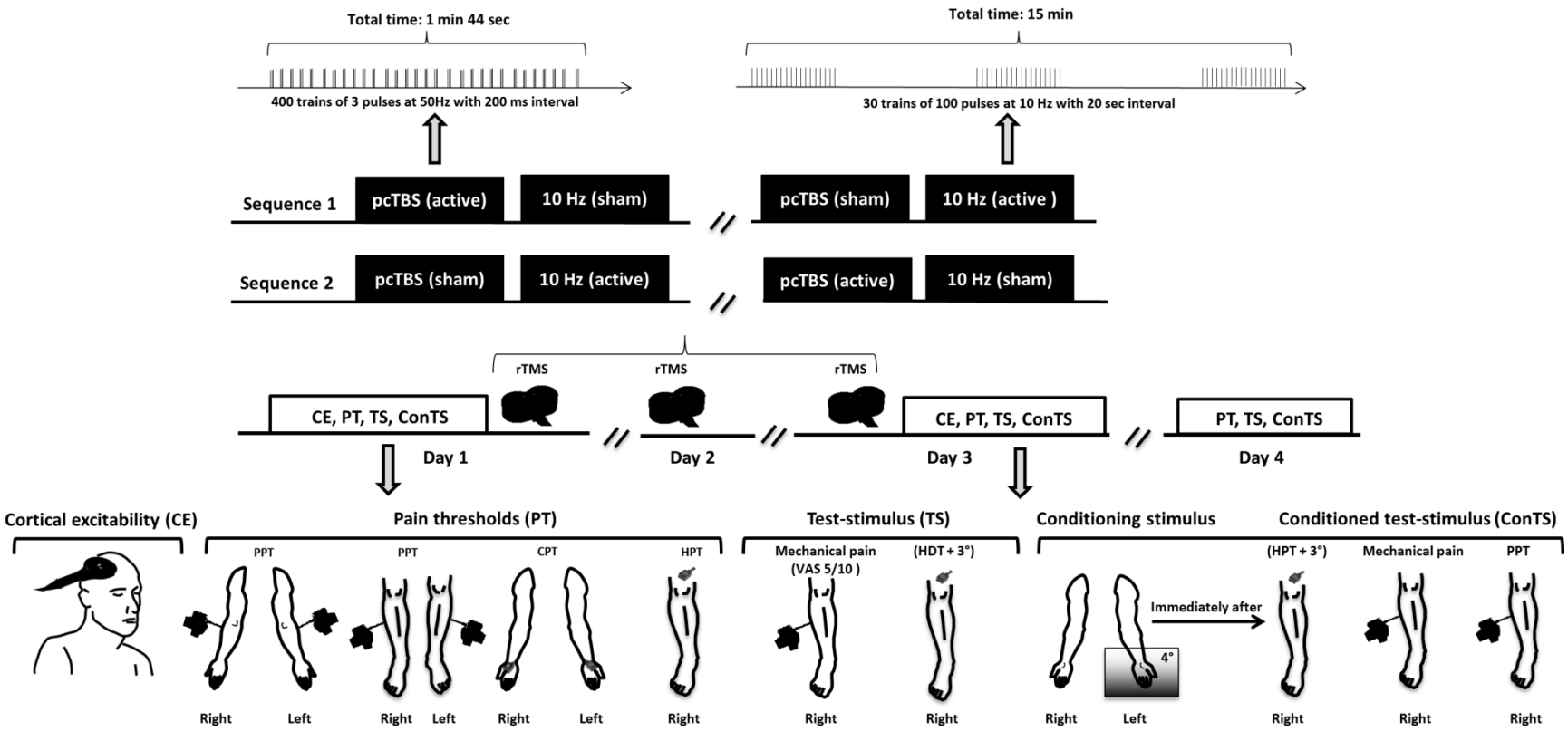
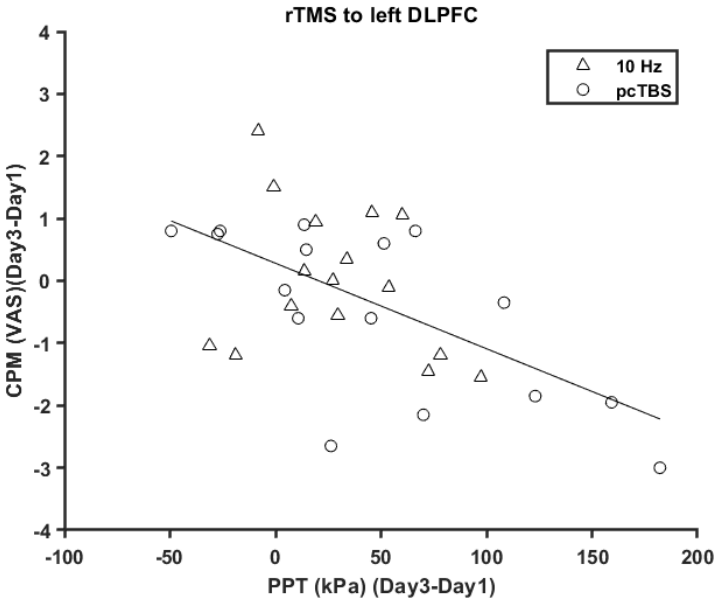
FIGURE LEGENDS

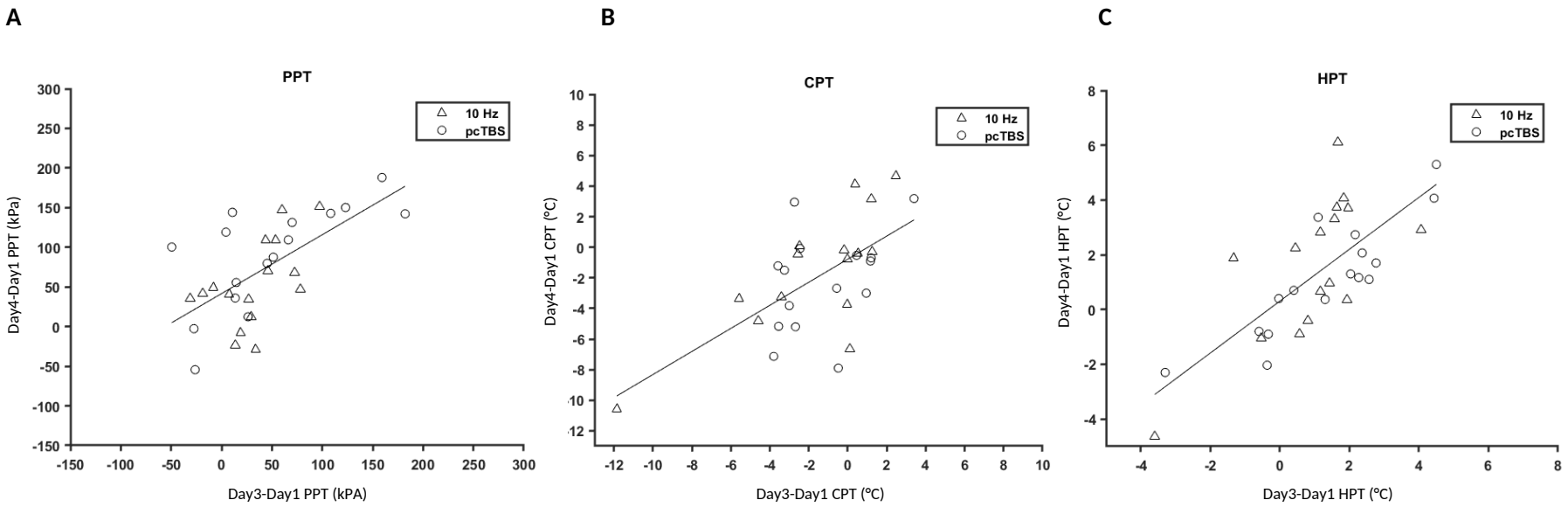
Figure 1: Three consecutive sessions of rTMS interventions (pcTBS and 10 Hz rTMS) to L-DLPFC were performed at Day1 (immediately after the measurements), Day2 and Day3 (before the measurements). Cortical excitability (CE) was assessed at Day1 and Day3. Mechanical and thermal pain thresholds (PT), Test-Stimulus (TS) and conditioned pain stimuli (ConTS) were assessed on Day1, Day3 and Day4.

Figure 2: Correlations between changes in pressure pain thresholds (PPT) and conditioning pain modulation (CPM; mean value of VAS reduction across heat and pressure test stimuli) at Day3. Data expressed as the difference relative to Day1. The triangle represents the 10 Hz stimulation and the circle the prolonged continuous theta burst stimulation (pcTBS).

Figure 3: Correlations between changes in pain thresholds at Day3 and Day4 (data expressed as the difference relative to Day 1). Effects on pressure (**A**, PPT), cold (**B**, CPT) and heat (**C**, HPT) pain thresholds are illustrated. The triangle represents the 10 Hz stimulation and the circle the prolonged continuous theta burst stimulation (pcTBS).


Total time: 1 min 44 sec
400 trains of 3 pulses at 50Hz with 200 ms interval
Total time: 15 min
30 trains of 100 pulses at 10 Hz with 20 sec interval
Sequence 1
pcTBS (active)
10 Hz (sham)
pcTBS (sham)
10 Hz (active)
Sequence 2
pcTBS (sham)
10 Hz (active)
pcTBS (active)
10 Hz (sham)
rTMS
rTMS
rTMS
CE, PT, TS, ConTS
CE, PT, TS, ConTS
PT, TS, ConTS
Day 1
Day 2
Day 3
Day 4
Cortical excitability (CE)
Pain thresholds (PT)
Test-stimulus (TS)
Conditioning stimulus
Conditioned test-stimulus (ConTS)
PPT
PPT
CPT
HPT
Mechanical pain
(VAS 5/10)
(HDT + 3°)
(HPT + 3°)
Mechanical pain
PPT
Immediately after
4°
Right
Left
Right
Left
Right
Left
Right
Right
Right
Right
Left
Right
Right
Right


rTMS to left DLPFC
10 Hz
pcTBS
CPM (VAS)(Day3-Day1)
PPT (kPa) (Day3-Day1)
4
3
2
1
0
-1
-2
-3
-4
-100
-50
0
50
100
150
200


A
PPT
10 Hz
pcTBS
Day4-Day1 PPT (kPa)
Day3-Day1 PPT (kPA)
B
CPT
10 Hz
pcTBS
Day4-Day1 CPT (°C)
Day3-Day1 CPT (°C)
C
HPT
10 Hz
pcTBS
Day4-Day1 HPT (°C)
Day3-Day1 HPT (°C)

Sample size (females)	16 (9)
Age (years)	30.9 ± 8.8
Height (cm)	168 ± 7.2
Weight (kg)	72 ± 17.1
BDI-II	4.6 ± 5.7
PCS	2.3 ± 3.8
PANAS-negative	14.7 ± 4.6
PANAS-positive	40.1 ± 7.5
Insomnia questionnaire	6.3 ± 5.4

Table 1. Participant characteristics. Beck Depression Inventory (BDI-II); Pain Catastrophizing Scale (PCS); positive and negative affective schedule (PANAS).

Modality	Site	Intervention	Day1	Day3	Day4
PPT (kPa)	Right ECRB	10Hz	256.3±59.2	315.6±67.7*	324.9±63.3*
		pcTBS	251.2±42.5	302.5±78.7*	334.5±75.9*
	Left ECRB	10Hz	278.6±83.6	304.6±63.1*	315.3±65.6*
		pcTBS	257.2±52.0	305.4±86.2*	331.9±93.3*
	Right TA	10Hz	564.8±162.2	569.5±177.9*	628.2±173.5*
		pcTBS	520.1±176.4	559.5±191.6*	636.4±170.5*
	Left TA	10Hz	507.4±167.2	537.2±135.9*	551.9±199.9*
		pcTBS	474.4±131.0	528.7±148.9*	560.3±139.1*
CPT (℃)	Right hand	10Hz	12.9±5.7	11.1±5.0	11.7±5.1
		pcTBS	11.9±4.8	11.2±5.7	10.3±4.9
	Left hand	10Hz	14.0±6.1	12.5±5.3	12.2±4.4
		pcTBS	15.8±3.5	14.0±4.0	12.9±5.1
HPT (℃)	Right thigh	10Hz	44.5±2.9	45.4±2.4*	46.1±1.7*
		pcTBS	45.1±2.3	46.4±1.5*	46.2±1.4*

Table 2. Mean (± SD, N = 16) pressure pain thresholds (PPTs) on extensor carpi radialis brevis (ECRB) and tibialis anterior (TA) muscles, cold pain threshold (CPT) on right and left hand, and heat pain threshold (HPT) on the right thigh recorded before (Day1) and after (Day3, Day4) repetitive transcranial magnetic stimulation (rTMS) protocols (10Hz and prolonged continuous theta burst stimulation [pcTBS]) to the L-DLPFC. Significantly increased compared with Day1 within the group (*, $P<0.05$).

Test stimuli	Intervention	Condition	Time		
			Day 1	Day 3	Day 4
HTS VAS (cm)	10Hz	Test-stimulus	6.4±2.1	5.5±2.1	6.3±2.0
		Conditioned test-stimulus	5.4±2.4	4.7±2.3	5.1±2.5
	pcTBS	Test-stimulus	6.4±2.2	7.0±2.5	6.2±2.3
		Conditioned test-stimulus	5.7±2.8	5.4±2.8	5.4±2.8
MTS VAS (cm)	10Hz	Test-stimulus	5.0±0.0	5.0±0.0	5.0±0.0
		Conditioned test-stimulus	3.5±1.9	3.1±1.9	3.3±1.7
	pcTBS	Test-stimulus	5.0±0.0	5.0±0.0	5.0±0.0
		Conditioned test-stimulus	3.7±1.8	3.6±1.7	3.2±1.8

Table 3: Mean (± SD, N = 16). Heat supra-pain threshold stimulus (HTS) and mechanical supra-pain threshold stimulus (MTS) before and after the cold pressor test. Recorded before (Day1) and after (Day3, Day4) repetitive transcranial magnetic stimulation (rTMS) protocols (10Hz and prolonged continuous theta burst stimulation [pcTBS]) to the L-DLPFC.

CPM	Intervention	Day 1	Day 3	Day 4
HTS VAS (cm)	10Hz	-1.2±1.2	-0.9±1.6	-1.1±1.4
	pcTBS	-0.7±1.6	-1.5±2.3	-0.8±2.1
MTS VAS (cm)	10Hz	-1.5±1.9	-1.9±1.9	-1.7±1.7
	pcTBS	-1.3±1.8	-1.5±1.7	-1.8±1.8
PPTs (kPa)	10Hz	105.8±96.5	108.6±94.0	73.5±153.7
	pcTBS	118.4±101.9	121.5±113.0	64.2±96.6

Table 4. Mean (± SD, N = 16). Conditioned pain modulation (CPM) effects (conditioned test-stimulus minus un-conditioned test stimulus) of heat supra-pain threshold stimulus (HTS), mechanical supra-pain threshold stimulus (MTS), and pressure pain thresholds (PPTs). Recorded before (Day1) and after (Day3 and Day4) repeated TMS protocols (10 Hz, and prolonged continuous theta burst stimulation [pcTBS]) on the L-DLPFC.

rTMS	Days	rMT (% MS0)	MEP 120% (mV)	MEP 140% (mV)	MEP Ratio (140/120%)	MEP 2 ms interval (mV)	MEP 4 ms interval (mV)	MEP 10 ms interval (mV)	MEP 15 ms interval (mV)
10Hz	Day 1	43.8±8.1	1.7±1.1	3.1±1.6	1.9±0.6	0.4±0.3	0.7±0.6	2.2±1.7	2.3±1.4
	Day 3	42.9±7.1	1.5±0.9	2.8±1.8	1.9±0.8	0.5±0.5	0.7±0.8	1.8±1.2	2.1±1.3
pcTBS	Day 1	42.7±6.7	1.4±0.9	3.1±2.1	2.2±1.1	0.5±0.5	0.9±0.7	2.2±1.4	2.2±1.1
	Day 3	43.3±7.8	1.7±1.0	3.2±2.1	1.9±0.6	0.6±0.5	1.0±8.8	2.3±1.7	2.1±1.4

Table 5. Mean (± SD, N = 16) cortical excitability parameters before (Day1) and after (Day3) rTMS (repetitive transcranial magnetic stimulation) on the L-DLPFC with either prolonged continuous theta burst stimulation (pcTBS) or 10 Hz rTMS (10Hz). rMT: resting motor threshold. MSO: maximum stimulator output. MEP: motor evoked potential.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page # where this item is located:
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5 and Figure 1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4,5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5,6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the	

		number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6,8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6,8
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	4

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10 and table 1
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*10-12*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	*10-12*

	(*b*) Report category boundaries when continuous variables were categorized
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	12,16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15,16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2