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'Nip it in the bud': Low-frequency rTMS of the prefrontal cortex disrupts threat memory consolidation in humans

Simone Battaglia [a,b,*], Claudio Nazzi [a], Miquel A. Fullana [c,d], Giuseppe di Pellegrino [a], Sara Borgomaneri [a,**]

[a] Center for Studies and Research in Cognitive Neuroscience, Department of Psychology "Renzo Canestrari", Cesena Campus, Alma Mater Studiorum Università di Bologna, 47521, Cesena, Italy
[b] Department of Psychology, University of Turin, 10124, Turin, Italy
[c] Adult Psychiatry and Psychology Department, Institute of Neurosciences, Hospital Clinic, 08036, Barcelona, Spain
[d] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), CIBERSAM, 08036, Barcelona, Spain

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ABSTRACT

It is still unclear how the human brain consolidates aversive (e.g., traumatic) memories and whether this process can be disrupted. We hypothesized that the dorsolateral prefrontal cortex (dlPFC) is crucially involved in threat memory consolidation. To test this, we used low-frequency repetitive transcranial magnetic stimulation (LF-rTMS) within the memory stabilization time window to disrupt the expression of threat memory. We combined a differential threat-conditioning paradigm with LF-rTMS targeting the dlPFC in the critical condition, and occipital cortex stimulation, delayed dlPFC stimulation, and sham stimulation as control conditions. In the critical condition, defensive reactions to threat were reduced immediately after brain stimulation, and 1 h and 24 h later. In stark contrast, no decrease was observed in the control conditions, thus showing both the anatomical and temporal specificity of our intervention. We provide causal evidence that selectively targeting the dlPFC within the early consolidation period prevents the persistence and return of conditioned responses. Furthermore, memory disruption lasted longer than the inhibitory window created by our TMS protocol, which suggests that we influenced dlPFC neural activity and hampered the underlying, time-dependent consolidation process. These results provide important insights for future clinical applications aimed at interfering with the consolidation of aversive, threat-related memories.

1. Introduction

In the last decade, neuroscientists have gathered crucial evidence that human threat memories are highly flexible and can be edited (Phelps & Hofmann, 2019). This has been possible mainly by interfering with the reconsolidation process, during which a previously consolidated memory, when reactivated, becomes unstable and thus malleable (Maddox, Hartmann, Ross, & Ressler, 2019). Pharmacological (M. R. Battaglia, Di Fazio, & Battaglia, 2023; S. Battaglia et al., 2023, 2024; Sevenster, Beckers, & Kindt, 2013; Tortora et al., 2023), behavioral (Schiller et al., 2010), and non-invasive brain stimulation techniques (Borgomaneri, Battaglia, Sciamanna, Tortora, & Laricchiuta, 2021; Borgomaneri et al., 2020) have been used to achieve this goal. One way to interfere with the formation and storage of an aversive memory might be to target its *consolidation process*, that is, to intervene when the memory trace is considered to be in a labile, unstable state, before it is consolidated, thus blocking retention of a newly acquired aversive learning.

Over the last few years, non-invasive brain stimulation has become a powerful method for modifying human threat memories (Asthana et al., 2013; Borgomaneri, Battaglia, Avenanti, & Pellegrino, 2021; Borgomaneri, Battaglia, Sciamanna, et al., 2021; Borgomaneri et al., 2020; Guhn et al., 2014; Raij et al., 2018). In one of these attempts, Ojala and colleagues (Ojala, Staib, Gerster, Ruff, & Bach, 2022) showed that continuous theta burst stimulation – cTBS, an inhibitory protocol of TMS – to the contralateral primary sensory cortex (S1) before threat acquisition decreased threat expression. However, while the somatosensory cortex may be involved in consolidating associative somatosensory aversive

* Corresponding author. Department of Psychology "Renzo Canestrari", University of Bologna, Viale Berti Pichat 5, 40127, Bologna, Italy
** Corresponding author. Department of Psychology "Renzo Canestrari", University of Bologna, Viale Berti Pichat 5, 40127, Bologna. Italy.
E-mail addresses: simone.battaglia@unibo.it (S. Battaglia), sara.borgomaneri@unibo.it (S. Borgomaneri).

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memories, other regions, in particular the prefrontal cortex, may be involved in consolidating aversive memories, regardless of their nature.

Within the prefrontal cortex, the dlPFC controls the recall and reactivation of memory traces (S. Battaglia, 2022; Cabeza & Nyberg, 2000; Eichenbaum, 2017; Moscovitch, 1992; Moscovitch & Winocur, 2002; Sandrini, Censor, Mishoe, & Cohen, 2013) and their progressive consolidation. Newer research suggests that this brain area is also involved in threat response reduction and threat memory modulation (Asthana et al., 2013; Mungee et al., 2014; van't Wout et al., 2016). Finally, the anatomical connectivity of the dlPFC with the amygdala (Delgado, Nearing, LeDoux, & Phelps, 2008), an essential region in threat processing, suggests a crucial role of the dlPFC in threat conditioning, as shown in our recent TMS study in which we demonstrated the crucial role of the dlPFC in the reconsolidation of aversive memories (Borgomaneri et al., 2020). However, several outstanding questions remain regarding the role of dlPFC in threat memory consolidation. Indeed, only one transcranial direct current stimulation (tDCS) study suggests that dlPFC may play a role in the initial stage of threat memory formation (Asthana et al., 2013), while another single tDCS study (Mungee et al., 2014) suggested that the dlPFC is involved in the reconsolidation process (i.e., the tDCS was applied 24 h after the consolidation following the presentation of a reminder). However, there is still a lack of understanding of the temporal boundaries of the consolidation process and within which time-window it can be modulated. Here, we aimed to answer these questions by targeting the dlPFC using low-frequency rTMS (LF-rTMS) at different times subsequent threat acquisition to test whether and when we could interfere with the consolidation of previously acquired aversive memory.

Based on these premises, we hypothesized that directly targeting the dlPFC with LF-rTMS within the consolidation window could impair threat memory recall, thereby revealing the crucial involvement of this area in the *consolidation* process. To this aim, we temporally interfered with the activity of the dlPFC by applying LF-rTMS within the memory stabilization time window, and subsequently assessed participants' threat memory recall at both recent and remote time points. If successful, our results could provide important insights into using neurostimulation as a treatment for fear-related pathologies, including anxiety and post-traumatic stress disorder (PTSD) patients (Rosson et al., 2022). Although some attempts have been made in this direction, current clinical interventions have not focused on interference with consolidation but rather on neurostimulation protocols after PTSD diagnosis, and without having participants perform a recall of the traumatic event, which is supposed to be critical for PFC activation (for a review, see Petrosino et al., 2021).

2. Materials and methods

2.1. Participants

Seventy-two healthy adult volunteers took part in the study. Participants were randomly assigned to one of four experimental groups: dlPFC (18 participants, 8 females, mean age ± SD: 22.4 ± 2.1), 6 h-dlPFC (18 participants, 9 females, mean age ± SD: 23.9 ± 3.0), Ctrl-Sham (18 participants, 10 females, 22.2 ± 1.8), or Ctrl-Occipital (18 participants, 11 females, 22.6 ± 1.7; see Fig. S1, panel A in Supplementary Material). All participants were right-handed and had normal or corrected-to-normal vision. None reported a current psychiatric, neurological or medical disorder nor any contraindication for rTMS (see the international TMS guidelines Rossi et al., 2009, 2021; Rossini et al., 2015). No discomfort or adverse effects of TMS were spontaneously reported by participants or noticed by the experimenter. Participants provided written informed consent before each session of the experiment. The procedures were approved by the University of Bologna Bioethics Committee and followed the ethical standards of the 1964 Declaration of Helsinki (World Health Organization, 2013).

2.2. Procedure and experimental design

The present study's design was adapted from our previous work (Borgomaneri et al., 2020). The study was performed at the Center for Studies and Research in Cognitive Neuroscience at the University of Bologna campus in Cesena, Italy. Participants were comfortably seated in a silent and dimly lit room, and their position was centered relative to the computer screen at a 100-cm viewing distance. Electrodes for skin conductance response (SCR) recording were attached to the distal phalanges of the second and third finger of the left hand, while shock electrodes were attached to the participant's right wrist. The SCR was continuously recorded while participants completed the task, and data were stored for offline analysis. Participants were asked to remain still during the task and focus their attention on the center of the screen. After verifying that SCR was recorded correctly, the shock intensity (US) was individually adjusted with a standard workup procedure. It was initially set at 0.5 mA (mA) and increased by 1 mA. At each step, the experimenter asked whether the administered shock was highly annoying. Thus, individual shock intensity was set when participants reported a highly annoying but not painful sensation (Dunsmoor, Murty, Davachi, & Phelps, 2015; Schiller, Levy, Niv, LeDoux, & Phelps, 2008). Finally, participants were informed that they could not influence the shock administration. The experiment used a delay differential threat conditioning paradigm (Fig. 1). The testing protocol involved different sessions administered over two consecutive days, during which the electrodes for the electric shock were attached to the participant's wrist (Kindt, Soeter, & Vervliet, 2009; Schiller et al., 2010; Sevenster et al., 2013). Each trial of the experiment consisted of the presentation of the conditioned stimulus for 4 s. The stimuli used as conditioned stimulus (CS+) and neutral stimulus (CS-) were counterbalanced among participants. The interstimulus interval (ISI) was a gray blank screen with a variable duration ranging from 14 to 17 s from stimulus offset to the following stimulus onset. The length of the ISI was chosen so that the SCR to the US in the preceding reinforced CS + trial would not overlap with the presentation of the following stimulus. At the end of the experiment, the State and Trait-Anxiety Inventory (STAI) (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) and the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983) were administered to participants.

2.3. Transcranial magnetic stimulation

TMS was applied with a Magstim super rapid^2 magnetic stimulator and a figure-of-eight coil with an outer winding diameter of 70 mm (Magstim Company Limited, Whiteland, UK). We set rTMS intensity at 110% of the rMT (see Supplementary Materials) and applied a single train of low-frequency rTMS at 1 Hz for a total duration of 15 min (900 pulses), a protocol that affects cortical excitability beyond the duration of the rTMS application itself (Chen et al., 1997). Moreover, low-frequency protocols are known for their ability to interfere with the activity of the targeted area (for a review see Sandrini, Umiltà, & Rusconi, 2011). For stimulation in the dlPFC and 6 h-dlPFC groups, the TMS coil was placed over F3 using the international 10–20 electroencephalogram (EEG) system, as in previous TMS studies (Beam, Borckardt, Reeves, & George, 2009; Borgomaneri et al., 2020; Rossi et al., 2001), corresponding to Brodmann area 9 (Fig. 2, I). The coil was held tangentially to the scalp with the handle positioned 45° with respect to the sagittal line. For sham stimulation, the coil was centered on F3 and positioned perpendicular to the scalp surface. As shown by previous experiments (Borgomaneri et al., 2020; Lisanby, Gutman, Luber, Schroeder, & Sackeim, 2001; Sandrini et al., 2011), this procedure ensures that no effective magnetic stimulation reaches the brain during the sham condition, while keeping the subject's feeling of coil–scalp contact and discharge noise similar to the real simulation (Fig. 2, II). In the case of occipital cortex stimulation (Ctrl-Occipital group), the coil was positioned horizontally over POz using the 10–20 EEG system

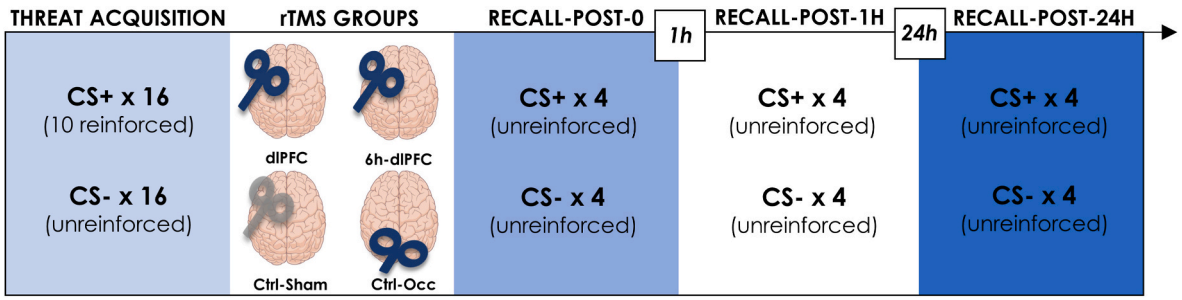

Fig. 1. Experimental design. On day 1, participants underwent threat acquisition with two visual stimuli (CS+ and CS-) and received repetitive transcranial magnetic stimulation (rTMS) or sham rTMS over the dlPFC or a control region. After rTMS, 4 CS+ and 4 CS- were presented to assess recall at four different time points (immediately, after 1 h, and after 24 h).

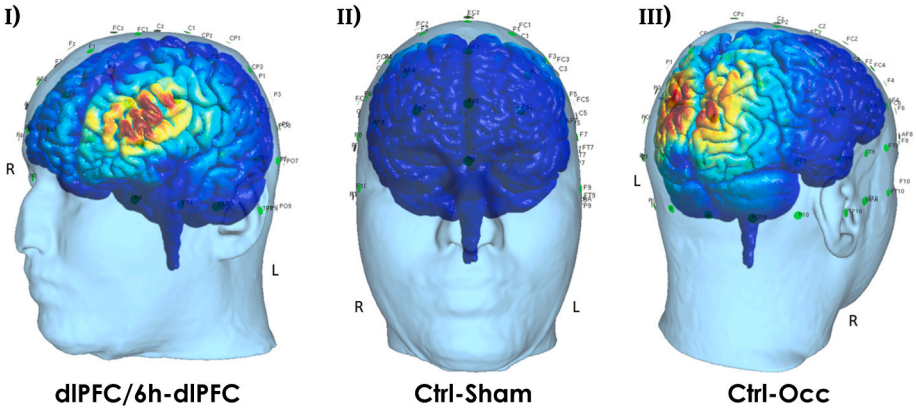

Fig. 2. Computational simulation of the estimated electric field distribution from rTMS targeting the brain. The volumetric spread of magnetic field simulation was created using SimNIBS v3.2.1. Conductivities for different tissue compartments were set as follows: 0.465 S per meter (S/m) (skin), 0.01 S/m (skull), 0.5 S/m (eyeballs), 1.654 S/m (cerebrospinal fluid), 0.275 S/m (gray matter) and 0.126 S/m (white matter). The estimation was carried out by simulating a figure-of-eight, 70 mm coil, with stimulation intensity set at 62%, the mean rMT for the dlPFC group. For dorsolateral prefrontal cortex (dlPFC) stimulation, the coil was placed over F3 using the international 10–20 electroencephalogram (EEG) system (panel I). For sham stimulation, the coil was placed over F3, but perpendicular to the scalp surface, and thus no magnetic stimulation reached the brain (panel II). The coil was placed over POz (panel III) for occipital cortex stimulation. The estimated computational simulation showed an accurate propagation of the rTMS stimulation over the dlPFC and the occipital cortex, hence supporting the rTMS setup used.

(Borgomaneri et al., 2020; Jacobs, de Graaf, Goebel, & Sack, 2012) (Fig. 2, III). Furthermore, SimNIBS v3.2.1 (Saturnino et al., 2019) was used to estimate the electric field distribution induced by TMS and for automatic skull segmentation from MR images (Nielsen et al., 2018) (Fig. 2).

2.4. Day 1: memory acquisition and neurostimulation

On day 1, four different sessions were performed: habituation (see Supplementary Materials), threat acquisition, neurostimulation (rTMS), and immediate and delayed (1 h) threat recall. At the beginning of the whole session, participants were informed that different stimuli would be presented on the screen and that they had to pay attention to the stimuli, as some might be paired with electric stimulation.

The threat acquisition session consisted of 16 CS+ and 16 CS- trials. One conditioned stimulus (CS+) was associated with the delivery of an electric shock (US) 60% of the times, 3.8 s after the CS + onset and co-terminated with the CS. The other CS (CS-) was never paired with the US. The trials were pseudo-randomly presented to participants such that no more than two identical CSs occurred in a row. Immediately after threat acquisition, dlPFC, Ctrl-Sham, and Ctrl-Occipital participants received 15 min at 1 Hz of rTMS. For all groups, the acquisition phase, stimulation, and test phase occurred in the morning, except for the 6 h-dlPFC group, where the acquisition phase occurred in the morning and stimulation and test occurred in the afternoon. We ensured that participants in this group did not sleep during the 6 h period. By applying rTMS stimulation 6 h after the acquisition session in the 6 h-dlPFC group, we were able to assess how the possibility of threat memory disruption is confined to the time window during which the memory is being consolidated. This suggests that the threat memory alteration observed in the dlPFC group is specifically fostered by the rTMS protocol, since all other control groups showed intact threat recalls. To assess conditioned responses to the CSs, SCR was measured during all experimental sessions, and the responses related to the CS+ were compared with those related to the CS- (see details in the SCR data analysis section).

After the rTMS, all participants were presented with an immediate recall session, in which they were informed they would see the same two stimuli (CSs). Importantly, the instructions did not reveal anything about the occurrence of the US. The recall session consisted of 4 CS+ and 4 CS-. The session was followed by a 1-h break, during which participants remained within the premises of the university campus and were free to use electronic devices. Moreover, the experimenter regularly checked on participants to make sure they were not sleeping. A subsequent recall session with the same characteristics as the one previously administered was performed 1 h later (delayed recall).

2.5. Day 2: memory recall

On day 2, 24 h after the threat acquisition session, a recall session was administered, identical to the other two on day 1. CS characteristics, trial order, and ISI were the same in all experimental sessions. The immediate and delayed recall phases were used for assessing rTMS effects within and without its inhibitory window, respectively, since the rTMS

effects are considered not to outlast the period of stimulation for more than 30 min (Eisenegger, Treyer, Fehr, & Knoch, 2008; Knecht, Ellger, Breitenstein, Ringelstein, & Henningsen, 2003; Lang et al., 2006), while the recall session after 24 h was used to evaluate the stability of the effects.

2.6. Data analysis

SCR data was analyzed offline using custom-made MATLAB scripts, and all statistical analyses were performed with STATISTICA (Dell Software, StatSoft STATISTICA, version 12.0, Round Rock, Texas, USA). Analyses of variance (ANOVA) were used to investigate differences within and between groups. Post hoc analyses were conducted with the Bonferroni test, and the significance threshold was $p < 0.05$. Moreover, effect size indices for main effects and interactions were computed using partial eta squared (η_p^2), whereas Cohen's d values were computed for post hoc comparisons (Cohen, 1977; Wolf, 1986). SCR data was extracted from the continuous signal and calculated for each trial as the base-to-peak amplitude of the minimum and largest deflection during the 0.5–4.5 s time window after stimulus onset (S. Battaglia, Garofalo, di Pellegrino, & Starita, 2020; Borgomaneri et al., 2020; Schiller et al., 2010). The minimum response criterion was 0.02, and smaller responses were coded as zero (S. Battaglia, Garofalo, & di Pellegrino, 2018; Boucsein et al., 2012). In the present study, none of the participants could be categorized as non-learners using the minimum amplitude cut-off of 0.02 μS in more than 50% of the CS + unreinforced trials (Lonsdorf et al., 2019). Regarding SCR to CSs, stimulus onset referred to the time of the CS appearance on the screen. SCRs were analyzed separately for each day, and only non-reinforced CS + trials were analyzed.

SCR following the CSs was analyzed to assess conditioned learning, whereas SCR following the US was analyzed to assess unconditioned responding. Raw SCR scores were square-root transformed to normalize the data distribution and scaled to each participant's square-root-transformed mean US response to account for inter-individual variability (Schiller et al., 2010).

3. Results

3.1. Threat acquisition

To ensure appropriate comparisons between groups regarding threat responses in the various recall sessions, we first assessed physiological activations in the acquisition phase. The analysis showed successful threat acquisition and no differences between groups, i.e., the four groups equally acquired threat conditioning. That is, a mixed-model ANOVA with Group (dlPFC, 6 h-dlPFC, Ctrl-Sham, and Ctrl-Occipital) as between-subject factor and Stimulus (CS+ and CS-) as within-subject factor revealed a significant effect of Stimulus ($F_{1,68} = 80.21$, $p < 0.001$, $\eta_p^2 = 0.54$), showing that SCR to the CS+ (mean ± SD: 0.29 μS ± 0.19) was higher than to the CS- (0.17 μS ± 0.12). Notably, this analysis did not reveal a significant main effect of Group nor a significant interaction between Group and Stimulus (all ps ≥ 0.81; all $\eta_p^2 \leq 0.02$; Table S1 in Supplementary Material; Fig. 3). Thus, the absence of Stimulus by Group interaction highlights that the average responses to the CS+ and CS- were similar across participants, irrespective of their group.

3.2. Threat recalls

Our main hypothesis was that rTMS of the dlPFC during threat memory consolidation would affect threat expression during each recall. The analysis of the recall sessions showed successful threat memory recall in all but the dlPFC group. A mixed-model ANOVA with Group, Stimulus, and Session (Recall-post-0, Recall-post-1h, and Recall-post-24 h) as within-subject factor revealed a significant effect of Stimulus ($F_{1,68}$ = 67.25, $p < 0.001$, $\eta_p^2 = 0.49$) and a significant Group by Stimulus interaction ($F_{3,68} = 2.83$, $p < 0.05$, $\eta_p^2 = 0.11$). Bonferroni comparisons revealed that only the dlPFC group showed impairment in threat memory recall (i.e., lack of differentiation between the CS+ and CS- during all Recall sessions; Recall-post-0: CS+: mean ± SD: 0.19 μS ± 0.19; CS-: 0.14 μS ± 0.16; Recall-post-1h: CS+: 0.23 μS ± 0.22; CS-: 0.18 μS ± 0.16; Recall-post-24 h: CS+: 0.27 μS ± 0.22; CS-: 0.21 μS ± 0.16; p = 1; Fig. 4). As expected, in the three control groups, greater SCR to the CS+ with respect to the CS- on all the recall sessions was observed (all ps ≤ 0.001; Table S1 in Supplementary Material). All in all, this suggests that, in the experimental group, rTMS successfully blocked the inception of the threat memory. Importantly, all the control groups (i.e., stimulation outside the consolidation time window, sham stimulation, and rTMS delivered on a control site) recalled the previously acquired memory, demonstrating no modification in the consolidation process.

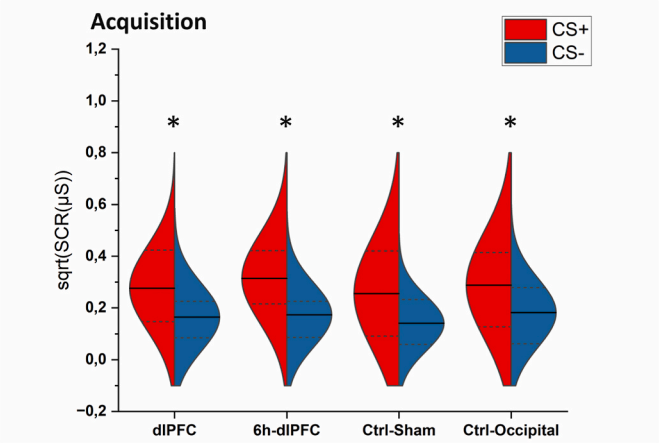

Fig. 3. Threat acquisition results. Split violin plots reporting the distribution of SCR amplitude of the two conditioned stimuli during Acquisition in the four groups. The solid line represents the median, while the dotted lines represent the 25th and the 75th percentile. * Indicates significant differences between the CS+ and the CS- ($p < 0.001$).

3.3. Questionnaire data

Anxiety and depressive symptoms may affect threat learning (Lissek et al., 2005; Nissen et al., 2010). Therefore, anxiety traits and anxiety and depressive symptoms were assessed with the State and Trait-Anxiety Inventory (STAI) (Spielberger et al., 1983) and the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). Due to a technical failure, the questionnaire data was available for only sixty-seven participants. A one-way ANOVA showed no significant effect of group on state anxiety scores ($F_{3,63} = 1.82$; $p = 0.15$; $\eta_p^2 = 0.08$; dlPFC, mean ± SD: 47.1 ± 13.1; 6 h-dlPFC: 40.2 ± 10.0; Ctrl-Sham, 48.4 ± 11.1; Ctrl-Occipital, 44.1 ± 9.1; see Fig. S1, panel E in Supplementary Material), trait anxiety scores ($F_{3,63} = 1.15$; $p = 0.34$; $\eta_p^2 = 0.05$; dlPFC, mean ± SD: 46.8 ± 10.8; 6 h-dlPFC: 46.1 ± 7.7; Ctrl-Sham, 49.2 ± 11.6; Ctrl-Occipital, 42.9 ± 7.7; see Fig. S1, panel F in Supplementary Material), depressive symptoms scores ($F_{3,63} = 1.82$; $p = 0.15$; $\eta_p^2 = 0.08$; dlPFC, mean ± SD: 4.5 ± 3.3; 6 h-dlPFC: 3.6 ± 2.2; Ctrl-Sham, 4.9 ± 2.5; Ctrl-Occipital, 3.1 ± 1.8; see Fig. S1, panel G in Supplementary Material), and anxious symptoms scores ($F_{3,63} = 0.96$; $p = 0.42$; $\eta_p^2 = 0.04$; dlPFC, mean ± SD: 7.2 ± 3.2; 6 h-dlPFC: 7.7 ± 3.0; Ctrl-Sham, 8.7 ± 4.1; Ctrl-Occipital, 6.8 ± 2.8; see Fig. S1, panel H in Supplementary Material). These results imply no difference between the four groups in anxiety and depression levels, suggesting that these factors did not influence the results.

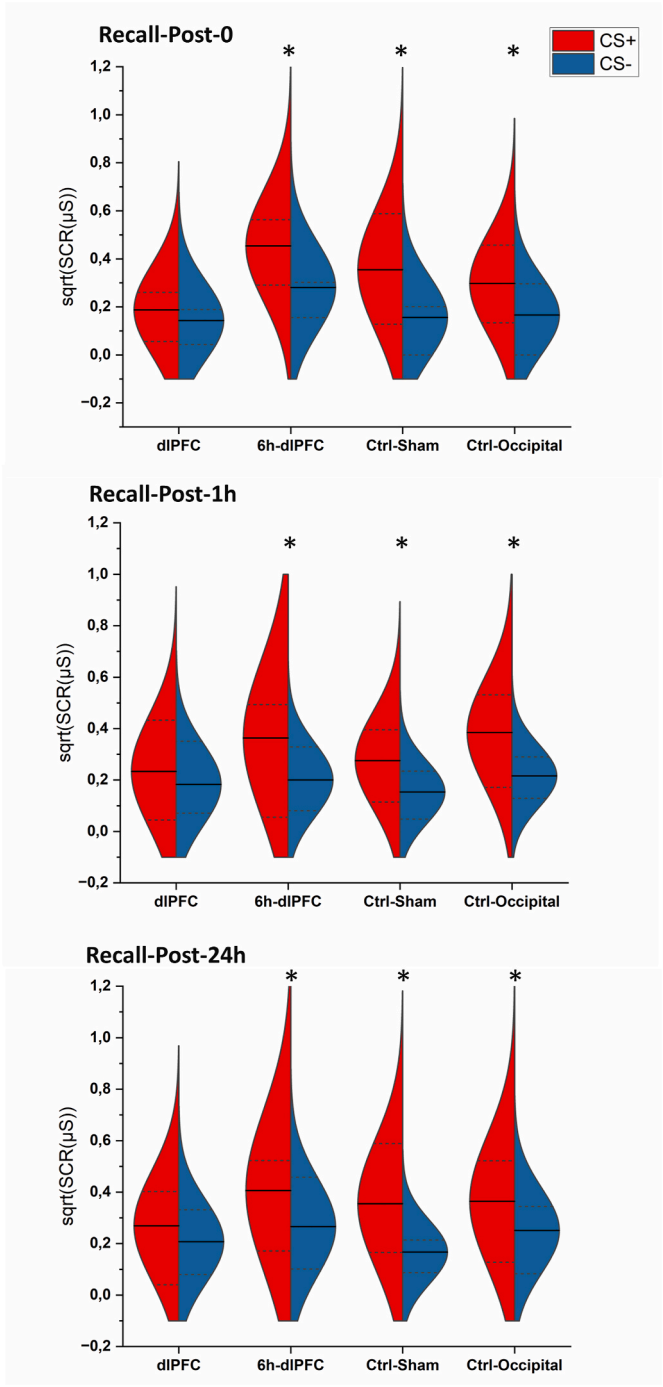

Fig. 4. Threat recall results. Split violin plots reporting the distribution of SCR amplitude of the two conditioned stimuli during Recall-post-0, Recall-post-1h, and Recall-post-24 h in the four groups. The solid line represents the median, while the dotted lines represent the 25th and the 75th percentile. * Indicates significant differences between the CS+ and the CS- ($p < 0.001$).

4. Discussion

Over the last decades, neuroscientific works provided evidence for the possibility of erasing maladaptive memories. The ability to regulate negative emotional responses is crucial for adaptive functions and is also essential in treating psychopathologies like PTSD, where intrusive traumatic memories significantly impact daily life. Here, we provided a more thorough understanding of the threat memory consolidation process in humans. In particular, we have shown that interfering with the dlPFC activation immediately after, but not 6 h later, memory acquisition impacts memory consolidation, suggesting the dlPFC to have a crucial role in consolidating threat memories. Using three separate control groups, we confirmed that our results were due to rTMS stimulation and that they were anatomically and temporally specific. Furthermore, we showed that the disruptive effect of inhibitory rTMS of the dlPFC interfered not only with the immediate recall of the memory but also with its recall after 1 h. These findings suggest that the threat memory consolidation has been successfully blocked. Notably, such effect lasted beyond the rTMS after-effect, confirming that the block was not due to the rTMS after-effect but that by inhibiting the dlPFC activity within the consolidation window (i.e., < 6 h), we hampered the memory formation before its consolidation. Crucially, this effect persisted also the following day, as evidenced by an impaired recall 24 h later. This is further corroborated by the finding that our intervention over the dlPFC disrupted the formation of threat memory in a temporally constrained manner. Thus, in the 6-h dlPFC group, threat memory recalls were still intact, meaning that rTMS specifically affected the consolidation process, which only lasts from minutes to hours after the encoding (Dudai, Karni, & Born, 2015).

Our findings are in line with recent correlational and clinical evidence supporting the pivotal role of the prefrontal cortex in threat acquisition (S. Battaglia et al., 2020; S. Battaglia, Harrison, & Fullana, 2022; Fullana et al., 2016; Harrison et al., 2017). Specifically, dlPFC regions have been found to be engaged in emotion regulation by modulating amygdala activity, ultimately diminishing threat responses (Amaral, 2002; Delgado et al., 2008; Groenewegen, Wright, & Uylings, 1997; Mcdonald, Mascagni, & Guo, 1996).

In support to our findings related to the temporal boundaries of the consolidation process, it has been demonstrated in animals that when protein synthesis is blocked 4 h after memory inception, there is no effect on learning, suggesting that consolidation likely ends before that time (Barrett & Sherry, 2012). However, no existing human studies have specifically tested the boundaries of the consolidation process. Here we confirm that after 6 h, the synaptic consolidation of aversive memories seems to be completed and no longer susceptible to modification by our rTMS protocol. Furthermore, neither the sham nor the occipital group recalls were impaired, suggesting that these findings were not the result of a placebo effect or of a general use of TMS. Therefore, our findings demonstrated that interfering with the dlPFC activity using rTMS immediately after threat acquisition interferes with the subsequent recall of the threat memory, highlighting that the PFC is a crucial node in the neurocircuitry underlying human memory synaptic consolidation. The classical view on memory consolidation suggests that memories are initially stored in subcortical circuitry, including the amygdala and hippocampal-entorhinal cortex, and are slowly consolidated over time in the PFC, thus excluding a possible role of the PFC in the first stages of memory consolidation (Kim & Fanselow, 1992; Kitamura et al., 2017; Nadel & Moscovitch, 1997; Squire, 1986). Our results suggest that the PFC may have a critical role in this process also at an initial stage. However, it is important to mention that TMS influences neural activity beyond the target region, reaching other functionally or anatomically linked areas, such as subcortical regions. Indeed, our stimulation protocol may have indirectly influenced the activity of the amygdala (Clarke et al., 2020; Feeser, Prehn, Kazzer, Mungee, & Bajbouj, 2014), which is involved, together with the dlPFC, in emotion regulation processes (i.e., by which individuals modulate the emotions they experience, including when and how they express such emotions and their intensity (Gross, 2015)). Nevertheless, our TMS protocol aimed to hamper the dlPFC activation, and can thus infer the crucial role of this area, in addition to other subcortical regions. This idea is supported by recent studies demonstrating that memory engram cells are formed quickly in the prefrontal cortex during the first stages of memory formation (Kitamura et al., 2017). Thus, we speculate that rTMS may have disrupted the inception of such activations, leading to impaired fear memories. In addition, memory information is relayed between the

hippocampus and the neocortex during the first stages of consolidation (Tambini, Ketz, & Davachi, 2010; van Kesteren, Fernández, Norris, & Hermans, 2010). Disruption of the dlPFC soon after fear memory acquisition may have hampered this process, resulting in compromised differential responding.

Furthermore, our group has recently demonstrated that the dlPFC is crucially involved in regulating threat expression during human *reconsolidation* (Borgomaneri et al., 2020), i.e., that interfering with dlPFC activity employing rTMS after memory reactivation disrupts threat expression. The present findings expand previous results, suggesting that dlPFC activity may be important even during the first instance of the consolidation of a memory, immediately after it is formed, thus highlighting the similarities in the neural and functional mechanisms mediating memory stabilization, both during the consolidation and reconsolidation of threat memories (see Alberini, 2005; S. Battaglia, Avenanti, Vécsei, & Tanaka, 2024a; Dudai, 2006; Lee, Nader, & Schiller, 2017 for reviews). Moreover, as shown by our results, this area is not critical when the memory has been stabilized (i.e., $>$ 6 h), and it becomes relevant anew only when memory becomes susceptible to changes after reactivation (Kindt et al., 2009). Notably, the effects on threat memory were still evident long after (24 h) rTMS was applied. This suggests that disrupting consolidation via dlPFC stimulation soon after a threat memory has been acquired could be used to treat aversive (e.g., traumatic) memories. In contrast to clinical protocols that target the dlPFC after PTSD has been diagnosed (and thus aim to interfere with system consolidation), our findings suggest that immediate intervention soon after the traumatic event (and before 6 h) could be used as an alternative therapeutic strategy to target synaptic consolidation.

A possible limitation of our study could be the sole use of SCR to assess fear responses. However, the SCR represents the most commonly used physiological index of human fear responding and is often the only measure considered when investigating changes in fear conditioning (Borgomaneri et al., 2020; Delgado et al., 2008; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Phelps, Delgado, Nearing, & Ledoux, 2004; Schiller et al., 2010). Combining different learning indices could have offered some advantages for interpretations. However, different learning measures may represent different dimensions of fear learning, which are not necessarily expected to converge. Moreover, the read-outs of multiple outcome measures (i.e., SCR and fear potential startle (FPS) reflex recordings) may not only interfere with each other but they may also interrupt and alter the process under study when simultaneously acquired (Lonsdorf et al., 2017). Future studies could endeavor to bridge this gap by incorporating alternative measurements, such as FPS or conditioned heart rate variations (S. Battaglia, Orsolini, et al., 2022; S. Battaglia & Thayer, 2022). Furthermore, it is important to note that in our study rTMS was exclusively administered to the left dlPFC and not the right dlPFC. Although targeting the right dlPFC may influence the consolidation of fear memories in a different manner, our prior research (Borgomaneri et al., 2020) demonstrated that both the right and left dlPFC play comparable roles in memory reconsolidation. Consequently, future studies should investigate more deeply whether a similar involvement of the left and right dlPFC can be observed in the consolidation process. Another limitation of our study is the absence of verbal indices i.e., subjective ratings. While subjective ratings are not mandatory for human threat conditioning protocols (Lonsdorf et al., 2017), we acknowledge that they may have offered valuable additional insights.

Although we can only speculate about the neural circuits involved in our behavioral effects, our results suggest that rTMS over the dlPFC disrupted its connectivity with the amygdala, leading to a lack of physiological responses even immediately afterwards, supporting the hypothesis that PFC may be a crucial neuroarchitecture node of the cortical and subcortical circuitry behind threat conditioning, at least in humans. This hypothesis needs to be tested in future studies combining TMS with neuroimaging.

In line with neuroimaging data showing that aversive memories are more resistant to suppression because of the greater prefrontal engagement during consolidation (Liu et al., 2016), our results support the possibility of a causal role of the dlPFC in threat memories synaptic consolidation, the process by which a temporary, labile memory is transformed into a more stable, long-lasting form. This suggests the need to revise the classical view about the brain network driving memory consolidation (Squire, 1986), according to which neocortical regions, particularly the prefrontal cortex, are necessary for the later, rather than initial, stages of memory consolidation.

Our findings have several implications that could enhance our knowledge on the neural dynamics of human threat learning, helping to refine existing clinical translational models of aberrant threat learning neural processes, and by suggesting the possibility to interfere with the aversive memory formation soon after (i.e., from minutes to hours) its acquisition. Unveiling the brain areas necessary for memory consolidation in the context of novel threat learning creates an engaging chance for non-invasive stimulation-based interventions, applicable to mental disorders such as anxiety and post-traumatic stress disorders. In this context, it is crucial to be aware that 20–60% of psychiatric populations show a poor response to classic pharmacological and behavioral therapies, putting a strain on the healthcare system (S. Battaglia, Nazzi, et al., 2023b; Battaglia, Nazzi, & Thayer, 2023c, 2024; Di Gregorio & Battaglia, 2024; Howes, Thase, & Pillinger, 2022; Battaglia, Avenanti, Vécsei, & Tanaka, 2024b). Further fundamental in-depth research applying a multi-method approach, followed by studies in clinical populations may possibly provide helpful insights for clinical applications (i.e., TMS-based therapy) (Battaglia, Schmidt, Hassel, & Tanaka, 2023). Thus, specific treatments resulting from new/combined approaches will also lead to a reduction in the financial concerns arising from the cost of psychiatric interventions.

CRediT authorship contribution statement

Simone Battaglia: Writing – original draft, Visualization, Supervision, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Claudio Nazzi:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Miquel A. Fullana:** Writing – review & editing. **Giuseppe di Pellegrino:** Writing – review & editing. **Sara Borgomaneri:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests.

Data availability

The link to the data is provided in the manuscript

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brat.2024.104548.

Abbreviations

CS	conditioned stimulus
dlPFC	dorsolateral prefrontal cortex
EEG	electroencephalogram
ISI	interstimulus interval
mA	milliampere
mPFC	medial prefrontal cortex
μS	microsiemens
mV	millivolts
rTMS	repetitive transcranial magnetic stimulation
rMT	resting motor threshold
S/m	Siemens per meter
SCR	skin conductance responses
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation
US	unconditioned stimulus
vmPFC	ventromedial prefrontal cortex

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