ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

State-Dependent TMS over Prefrontal Cortex Disrupts Fear-Memory Reconsolidation and Prevents the Return of Fear

This is the final peer-reviewed author’s accepted manuscript (postprint) of the following publication:

Published Version:
Borgomaneri S., Battaglia S., Garofalo S., Tortora F., Avenanti A., di Pellegrino G. (2020). State-Dependent TMS over Prefrontal Cortex Disrupts Fear-Memory Reconsolidation and Prevents the Return of Fear. CURRENT BIOLOGY, 30(18), 3672-3679 [10.1016/j.cub.2020.06.091].

Availability:
This version is available at: https://hdl.handle.net/11585/780376 since: 2020-11-13

Published:
DOI: http://doi.org/10.1016/j.cub.2020.06.091

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

07 August 2024

Manuscript

State-dependent TMS over prefrontal cortex disrupts fear memory reconsolidation and prevents the return of fear

Sara Borgomaneri[1,2#], Simone Battaglia[1,3#], Sara Garofalo[1,3], Francesco Tortora[1], Alessio Avenanti[1,4] and Giuseppe di Pellegrino[1,3]*

[1] Center for Studies and Research in Cognitive Neuroscience, Department of Psychology, University of Bologna, Cesena Campus, Cesena 47521, Italy

[2] IRCCS Fondazione Santa Lucia, Rome, 00179, Italy

[3] Department of Psychology, University of Bologna, Bologna, 40127, Italy

[4] Centro de Investigación en Neuropsicología y Neurociencias Cognitivas, Universidad Católica del Maule, Talca 3460000, Chile.

[#]Authors equally contributed to the present work.

*Correspondence should be addressed to: Giuseppe di Pellegrino. Department of Psychology, University of Bologna, Viale Berti Pichat 5, Bologna, 40127, Italy. e-mail: g.dipellegrino@unibo.it

In Brief

Borgomaneri et al., show that the noninvasive stimulation of the prefrontal cortex following memory reactivation disrupts physiological responding to learned fear, and argues in favour of a critical role of the dlPFC in the neural network that mediates the reconsolidation of fear memories in humans.

Highlights

- Post-retrieval rTMS over dlPFC reduces physiological responding to learned fear
- Post-retrieval rTMS over dlPFC prevents the return of fear after reinstatement
- Post-retrieval rTMS over dlPFC disrupts fear responses, not declarative memory
- The dlPFC plays a key role in fear memory reconsolidation

Summary

Erasing maladaptive memories has been a challenge for years. A way to change fear memories is to target the process of reconsolidation, during which a retrieved memory transiently returns to a labile state, amenable to modification [1,2]. Disruption of human fear memory reconsolidation has been classically attempted with pharmacological [3], or behavioral (e.g., extinction) [4] treatments which, however, do not clarify the underlying brain mechanism. To address this issue, here, in 84 healthy humans submitted to six experiments, we combined a differential fear conditioning paradigm with repetitive transcranial magnetic stimulation (rTMS) administered in a state-dependent manner. In a critical condition, we stimulated the dorsolateral prefrontal cortex (dlPFC) 10 minutes after a reminder cue that reactivated a fear memory acquired one day before. At testing, twenty-four hours after rTMS, participants exhibited decreased physiological expression of fear, as shown by their skin conductance response. Similar reductions were observed when targeting the left and the right dlPFC. In contrast, no decrease was observed in participants tested immediately after dlPFC-rTMS or in participants receiving either control rTMS (i.e., active control site and sham stimulations), or dlPFC-rTMS without preceding fear memory reactivation, thus showing both the site/time-specificity and state-dependency of our rTMS intervention. Expression of fear was indeed reduced only when dlPFC-rTMS was administered within the reconsolidation time-window. Moreover, dlPFC-rTMS prevented subsequent return of fear after extinction training. These findings highlight the causal role of dlPFC in fear memory reconsolidation and suggest that rTMS can be used in humans to prevent the return of fear.

Keywords

Reconsolidation, Fear Conditioning, State-Dependency, Transcranial Magnetic Stimulation, Dorsolateral Prefrontal Cortex, Reinstatement

Results and Discussion

The neural circuitry underlying fear memory reconsolidation in humans remain largely unknown. Since retrieval or reactivation by a reminder cue can induce reconsolidation, here, we focus on the dlPFC, a neocortical region crucially involved in controlling the retrieval and reactivation of memory traces [5–9], and in their gradual consolidation.

While previous human neuroimaging research has primarily implicated the dlPFC in the cognitive regulation of emotional processes [10,11], more recent studies have suggested that this brain region is also involved in some aspects of threat response reduction and fear memory modulation [12–14]. There remain, however, several outstanding questions regarding the role of the dlPFC in fear memory modification. A challenge moving forward is to understand: i) whether disruption of dlPFC activity by noninvasive stimulation impairs reconsolidation of fear memories in humans, ii) whether dlPFC has functional laterality specificities, and iii) to what extent noninvasive brain stimulation interventions could prevent the return of fear (reinstatement).

To these aims, we applied low frequency rTMS – a noninvasive stimulation technique used to evaluate the causal roles of focal brain regions – to the dlPFC during reconsolidation of a previously acquired fear memory. Animal and human studies revealed that the retrieval cue engages a time-limited plasticity window (thought to last at most 6 h after reactivation [4,15–17]) in which reconsolidation operates. Accordingly, we reactivated existing fear memories using a reminder cue able to trigger reconsolidation [4,18,19], and, 10 minutes later, we administered rTMS for 15 minutes to disrupt dlPFC function. In order to investigate a possible hemispheric laterality in the role of the dlPFC in reconsolidation of fear memories, in two experimental groups, we administered rTMS to the left (l-dlPFC) or right (r-dlPFC) dlPFC.

To establish the causal role of the dlPFC in the reconsolidation of fear memories, we designed four control conditions. First, to test the state-dependent efficacy of the above treatments and ensure they were specific to reconsolidation, we applied l-dlPFC rTMS without memory reactivation (Ctrl-NoRem). Moreover, to test whether the rTMS effect is time-dependent, such that reduction of fear memory post-reactivation is only observable at long-term testing (i.e., after 24 hours) but not shortly after the reconsolidation intervention, in an additional control group (Ctrl-Day2), fear memory was assessed on day 2, immediately after the administration of state-dependent rTMS over l-dlPFC. Finally, to determine whether the rTMS effect was topographically specific and control for potential nonspecific effects (e.g., discomfort) of noninvasive brain stimulation, in two further control groups, we applied state-dependent rTMS over the occipital cortex (Ctrl-Occipital) as an active control site or in sham modality (Ctrl-Sham). A total of eighty-four participants took part in

the main study (see Data Replicability section for a replication study in a further, separate experimental group). Seventy participants (i.e., in the l-dlPFC, r-dlPFC, Ctrl-NoRem, Ctrl-Occipital and Ctrl-Sham groups) were tested across three days, while fourteen participants (Ctrl-Day2 group) were tested across two days. We followed established procedures to ensure acquisition, reconsolidation, extinction, and reinstatement of fear memory (see Figure 1) [2,4,20]. A physiological measure (i.e., SCR), and subjective reports (i.e., CS-US contingency ratings) of fear learning and memory were collected throughout the experiment as dependent measures.

Please insert Figure 1 near here

On day 1 (fear acquisition), all participants underwent a differential fear conditioning procedure, during which two neutral visual scenes were used as conditioned stimuli (CS+ and CS-). The CS+ was associated with an unconditioned aversive stimulus (US) – a mild wrist shock – while the CS- was never associated with a shock (i.e., the CS did not predict the occurrence of the US) [21].
The analysis of the SCRs showed successful fear learning as indexed by the emergence and development of SCR amplitudes that discriminated between CS+ and CS- [21]. That is, the stimulus (CS+ and CS-) by phase (early and late phase) interaction was significant ($F_{1,78} = 21.97$; $p < 0.0001$; $\eta_p^2 = 0.22$). Follow-up tests revealed a larger SCR to CS+ than to CS− trials during the early phase (mean SCR ± standard deviation, SD, for CS+: 0.51 µS ± 0.21; for CS-: 0.38 µS ± 0.21; $p = 0.0001$; $d = 0.85$) and the late phase of acquisition (CS+: 0.47 µS ± 0.26; CS-: 0.24 µS ± 0.18; $p = 0.0001$; $d = 1.36$) across all groups, and the difference between the SCR to CS+ and CS− trials was greater in the late phase than in the early phase (early phase: 0.13 µS ± 0.15; late phase: 0.23 µS ± 0.17; $p < 0.001$; $d = 0.52$). Importantly, the analysis revealed neither a significant main effect of group, nor any interactions between group and either stimulus or phase (all p-values > 0.33; all $\eta_p^2 < 0.07$; see Figure 2 and Table S1 for further statistics), indicating similar fear learning effects across groups.

Please insert Figure 2 near here

Likewise, CS-US contingency ratings – assessed on a 0-100 visual analog scale (VAS) at the beginning and the end of the session – revealed a significant stimulus x phase (pre- and post-fear acquisition) interaction ($F_{1,78} = 88.02$; $p < 0.0001$; $\eta_p^2 = 0.53$). Follow-up tests showed that the CS+

elicited significantly larger shock-expectancy ratings than the CS- did after fear conditioning (mean ratings ± SD for CS+: 36.49 ± 30.12; CS-: 5.94 ± 11.74; $p < 0.001$; $d = 1.01$), but not before fear conditioning (CS+: 9.22 ± 15.12; CS-: 9.49 ± 15.67; $p = 0.91$; $d = 0.01$). There were no significant differences between groups (see Table 1 and Table S1 for further statistics). Overall, these data demonstrate that fear learning took place equivalently across all groups of participants.

On day 2 (fear memory reactivation and brain stimulation), in the five state-dependent rTMS groups, we provided reactivation trials: the CS+ was presented twice without the US to act as a reminder and reactivate the memory trace [2,4,20]. Afterwards, low frequency rTMS at 1Hz was applied for 15 minutes to a specific brain region, according to the assigned group: l-dlPFC, r-dlPFC, Ctrl-Day2, Ctrl-Occipital, Ctrl-Sham. For the additional control group (Ctrl-NoRem), rTMS was administered to the left dlPFC without memory reactivation.

The five state-dependent groups expressed comparable levels of SCR during reactivation of fear memory (Ctrl-Sham: mean SCR to CS+ presentations ± SD: 0.71 µS ± 0.44; Ctrl-Occipital: 0.67 µS ± 0.28; Ctrl-Day2: 0.78 µS ± 0.40; r-dlPFC: 0.61 µS ± 0.32 and l-dlPFC: 0.75 µS ± 0.49; $F_{4,65} = 0.40$; $p = 0.81$; $\eta_p^2 = 0.02$). In addition, fear memory was equally well consolidated in the five groups, as revealed by the absence of a main effect of group ($F_{4,65} = 0.49$; $p = 0.74$; $\eta_p^2 = 0.03$) and the absence of an interaction effect between group and phase ($F_{4,65} = 0.47$; $p = 0.76$; $\eta_p^2 = 0.03$). That is, there was no effect of group on SCR that differed between the last four acquisition trials (day 1) and the two reactivation trials (day 2). These data demonstrate that, before the reconsolidation manipulation, the conditioned response was equally expressed across groups.

The last session (memory recall, extinction, and reinstatement) occurred on day 3 for all groups except for the control group Ctrl-Day2, which was tested on day 2, immediately after the rTMS. In the last session, all groups first performed a memory recall test consisting of 4 unreinforced CS+ and 4 CS- presentations. Immediately afterwards, participants underwent an extinction training procedure, in which they were exposed to both the CSs (12 trials each) without the US. Then, participants received 3 unsignaled USs (reinstatement of extinguished fear [3,4,22]), and subsequently underwent a test for fear memory reinstatement consisting of 4 unreinforced CS+ and 4 CS-.

The analysis revealed a significant interaction ($F_{10,156} = 1.91$; $p = 0.048$; $\eta_p^2 = 0.11$) between group (r-dlPFC, l-dlPFC, Ctrl-Sham, Ctrl-Occipital, Ctrl-Day2 and Ctrl-NoRem), stimulus (CS+ and CS-) and phase (memory recall, extinction, and reinstatement).

Specifically, in the memory recall test (48h after fear acquisition), the administration of state-dependent rTMS over both right and left dlPFC significantly decreased SCR differences between CS+ and CS- (r-dlPFC, CS+: 0.60 µS ± 0.40; CS-: 0.51 µS ± 0.38; $p = 0.46$; $d = 0.45$; l-dlPFC, CS+:

0.65 µS ± 0.30; CS-: 0.54 µS ± 0.32; $p = 0.34$; $d = 0.56$). Conversely, the expression of fear memory remained stable in the four control groups, with significantly larger SCRs to the CS+ than to the CS- (Ctrl-Sham, CS+: 0.63 µS ± 0.42; CS-: 0.46 µS ± 0.27; $p = 0.043$; $d = 0.74$; Ctrl-Occipital, CS+: 0.70 µS ± 0.29; CS-: 0.44 µS ± 0.28; $p < 0.001$; $d = 0.99$; Ctrl-Day2: CS+: 0.70 µS ± 0.36; CS-: 0.53 µS ± 0.37; $p = 0.03$; $d = 1.41$; Ctrl-NoRem, CS+: 0.80 µS ± 0.25; CS-: 0.67 µS ± 0.28; $p = 0.03$; $d = 0.92$).

In the extinction training phase, no significant SCR differences between CS+ and CS- trials were observed in any group (Ctrl-Sham, CS+: 0.32 µS ± 0.41; CS-: 0.26 µS ± 0.31; $p = 0.83$; $d = 0.39$; Ctrl-Occipital, CS+: 0.36 µS ± 0.23; CS-: 0.23 µS ± 0.16; $p = 0.26$; $d = 0.62$; Ctrl-Day2: CS+: 0.43 µS ± 0.33; CS-: 0.30 µS ± 0.23; $p = 0.35$; $d = 0.69$; Ctrl-NoRem, CS+: 0.40 µS ± 0.24; CS-: 0.23 µS ± 0.14; $p = 0.053$; $d = 0.95$; r-dlPFC, CS+: 0.38 µS ± 0.33; CS-: 0.33 µS ± 0.24; $p = 0.88$; $d = 0.32$; l-dlPFC, CS+: 0.34 µS ± 0.24; CS-: 0.27 µS ± 0.12; $p = 0.74$; $d = 0.39$; see Figure 3). Note that the differential fear response was already eliminated during the recall phase in the groups that received rTMS over the right and left dlPFC.

Please insert Figure 3 near here

This result ensures that the six groups were in a similar state of extinction. Namely, the conditioned fear response was equally reduced after the extinction training in all groups.

Exposure to the aversive stimulus (i.e., the shock) after fear extinction has been shown to reinstate the expression of the original fear memory in animals [23] and humans [24]. Accordingly, following fear memory reinstatement, we observed different SCRs between CS+ and CS- in the four control groups (Ctrl-Sham, CS+: 0.62 µS ± 0.46; CS-: 0.31 µS ± 0.30; $p < 0.001$; $d = 0.73$; Ctrl-Occipital, CS+: 0.52 µS ± 0.24; CS-: 0.36 µS ± 0.17; $p = 0.046$; $d = 0.67$; Ctrl-Day2, CS+: 0.62 µS ± 0.34; CS-: 0.35 µS ± 0.23; $p < 0.001$; $d = 1.31$; Ctrl-NoRem, CS+: 0.61 µS ± 0.32; CS-: 0.32 µS ± 0.21; $p < 0.001$; $d = 0.86$; see Table S1 for further statistics). Crucially, reinstatement was unsuccessful in the right and the left dlPFC groups, in which we observed no difference between CS+ and CS- (r-dlPFC, CS+: 0.59 µS ± 0.41; CS-: 0.48 µS ± 0.29; $p = 0.22$; $d = 0.49$; l-dlPFC, CS+: 0.46 µS ± 0.24; CS-: 0.44 µS ± 0.31; $p = 0.93$; $d = 0.09$).

Taken together, these data indicate that state-dependent rTMS over the dlPFC (after fear memory reactivation) not only diminished fear expression at recall, but also prevented the return of fear following the reinstatement procedure. Remarkably, we successfully replicated this finding in an additional experimental group of 14 participants, in which we administered state-dependent dlPFC

rTMS and observed no fear expression at recall or following the reinstatement procedure (see Data replicability section).

Interestingly, subjective US-expectancy, collected at the end of day 3 (or day 2 in the Ctrl-Day2 group), revealed a different pattern. The analysis showed significantly a higher shock-expectancy ($F_{1,78} = 25.60$; $p < 0.001$; $\eta_p^2 = 0.25$) for CS+ (mean ratings ± SD: 19.17 ± 22.61) than CS- (6.49 ± 12.20) that was equally present in all groups ($F_{5,78} = 1.96$; $p = 0.09$; $\eta_p^2 = 0.11$), thereby indicating no effect of dlPFC stimulation on participants' learned expectations of the unconditioned stimulus. An additional analysis investigating possible changes among groups from day 1 to day 3 revealed no main effect of, or interactions with, the factor group (all p-values > 0.12).

Please insert Table 1 near here

Several alternative explanations of the present findings can be discarded. First, the results cannot be explained by a general amnestic effect of brain stimulation, as the group receiving rTMS to the control brain area (occipital cortex) continued to express fear (higher SCR to CS+ compared to CS-) at recall and following reinstatement. Second, only the stimulation of the right and left dlPFC was causally associated with no fear response in both testing phases. Third, the evidence that participants persistently expressed fear (in terms of both psychophysiological reactions and subjective ratings) when the memory was not reactivated by presentation of the CS+ (i.e., in the Ctrl-NoRem group) confirms that the dlPFC manipulation via rTMS was state-dependent, and specifically acted on the memory reconsolidation process [2]. Finally, we found intact fear memory expression when the testing phase took place shortly after reactivation and dlPFC-rTMS treatment (i.e., in the Ctrl-Day2 group), but substantially impaired fear responses 24h later (i.e., in the l-dlPFC and r-dlPFC groups).

Thus, in line with the reconsolidation hypothesis [25,26], the effect of rTMS over the dlPFC evolves over time and depends on memory reactivation. These results, together with the absence of fear recovery following reinstatement [27], argue in favor of a direct modification of the original memory trace rather than the formation of a new memory, as occurs in extinction [23,28].

The reconsolidation hypothesis assumes that, when reactivated, memories transiently enter into a labile and changeable state, necessitating a process of restabilization in order to persist [29]. In this study, we demonstrate the critical role of the dlPFC in the reconsolidation process, and the

possibility of modifying existing fear memories with noninvasive stimulation of this brain region during the reconsolidation time window.

This is the first study to directly compare the roles of the right and left dlPFC [30]. Since we found no overall difference between right and left dlPFC stimulation, our results suggest the absence of any functionally relevant lateralization in the role of the dlPFC in fear memory reconsolidation.

Regarding the putative brain mechanisms underlying our findings, the dlPFC is particularly central to the control of memory retrieval with respect to the actual context, the maintenance and processing of retrieved information in working memory, and the evaluation and monitoring of reactivated memories. At a cognitive level, reconsolidation has been suggested to critically depend on active working memory processing (e.g., rehearsal [31,32]; but see also [33]). Accordingly, in the present study, interference with dlPFC activity during the reconsolidation window may have substantially reduced the allocation of working memory resources needed for the re-storage and retention of the destabilized memory, thus leading to disruption of the original memory trace. Indeed, our finding that only the experimental groups failed to discriminate between fearful and neutral stimuli (differential SCR in CS+ and CS- trials) during the recall test indicates that the dlPFC is critical for restabilizing the memory through reconsolidation.

rTMS not only affects the targeted local region but also changes activity in anatomically or functionally interconnected distal cerebral regions. Thus, perturbation of the dlPFC during the reconsolidation time-window may have altered hippocampal-prefrontal connectivity, as already postulated for non-emotional memories [8,34], and, crucially, amygdala-prefrontal coupling. Interestingly, dlPFC regions engaged in emotion regulation have been reported to influence the amygdala, diminishing fear through connections to ventromedial prefrontal (vmPFC) regions [35–39]. Future work combining rTMS and fMRI may shed light on how functional interactions between remote but interconnected brain regions may support reconsolidation of fear memories.

Notably, dlPFC stimulation had no effect on the declarative memory about which conditioned stimulus had been paired with the shock, although this factual knowledge no longer accounted for reliable fear responses in those participants. This finding suggests that post-retrieval stimulation of the prefrontal cortices reduces the reconsolidation of implicit fear memories, as measured by physiological responses, while leaving the cognitive component of prior contingency learning untouched.

In fact, a memory trace may involve multiple, distinct representations encoded by different brain systems. Following reactivation, the destabilization and disruption induced by a reconsolidation-based treatment may not affect the entire network supporting memory, but only a portion of it [40]. It is therefore possible that a residual, non-destabilized component of the memory trace may

support some degree of performance during a retention test. It has to be acknowledged that the idea that brain stimulation can interfere with memory is not completely new. In 1968, two influential papers reported, in rodents, an elimination of the fear response by pairing a brief presentation of the conditioned stimulus with an electroconvulsive shock [41,42]. Even if impressive, such an approach could not be easily translated to humans. Crucially, our study identified a potential target for interfering with the memory consolidation process, which represents a clinical priority. More recent noninvasive approaches to brain stimulation tried to tackle this issue [8,12,14,28,34,43–46]. However, findings from these studies have scarcely been replicated and, critically, none of the aforementioned studies was designed to reduce fear memories by directly interfering with the reconsolidation process. Moreover, they failed to investigate the critical role of the dlPFC in the reconsolidation process, and whether targeting the right or left dlPFC similarly impacts fear memory – a critical point in the design of clinical TMS protocols [47]. Finally, none of the existing noninvasive brain stimulation studies tested the strength of the neuromodulation by means of a reinstatement procedure.

We did not investigate the boundaries of the reconsolidation window, that is, we did not assess the effect of the dlPFC stimulation administered after the end of the reconsolidation window. Note, however, that the length of such temporal window is still speculative: reconsolidation processes are thought to last approximately 6 hours after the reminder in animal models [2], but their duration may vary depending on the paradigm [48–50]. Moreover, an additional control group testing the reconsolidation window boundaries would be systematically different in testing time (afternoon vs morning); therefore a null effect would be inconclusive, as the time of day or gap between the intervention and recall test may have intervened. Notwithstanding, examining the length of the reconsolidation window remains crucial, and a follow-up study, including multiple time windows after reactivation (e.g., 1 hour, 6 hours, 8 hours) each with the appropriate controls, will be required to address this issue directly. A further potential limitation is that we limited our physiological recordings to SCRs. Future studies will test whether disruption of fear recall can be traced using additional physiological measures of emotional responses.

To summarize, these results demonstrate that noninvasive stimulation of the prefrontal cortex following memory reactivation disrupts the expression of fear to a previously conditioned threatening stimulus, and argue in favor of a critical role of the dlPFC in the neural network that mediates the reconsolidation of fear memories in humans. These findings provide a step forward toward understanding the mechanisms underlying fear memory reconsolidation, and they have potential clinical implications for targeting emotional, maladaptive memories [51]. Uncovering the brain regions involved in the reconsolidation of emotional memories constitutes a challenging

opportunity for noninvasive brain stimulation and reconsolidation-based interventions, which are increasingly applied to conditions like phobia, addiction, post-traumatic stress disorder, obsessive-compulsive disorder and many others [52].

Acknowledgments

This work was supported by a RFO Grant from the University of Bologna to G.d.P. and by grants from the Ministero della Salute, Italy [GR-2010-2319335], Cogito Foundation, Switzerland [R-117/13 and 14-139-R], Fondazione del Monte di Bologna e Ravenna, Italy [339bis/2017], Bial Foundation [347/18] awarded to A.A., and by grant from Ministero della Salute, Italy [GR-2018-12365733] awarded to Sa.B. The authors thank Brianna Beck for proofreading the manuscript and Santino Aprile, and Claudio Nazzi for their help with the experimental sessions and participant recruitment.

Author Contributions

All authors developed the study concept and contributed to the study design; Sa.B. and S.B. performed testing and data collection; S.B. performed the data analysis; Sa.B., S.B. and S.G. wrote the manuscript; all authors approved the final version of the manuscript for submission.

Declaration of Interests

The authors declare no competing interests.

References

1. Besnard, A., Caboche, J., and Laroche, S. (2012). Reconsolidation of memory: A decade of debate. Prog. Neurobiol. *99*, 61–80.

2. Elsey, J.W.B., Van Ast, V.A., and Kindt, M. (2018). Human memory reconsolidation: A guiding framework and critical review of the evidence. Psychol. Bull. *144*, 797–848.

3. Kindt, M., Soeter, M., and Vervliet, B. (2009). Beyond extinction: erasing human fear responses and preventing the return of fear. Nat. Neurosci. *12*, 256–258.

4. Schiller, D., Monfils, M.-H., Raio, C.M., Johnson, D.C., Ledoux, J.E., and Phelps, E.A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. Nature *463*, 49–53.

5. Cabeza, R., and Nyberg, L. (2000). Neural bases of learning and memory: Functional neuroimaging evidence. Curr. Opin. Neurol. *13*, 415–421.

6. Eichenbaum, H. (2017). Prefrontal-hippocampal interactions in episodic memory. Nat. Rev. Neurosci. *18*, 547–558.

7. Moscovitch, M., and Winocur, G. (2002). The frontal cortex and working with memory. In Principles of Frontal Lobe Function (Oxford University Press), pp. 188–209.

8. Sandrini, M., Censor, N., Mishoe, J., and Cohen, L.G. (2013). Causal role of prefrontal cortex in strengthening of episodic memories through reconsolidation. Curr. Biol. *23*, 2181–2184.

9. Simons, J.S., and Spiers, H.J. (2003). Prefrontal and medial temporal lobe interactions in long-term memory. Nat. Rev. Neurosci. *4*, 637–648.

10. Fullana, M.A., Harrison, B.J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Àvila-Parcet, A., and Radua, J. (2016). Neural signatures of human fear conditioning: An updated and extended meta-analysis of fMRI studies. Mol. Psychiatry *21*, 500–508.

11. Ochsner, K.N., Silvers, J.A., and Buhle, J.T. (2012). Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. Ann. N. Y. Acad. Sci. *1251*, E1–E24.

12. Asthana, M., Nueckel, K., Mühlberger, A., Neueder, D., Polak, T., Domschke, K., Deckert, J., and Herrmann, M.J. (2013). Effects of transcranial direct current stimulation on

consolidation of fear memory. Front. Psychiatry *4*, 107.

13. Van 't Wout, M., Mariano, T.Y., Garnaat, S.L., Reddy, M.K., Rasmussen, S.A., and Greenberg, B.D. (2016). Can transcranial direct current stimulation augment extinction of conditioned fear? Brain Stimul. *9*, 529–536.

14. Mungee, A., Kazzer, P., Feeser, M., Nitsche, M.A., Schiller, D., and Bajbouj, M. (2014). Transcranial direct current stimulation of the prefrontal cortex: A means to modulate fear memories. Neuroreport *25*, 480–484.

15. Kindt, M., Soeter, M., and Vervliet, B. (2009). Beyond extinction: Erasing human fear responses and preventing the return of fear. Nat. Neurosci. *12*, 256–258.

16. Monfils, M.H., Cowansage, K.K., Klann, E., and LeDoux, J.E. (2009). Extinction-reconsolidation boundaries: Key to persistent attenuation of fear memories. Science *324*, 951–955.

17. Nader, K., Schafe, G.E., and Le Doux, J.E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature *406*, 722–726.

18. Sevenster, D., Beckers, T., and Kindt, M. (2013). Prediction error governs pharmacologically induced amnesia for learned fear. Science *339*, 830–833.

19. Merlo, E., Milton, A.L., Goozée, Z.Y., Theobald, D.E., and Everitt, B.J. (2014). Reconsolidation and extinction are dissociable and mutually exclusive processes: Behavioral and molecular evidence. J Neurosci *34*, 2422–2431.

20. Kindt, M., Soeter, M., and Sevenster, D. (2014). Disrupting reconsolidation of fear memory in humans by a noradrenergic β-blocker. J. Vis. Exp. *94*, 1–8.

21. Lonsdorf, T.B., Menz, M.M., Andreatta, M., Fullana, M.A., Golkar, A., Haaker, J., Heitland, I., Hermann, A., Kuhn, M., Kruse, O., *et al.* (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. Neurosci. Biobehav. Rev. *77*, 247–285.

22. Haaker, J., Golkar, A., Hermans, D., and Lonsdorf, T.B. (2014). A review on human reinstatement studies: An overview and methodological challenges. Learn. Mem. *21*, 424–440.

23. Bouton, M.E. (2002). Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biol Psychiatry *52*, 976–986.

24. Norrholm, S.D., Jovanovic, T., Vervliet, B., Myers, K.M., Davis, M., Rothbaum, B.O., and Duncan, E.J. (2006). Conditioned fear extinction and reinstatement in a human fear-potentiated startle paradigm. Learn. Mem. *13*, 681–685.

25. Nader, K., and Hardt, O. (2009). A single standard for memory: The case for reconsolidation. Nat. Rev. Neurosci. *10*, 224–234.

26. Debiec, J., LeDoux, J.E., and Nader, K. (2002). Cellular and systems reconsolidation in the hippocampus. Neuron *36*, 527–538.

27. Barak, S., and Ben Hamida, S. (2012). Memory erasure, enhanced extinction and disrupted reconsolidation. J. Neurosci. *32*, 2250–2251.

28. Raij, T., Nummenmaa, A., Marin, M.F., Porter, D., Furtak, S., Setsompop, K., and Milad, M.R. (2018). Prefrontal cortex stimulation enhances fear extinction memory in humans. Biol. Psychiatry *84*, 129–137.

29. Agren, T. (2014). Human reconsolidation: A reactivation and update. Brain Res. Bull. *105*, 70–82.

30. Sandrini, M., Cohen, L.G., and Censor, N. (2015). Modulating reconsolidation: A link to causal systems-level dynamics of human memories. Trends Cogn. Sci. *19*, 475–482.

31. James, E.L., Bonsall, M.B., Hoppitt, L., Tunbridge, E.M., Geddes, J.R., Milton, A.L., and Holmes, E.A. (2015). Computer game play reduces intrusive memories of experimental trauma via reconsolidation-update mechanisms. Psychol. Sci. *26*, 1201–1215.

32. Diekelmann, S., Büchel, C., Born, J., and Rasch, B. (2011). Labile or stable: Opposing consequences for memory when reactivated during waking and sleep. Nat. Neurosci. *14*, 381–386.

33. Chalkia, A., Vanaken, L., Fonteyne, R., and Beckers, T. (2019). Interfering with emotional processing resources upon associative threat memory reactivation does not affect memory retention. Sci. Rep. *9*, 1–11.

34. Sandrini, M., Brambilla, M., Manenti, R., Rosini, S., Cohen, L.G., and Cotelli, M. (2014). Noninvasive stimulation of prefrontal cortex strengthens existing episodic memories and reduces forgetting in the elderly. Front. Aging Neurosci. *6*, 1–9.

35. Delgado, M.R., Nearing, K.I., LeDoux, J.E., and Phelps, E.A. (2008). Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. Neuron *59*, 829–

838.

36. Milad, M.R., and Quirk, G.J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. Nature *420*, 70–74.

37. Amaral, D.G. (2002). The primate amygdala and the neurobiology of social behavior: implications for understanding social anxiety. Biol. Psychiatry *51*, 11–7.

38. Groenewegen, H.J., Wright, C.I., and Uylings, H.B. (1997). The anatomical relationships of the prefrontal cortex with limbic structures and the basal ganglia. J. Psychopharmacol. *11*, 99–106.

39. McDonald, A.J., Mascagni, F., and Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. Neuroscience *71*, 55–75.

40. Soeter, M., and Kindt, M. (2010). Dissociating response systems: Erasing fear from memory. Neurobiol. Learn. Mem. *94*, 30–41.

41. Misanin, J.R., Miller, R.R., and Lewis, D.J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science *160*, 554–555.

42. Schneider, A.M., and Sherman, W. (1968). Amnesia: A function of the temporal relation of footshock to electroconvulsive shock. Science *159*, 219–221.

43. Censor, N., Dimyan, M.A., and Cohen, L.G. (2010). Modification of existing human motor memories is enabled by primary cortical processing during memory reactivation. Curr. Biol. *20*, 1545–1549.

44. Javadi, A.H., and Walsh, V. (2012). Transcranial direct current stimulation (tDCS) of the left dorsolateral prefrontal cortex modulates declarative memory. Brain Stimul. *5*, 231–241.

45. Javadi, A.H., and Cheng, P. (2013). Transcranial direct current stimulation (tDCS) enhances reconsolidation of long-term memory. Brain Stimul. *6*, 668–674.

46. Guhn, A., Dresler, T., Hahn, T., Mühlberger, A., Ströhle, A., Deckert, J., and Herrmann, M.J. (2012). Medial prefrontal cortex activity during the extinction of conditioned fear: An investigation using functional near-infrared spectroscopy. Neuropsychobiology *65*, 173–182.

47. Karsen, E.F., Watts, B. V., and Holtzheimer, P.E. (2014). Review of the effectiveness of

transcranial magnetic stimulation for post-traumatic stress disorder. Brain Stimul. *7*, 151–157.

48. Kroes, M.C.W., Schiller, D., LeDoux, J.E., and Phelps, E.A. (2016). Translational approaches targeting reconsolidation. Curr. Top. Behav. Neurosci. *28*, 197–230.

49. Phelps, E.A., and Schiller, D. (2013). Reconsolidation in humans. In Memory Reconsolidation (Waltham, MA: Academic Press), pp. 185–212.

50. Lee, J.L.C., Amorim, F.E., Cassini, L.F., and Amaral, O.B. (2019). Different temporal windows for CB1 receptor involvement in contextual fear memory destabilisation in the amygdala and hippocampus. PLoS One *14*, e0205781.

51. Pennington, Z.T., and Fanselow, M.S. (2018). Indirect targeting of subsuperficial brain structures with Transcranial Magnetic Stimulation reveals a promising way forward in the treatment of fear. Biol. Psychiatry *84*, 80–81.

52. Schwabe, L., Nader, K., and Pruessner, J.C. (2014). Reconsolidation of human memory: Brain mechanisms and clinical relevance. Biol. Psychiatry *76*, 274–280.

53. Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A., The Safety of TMS Consensus Group, Nasreldin, M., Nakatsuka, M., Koganemaru, S., Fawi, G., Avanzini, G., *et al.* (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin. Neurophysiol. *120*, 2008–39.

54. Rossini, P.M., Burke, D., Chen, R., Cohen, L.G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P.B., George, M.S., *et al.* (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin. Neurophysiol. *126*, 1071–1107.

55. Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., and Pine, D.S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. Behav. Res. Ther. *43*, 1391–1424.

56. Spielberger, C.D. (1983). Manual for the State-Trait Anxiety Inventory (Palo Alto, CA: Consulting Psychologist Press).

57. Zigmond, A.S., and Snaith, R.P. (1983). The Hospital Anxiety and Depression Scale. Acta Psychiatr. Scand. *67*, 361–370.

58. Brainard, D.H. (1997). The Psychophysics Toolbox. Spat. Vis. *10*, 433–6.

59. Battaglia, S., Garofalo, S., and di Pellegrino, G. (2018). Context-dependent extinction of threat memories: influences of healthy aging. Sci. Rep. *8*, 12592.

60. Schiller, D., Levy, I., Niv, Y., LeDoux, J.E., and Phelps, E.A. (2008). From fear to safety and back: Reversal of fear in the human brain. J. Neurosci. *28*, 11517–11525.

61. Unsworth, N., Heitz, R.P., Schrock, J.C., and Engle, R.W. (2005). An automated version of the operation span task. Behav. Res. Methods *37*, 498–505.

62. Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., *et al.* (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr. Clin. Neurophysiol. *91*, 79–92.

63. Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E.M., Hallett, M., and Cohen, L.G. (1997). Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology *48*, 1398–1403.

64. Rossi, S., Cappa, S.F., Babiloni, C., Pasqualetti, P., Miniussi, C., Carducci, F., Babiloni, F., and Rossini, P.M. (2001). Prefontal cortex in long-term memory: An 'interference' approach using magnetic stimulation. Nat. Neurosci. *4*, 948–952.

65. Jacobs, C., de Graaf, T.A., Goebel, R., and Sack, A.T. (2012). The temporal dynamics of early visual cortex involvement in behavioral priming. PLoS One *7*.

66. Lisanby, S.H., Gutman, D., Luber, B., Schroeder, C., and Sackeim, H.A. (2001). Sham TMS: Intracerebral measurement of the induced electrical field and the induction of motor-evoked potentials. Biol. Psychiatry *49*, 460–3.

67. Sandrini, M., Umiltà, C., and Rusconi, E. (2011). The use of transcranial magnetic stimulation in cognitive neuroscience: A new synthesis of methodological issues. Neurosci. Biobehav. Rev. *35*, 516–536.

68. Cohen, J. (1977). Statistical power analysis for the behavioral sciences (New York: Academic Press.).

69. Wolf, F.M. (1986). Meta-Analysis: Quantitative Methods for Research Synthesis (Beverly Hills, CA: Sage.).

70. Schiller, D., Kanen, J.W., LeDoux, J.E., Monfils, M.H., and Phelps, E.A. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. Proc. Natl. Acad. Sci. U. S. A. *110*, 20040–20045.

71. Boucsein, W., Fowles, D.C., Grimnes, S., Ben-Shakhar, G., Roth, W.T., Dawson, M.E., and Filion, D.L. (2012). Publication recommendations for electrodermal measurements. Psychophysiology *49*, 1017–1034.

FIGURE LEGEND

Figure 1. **Schematic representation of the experimental design and procedure for the fear memory reconsolidation experiment.** On separate days, participants performed a differential fear conditioning task. Images of two indoor scenes were used as conditioned stimuli (CS+ and CS-) presented in pseudorandom order. On day 1, during acquisition, the CS+ stimulus terminated with a shock (US, depicted as a lightning bolt) on 60% of the trials. On day 2, fear memory was reactivated with two presentations of unreinforced CS+ (reminder), except for the No-reminder group. Ten minutes after memory reactivation, participants received rTMS over either the dorsolateral prefrontal cortices (l-dlPFC and r-dlPFC), or placebo rTMS (Ctrl-Sham), or over a control site (Crtl-Occipital). For the Ctrl-Day2 group, fear memory was tested at day 2, while all the other groups were exposed to both the CSs without the US at day 3. For the Ctr-NoRem group, rTMS was applied over the left dlPFC without memory reactivation. After the extinction phase, and before reinstatement, participants received three unsignaled USs.

Figure 2. **SCR during fear acquisition (Day 1).** Data are represented as mean ± SEM of the SCR amplitude recorded during acquisition (Day 1) in the six groups. * denotes significant comparisons ($p < 0.05$).

Figure 3. **SCR during memory recall, extinction, and reinstatement phases (Day 3).** Data are represented as mean ± SEM of the SCR amplitude recorded during memory recall, extinction, and reinstatement phases (Day 3) in the six groups. * denotes significant comparisons ($p < 0.05$).

TABLE

Groups	Day 1		Day 3	
	CS+	CS-	CS+	CS-
r-dlPFC	30 ± 31	1 ± 2	13 ± 19	2 ± 4
l-dlPFC	37 ± 32	12 ± 19	20 ± 24	14 ± 21
Ctrl-Sham	44 ± 32	2 ± 4	17 ± 17	6 ± 11
Ctrl-Occipital	46 ± 27	7 ± 13	32 ± 31	1 ± 2
Ctrl-Day2	24 ± 30	4 ± 8	7 ± 9	4 ± 5
Ctrl-NoRem	36 ± 25	7 ± 10	22 ± 23	12 ± 10

Table 1. **CS-US contingency ratings.** Data are reported as mean ± SD contingency ratings for the CS+ and the CS- stimulus assessed on 0-100 visual analog scale (VAS) at day 1 and day 3.

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Giuseppe di Pellegrino (g.dipellegrino@unibo.it).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The datasets generated during this study are available at Open Science Framework Repository https://osf.io/jt52r/. The published article includes all datasets generated or analysed during this study.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Participants

Eighty-four healthy volunteers took part in the study. Participants were randomly assigned to one of six experimental groups: Ctrl-Sham (14 participants, 8 females, mean age ± SD: 23.2 ± 1.8), Ctrl-Occipital (14 participants, 9 females, 24.4 ± 3.1), Ctrl-NoRem (14 participants, 11 females, 21.6 ± 2.0), Ctrl-Day2 (14 participants, 6 females, 22.4 ± 3.7), r-dlPFC (14 participants, 9 females, 23.1 ± 2.6), and l-dlPFC (14 participants, 8 females, 23.9 ± 2.3). All participants were right-handed, had normal or corrected-to-normal visual acuity in both eyes, and were naïve about the purposes of the experiment. None of the participants had neurological, psychiatric or other medical problems, nor any contraindication to TMS [53,54]. Participants provided written informed consent. The procedures were approved by the University of Bologna Bioethics Committee, and were in accordance with the ethical standards of the 1964 Declaration of Helsinki (World Health Organisation, 2013). No discomfort or adverse effects of TMS were spontaneously reported by participants or noticed by the experimenter. It is widely known that anxiety and depression may affect the skin conductance response (SCR) in classical fear conditioning [55]. To account for such variability, anxiety traits were measured by means of trait-anxiety scores using form Y2 of the State and Trait-Anxiety Inventory (STAI-Y2) [56], whereas depression was assessed by means of the Hospital Anxiety and Depression Scale (HADS) [57]. A one-way ANOVA showed no significant effect of group on anxiety scores ($F_{5,78} = 1.17$; $p = 0.33$; $\eta_p2 = 0.07$; Ctrl-Sham, mean ± SD: 46.7 ± 10.0; Ctrl-Occipital, 42.9 ± 7.8; Ctrl-Day2, 42.6 ± 7.9; Ctrl-NoRem, 42.7 ± 8.1; r-dlPFC, 40.2 ±

8.0; l-dlPFC, 39.6 ± 11.1), or depression ($F_{5,78} = 0.49$; $p = 0.78$; $\eta_p 2 = 0.03$; Ctrl-Sham, 3.1 ± 2.2; Ctrl-Occipital, 2.9 ± 1.4; Ctrl-Day2, 3.6 ± 2.2; Ctrl-NoRem, 3.7 ± 2.9; r-dlPFC, 3.1 ± 3.1 and l-dlPFC, 2.5 ± 2.7).

METHOD DETAILS

Materials

The study was implemented in Matlab R2016 software (The MathWorks, Inc., Natick, Massachusetts, United States) and stimuli presentation and shock administration were controlled by PsychToolbox [58], running on a Windows-based PC (Lenovo ThinkCentre Desktop Computer). Stimuli were created with Blender (Blender Foundation, Amsterdam, Netherlands) and Cinema 4D R17 software (MAXON Computer GmbH, Friedrichsdorf, Germany), and were presented on a computer screen (screen size: 43 inches; resolution: 1920 x 1080; refresh rate: 60Hz). Stimuli consisted of images of two different 3D indoor scenes (i.e., a yellow-blue room and a grey-red room), representing the conditioned stimuli (CSs) of the study [59]. Stimulus presentation and assignment to the experimental condition was counterbalanced across participants, and the reinforced CS+ and the unreinforced CS- were counterbalanced, as well. The unconditioned stimulus (US) was a 200-ms train of electric square pulses (individual pulse width of 1 ms, frequency 50 Hz), generated by a constant-current stimulator (DS7A, Digitimer Ltd., UK) delivered to the participants' left inner wrist. The intensity of the stimulation was set with a standard workup procedure. It was initially set at 0.5 mA and increased by 1 mA. At each step, the experimenter asked whether or not the administered shock was highly annoying. Thus, individual shock intensity was set when participants reported highly annoying, but not painful, sensation [60]. A one-way ANOVA on shock intensity showed no significant differences between groups ($F_{5,78} = 1.84$; $p = 0.11$; $\eta_p 2 = 0.11$; Ctrl-Sham, mean ± SD: 9.1 mA ± 1.9; Ctrl-Occipital, 7.6 mA ± 2.6; Ctrl-Day2, 7.68 mA ± 2.51; Ctrl-NoRem, 8.8 mA ± 1.2; r-dlPFC, 8.1 mA ± 1.7; l-dlPFC, 9.2 mA ± 1.5).

SCR recording

The skin conductance response (SCR) was recorded with two Ag/AgCl electrodes (Biopac TSD203 electrodes). The electrodes have a 6-mm diameter contact area with a 1.6-mm cavity that was filled with an isotonic conductive gel and attached to the distal phalanges of the second and the third fingers of the participant's left hand. A DC amplifier (Biopac EDA100C) was used while recording the SCR. The gain factor was 5μS/V and the low-pass filter was set at 10 Hz. The analog signal was then passed through a Biopac MP-150 digital converter at a 200-Hz rate. The signal was recorded with AcqKnowledge 3.9 (BIOPAC Systems, Inc., Goleta, California) and converted to microsiemens (μS) for offline analysis.

Procedure and experimental design

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 tested individually. The procedure was the same for all participants. 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 SCR recording and for shock pulse administration were attached to the participant. The SCR was recorded continuously while participants completed the task, and data were stored for offline analysis. Participants were asked to remain as quiet and still as possible during the task and to keep their attention on the center of the screen. After verifying that SCR was being properly recorded, the intensity of the shock pulse, to be used as the unconditioned stimulus (US), was adjusted for each participant as described above. Finally, participants were informed that they had no effect on shock administration.

The experiment used a differential fear conditioning paradigm. The testing protocol involved different phases administered over three consecutive days, during which the electrodes for the

electric shock were attached to the participant's wrist [4,15,18]. During the experiment, regardless of the phase, each trial consisted of the presentation of the conditioned stimulus for 4 s. The interstimulus interval (ISI) was a grey blank screen, with a variable duration ranging from 14 to 17 s from stimulus offset to the next stimulus onset. The length of the ISI was chosen to avoid complete masking of conditioned SCRs by the unconditioned SCR to the shock in the preceding trial.

On day 1, two different phases were performed: habituation and fear acquisition. At the beginning of the session, participants were informed that different stimuli would be presented on the screen, and the participant's assignment would be to carefully observe the stimuli, as some of them might be paired with electric stimulation. During the habituation phase, the CS+ and the CS- were presented 2 times each in a random order. To ensure the absence of baseline differences within and between groups in response to the CSs stimuli before conditioning, we performed a Group x Stimulus ANOVA on SCR data collected during habituation, which showed neither significant main effects, nor a significant interaction (all $F < 1.30$; all p-values > 0.26; all $\eta_p^2 < 0.06$).

The fear acquisition phase consisted of 16 CS+ and 16 CS- trials. One CS was associated with the administration of a shock pulse, resulting in the conditioned stimulus (CS+), while the other CS was never paired with any consequence (CS-). In CS+ trials, the US (shock pulse) was administered 60% of the time (10 out of 16 trials), 3.8 s after the CS+ onset, and co-terminated with the CS+. In CS- trials, the US was never administered. The trials were pseudo-randomly presented to participants such that no more than two identical CSs occurred in a row. At the beginning of each day, participants received the instruction: "Press the spacebar every time a stimulus is presented on the screen". This was done in order to focus their attention on the screen and facilitate learning of the CS–US contingencies.

On day 2, 24 hours after the fear acquisition phase, fear memory reactivation was performed, except for the Ctrl-NoRem group. Participants were told that the same stimuli would be presented, and they were explicitly instructed to remember what they had learned the day before [18]. The memory

was reactivated with two presentations of unreinforced (without US) conditioned stimuli (CS+). The ISI was the same as in the other days, namely, 14 to 17 s from stimulus offset to the next stimulus onset. Based on previous findings showing that the reconsolidation process seems to begin between 3 and 10 min after memory reactivation [16], participants received rTMS (see details below) 10 min after reactivation by presentation of the reminder cues. For the Ctrl-NoRem group, participants were tested in a different room with a different experimenter, and they underwent a single session of rTMS over left-dlPFC without any reactivation procedure.

To assess whether the unpleasantness of the stimulation could directly affect our results, at the end of the TMS session, participants were asked to provide subjective unpleasantness ratings of the sensations caused by the magnetic stimulation, using a 5-point Likert scale ranging from 1 (“not unpleasant at all”) to 5 (“extremely unpleasant”). A one-way ANOVA on unpleasantness ratings showed no significant effect of group ($F_{5,78} = 1.45$; $p = 0.22$; $\eta_p^2 = 0.08$; Ctrl-Sham, mean ± SD: 1.1 ± 0.3; Ctrl-Occipital, 1.5 ± 0.8; Ctrl-Day2, 1.8 ± 0.7; Ctrl-NoRem, 1.6 ± 0.8; r-dlPFC, 1.5 ± 0.7; l-dlPFC, 1.5 ± 0.9).

On day 3, memory recall and extinction-reinstatement took place 24h after memory reactivation (day 2) – that is to say, 48h after the acquisition phase (day 1).

Participants were instructed that they would see the same two stimuli (CSs) from the first day. Importantly, the instructions did not reveal anything about the occurrence of the US. The memory recall phase consisted of 4 CS+ and 4 CS-, and the following extinction phase consisted of 12 CS+ and 12 CS- trials (the same that were presented during the fear acquisition phase), no longer followed by the US. After extinction learning, 3 unsignaled shocks (USs) were delivered to the wrist as a reinstatement procedure, followed by a memory recall test. During this last phase, 4 CS+ and 4 CS- trials without any US were presented to participants. CSs characteristics, trial order, and ISI were the same in all experimental phases.

To assess conditioned responses to the CSs, SCR was measured during all the experimental phases, and the responses related to the CS+ were contrasted with those related to the CS-. Moreover, at the

end of day 1 and day 3, participants were asked to rate, for each of the two CS stimuli, their expectancy of the US by marking a horizontal 10-cm Visual Analogue Scale (VAS) ranging from 0 ("not at all") to 100 ("extremely").

Finally, to rule out the possibility that the observed effects of rTMS over the left and right dlPFC were simply due to a decline in higher-level cognitive processes such as working memory abilities, participants' working memory capacity (WMC) was assessed through the automated version of the operation span task (AOSPAN; [61] at the end the experiment (day 3). A one-way ANOVA on WMC scores showed no significant effect of group ($F_{5,78} = 1.16$; $p = 0.34$; $\eta_p^2 = 0.07$).

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 70mm (Magstim Company Limited, Whiteland, UK). After the memory reactivation phase on day 2 (or at the beginning of the day 2 session, in the case of the Ctrl-NoRem group), we determined the intensity of the rTMS protocol by assessing the individual resting motor threshold (rMT). We placed the coil tangentially to the scalp on the region overlying the motor cortex ipsilateral to the targeted dlPFC (for Ctrl-Sham and Ctrl-Occipital, we considered the left motor cortex) with the coil handle pointing backward and laterally at a 45° angle away from the midline. Using a suprathreshold pulse intensity (approximately 120-130% of the rMT), the coil was moved on the scalp to determine the optimal position from which maximal MEP amplitudes could be elicited in the contralateral first dorsal interosseous (FDI) muscle – corresponding to the hand area in the motor cortex. From that position, we assessed the rMT, which was defined as the minimal intensity of the stimulator output that produces MEPs with amplitudes of at least 50μV with 50% probability [62]. A one-way ANOVA on rMT intensity showed no significant effect of group ($F_{5,78} = 1.00$; $p = 0.42$; $\eta_p^2 = 0.06$; Ctrl-Sham, mean ± SD: 69.8 ± 10.0; Ctrl-Occipital, 68.9 ± 13.7; Ctrl-Day2, 76.2 ± 13.8; Ctrl-NoRem, 66.3 ± 13.0; r-dlPFC, 72.6 ± 16.4; l-dlPFC, 72.9 ± 10.5).

After determination of each individual's rMT, we set rTMS intensity at 110% of the rMT and applied a single train of low-frequency rTMS at 1 Hz for a total duration of 15 min (900 pulses), a protocol that has been shown to affect cortical excitability beyond the duration of the rTMS application itself [63]. For stimulation of the left lateral PFC in the l-dlPFC, Ctrl-Day2 and Ctrl-NoRem groups, the TMS coil was placed over F3 using the international 10–20 electroencephalogram (EEG) system, while electrode F4 was chosen for the right lateral PFC, as in previous TMS studies [8,64], corresponding to Brodmann area 9. The coil was held tangentially to the scalp with the handle positioned 45° with respect to the sagittal line. In the case of occipital cortex stimulation (Ctrl-Occipital group), the coil was placed positioned horizontally over POz using the 10–20 EEG system [65]. For sham stimulation, the coil was centered on CPZ and positioned perpendicular to the scalp surface. As shown by previous experiments [66,67], 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.

QUANTIFICATION AND STATISTICAL ANALYSIS

SCR and subjective data analysis

Data were 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). Analysis of variance (ANOVA) was used to investigate differences within and between groups. Post-hoc analyses were conducted with Newman-Keuls 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 [68,69]. SCR data were 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 to 4.5 s time window after stimulus onset [4,70]. The minimum response criterion was 0.02, and smaller

responses were encoded as zero [71]. In the present study, none of the participants could be categorized as non-responders using the minimum amplitude cut-off of 0.02 uS in more than 50% of the CS+ unreinforced trials. Regarding SCR to CSs, stimulus onset referred to the time of the CS appearance on the screen. Regarding SCR to the US, stimulus onset was represented by the time of shock administration (3.8s after the onset of the CS). 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 mean square-root-transformed US response, to account for inter-individual variability [4]. SCRs were analyzed separately for each day. On day 1, to assess conditioned responses to the CS+, we separated CS+ from unconditioned responses to the shocks themselves. Hence, only non-reinforced CS+ trials were analyzed.

Data Replicability

In order to replicate our findings, a new sample of 14 participants (7 females, mean age ± SD: 23.3 ± 2.6) was tested in a further experiment that targeted the dlPFC in the left hemisphere. These data were directly compared with the data from the other experimental groups (r-dlPFC and l-dlPFC) to confirm the strength of our results.

The group x stimulus x phase ANOVA performed on day 1 (fear acquisition) showed successful fear learning. That is, the stimulus (CS+/CS-) by phase (early/late phase) interaction was significant ($F_{1,39} = 14.16$; $p < 0.001$; $\eta_p^2 = 0.27$). Follow-up tests revealed larger SCRs to CS+ than to CS− trials during the early phase (mean SCR ± SD for CS+: 0.45 μS± 0.19; for CS-: 0.37 μS ± 0.20; p = 0.001; d = 0.46) and the late phase of acquisition (CS+: 0.43 μS ± 0.23; CS-: 0.23 μS ± 0.16; p = 0.0001; d = 1.60) across all groups, and the difference between SCRs to CS+ and CS− trials was greater in the late phase than in the early phase (early phase: 0.09 μS ± 0.18; late phase: 0.20 μS ± 0.13; $p < 0.001$; d = 0.57). Importantly, the analysis revealed a significant stimulus x group interaction ($F_{2,39} = 3.51$; $p = 0.04$; $\eta_p^2 = 0.15$). However, follow-up tests revealed no significant

differences across groups (all p-values > 0.06). No significant main effect of, or interactions with, the factor group were found (all p-values > 0.29; all $\eta_p^2 < 0.6$).

CS-US contingency ratings revealed a significant stimulus x phase (pre-/post-fear acquisition) interaction ($F_{1,39} = 23.77$; $p < 0.0001$; $\eta_p^2 = 0.38$). Crucially, the analysis revealed neither a significant main effect of group nor an interaction with the factor group (all p-values > 0.18; all $\eta_p^2 < 0.08$). Follow-up tests showed that the CS+ elicited significantly larger shock-expectancy ratings than the CS- did after fear conditioning (mean ratings ± SD for CS+: 31.27 ± 29.47; CS-: 9.40 ± 17.86; p = 0.0001; d = 0.62), but not before fear conditioning (CS+: 8.57 ± 12.32; CS-: 10.15 ± 16.65; p = 0.89; d = 0.09). Overall, these data demonstrate that fear learning took place similarly across the three dlPFC groups of participants.

The three experimental groups showed comparable levels of SCR during reactivation of fear memories (r-dlPFC: 0.61 μS ± 0.32; l-dlPFC: 0.75 μS ± 0.49; l-dlPFC2: 0.67 μS ± 0.22; $F_{2,39} = 0.50$; $p = 0.61$; $\eta_p^2 = 0.03$). In addition, the group x phase (late acquisition/reactivation) ANOVA performed on day 2 (fear memory reactivation and brain stimulation) showed that fear memories were equally well consolidated across the three groups, as revealed by both the absence of a main effect of group ($F_{2,39} = 0.26$; $p = 0.77$; $\eta_p^2 = 0.01$) and the absence of an interaction effect between group and phase ($F_{2,39} = 0.55$; $p = 0.58$; $\eta_p^2 = 0.03$). That is, there was no effect of group on SCR that differed between the last four acquisition trials (day 1) and the two reactivation trials (day 2). These data demonstrate that, before the reconsolidation manipulation, the conditioned response was equally expressed across groups.

The group x stimulus x phase ANOVA performed on day 3 (memory recall, extinction, and reinstatement) did not reveal a significant three-way interaction ($F_{4,78} = 0.48$; $p = 0.75$; $\eta_p^2 = 0.02$) between group (r-dlPFC, l-dlPFC, l-dlPFC2), stimulus (CS+ and CS-) and phase (memory recall, extinction, and reinstatement). Crucially, the analysis revealed neither a significant main effect of group nor any interaction with the factor group (all p-values > 0.24; all $\eta_p^2 < 0.6$). These results confirm that, following administration of rTMS over the dlPFC, there were no significant

differences between CS+ and CS- in any group in the memory recall phase (r-dlPFC, CS+: 0.60 μS ± 0.40; CS-: 0.51 μS ± 0.38; l-dlPFC, CS+: 0.65 μS ± 0.30; CS-: 0.54 μS ± 0.32; l-dlPFC2, CS+: 0.62 μS ± 0.33; CS-: 0.44 μS ± 0.31). In the extinction phase, no differences between CS+ and CS- were observed in any group (r-dlPFC, CS+: 0.38 μS ± 0.33; CS-: 0.33 μS ± 0.24; l-dlPFC, CS+: 0.34 μS ± 0.24; CS-: 0.27 μS ± 0.12; l-dlPFC2, CS+: 0.47 μS ± 0.32; CS-: 0.37 μS ± 0.32). Finally, fear memory reinstatement was unsuccessful in all dlPFC groups (r-dlPFC, CS+: 0.59 μS ± 0.41; CS-: 0.48 μS ± 0.29; l-dlPFC, CS+: 0.46 μS ± 0.24; CS-: 0.44 μS ± 0.31; l-dlPFC2, CS+: 0.59 μS ± 0.34; CS-: 0.50 μS ± 0.38).

Finally, subjective US-expectancy collected at the end of day 3 confirmed previous findings of a significantly higher shock-expectancy ($F_{1,39} = 6.06$; $p = 0.018$; $\eta_p^2 = 0.134$) for CS+ (mean ratings ± SD: 16.78 ± 22.80) than CS- (6.99 ± 14.43). Moreover, we observed no main effect of group ($F_{2,39} = 1.64$; $p = 0.21$; $\eta_p^2 = 0.07$) or interaction between stimulus and group ($F_{2,39} = 0.19$; $p = 0.82$; $\eta_p^2 = 0.01$) on US-expectancy. Thus, these data indicate no effect of dlPFC stimulation on participants' learned expectations of the unconditioned stimulus.

Furthermore, a series of one-way ANOVAs showed no significant effect of group on shock intensity ($F_{2,39} = 1.79$; $p = 0.18$; $\eta_p^2 = 0.08$; r-dlPFC, 8.1 mA ± 1.7 mA; l-dlPFC, 9.2 mA ± 1.5 mA; l-dlPFC2, 8.4 mA ± 1.6 mA), unpleasantness ratings ($F_{2,39} = 2.54$; $p = 0.09$; $\eta_p^2 = 0.11$; r-dlPFC, 1.5 ± 0.7; l-dlPFC, 1.5 ± 0.9; l-dlPFC2, 2.07 ± 069), rMT intensity ($F_{2,39} = 0.19$; $p = 0.82$; $\eta_p^2 = 0.009$; r-dlPFC, 72.6 ± 16.4; l-dlPFC, 72.9 ± 10.5; l-dlPFC2, 70.3 ± 7.5), or WMC scores ($F_{2,39} = 2.24$; $p = 0.12$; $\eta_p^2 = 0.10$).

CellPress

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Raw Datasets	This Paper	https://osf.io/jt52r/
Software and Algorithms		
AcqKnowledge (version 3.9)	BIOPAC Systems, Inc.	https://www.biopac.com/
Blender (version 2.77)	Blender Foundation	https://www.blender.org/download/
Cinema 4D (version R17)	MAXON Computer GmbH	https://www.maxon.net/it/prodotti/cinema-4d
MATLAB (version r2016a)	MathWorks, Inc.	https://mathworks.com/products/matlab.html
Psychtoolbox (version 3.0.14)	Open Source software	http://psychtoolbox.org/
STATISTICA (version 12)	Dell Software	http://www.statsoft.com/Products/STATISTICA-Features
Other		
Biopac MP-150 and EDA100C	BIOPAC Systems, Inc.	https://www.biopac.com/
Digitimer Stimulator (model DS7)	Digitimer Ltd	https://digitimer.com/
Magstim TMS Super Rapid2	Magstim Company Limited	https://www.magstim.com/product/rapid-family/

Figure1 Click here to access/download;Figure;Fig1.tif

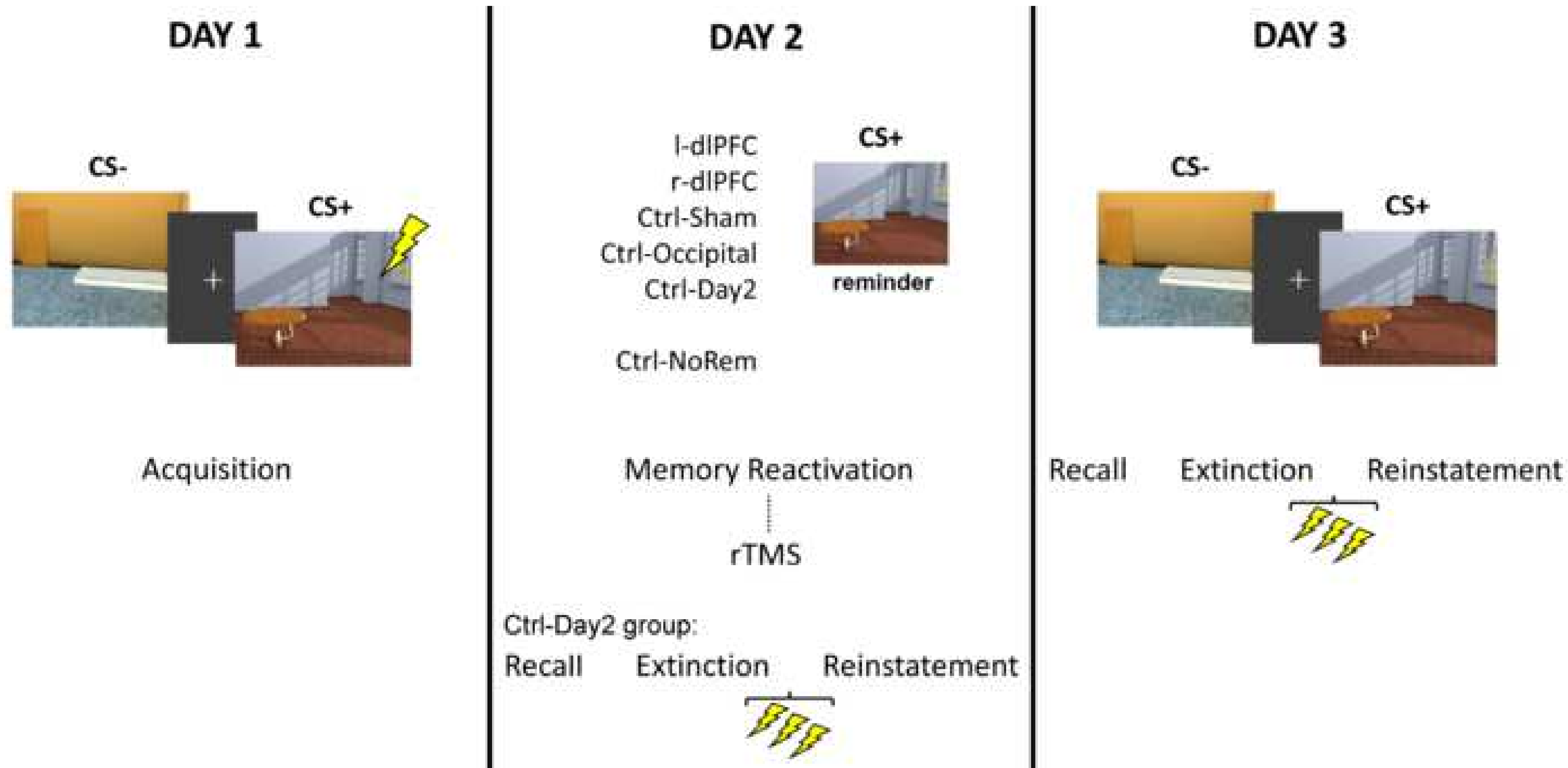


Figure2

Click here to access/download;Figure;Fig2.tif

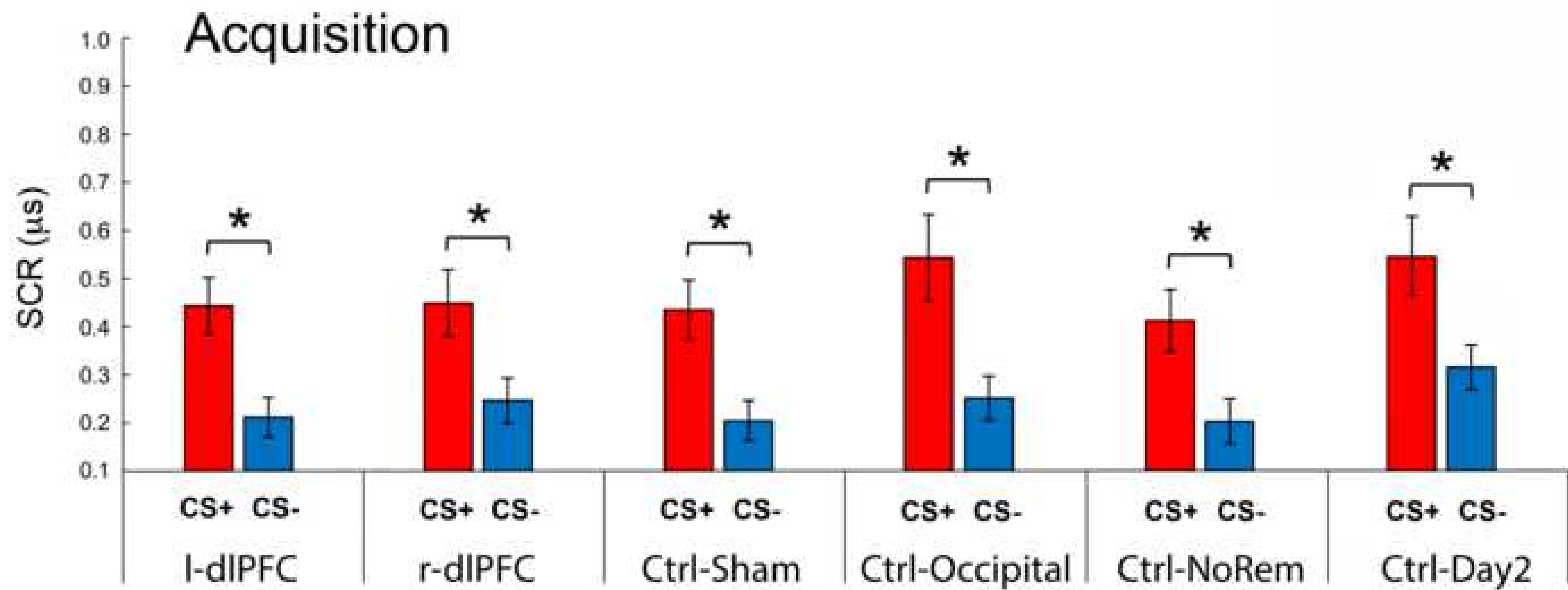
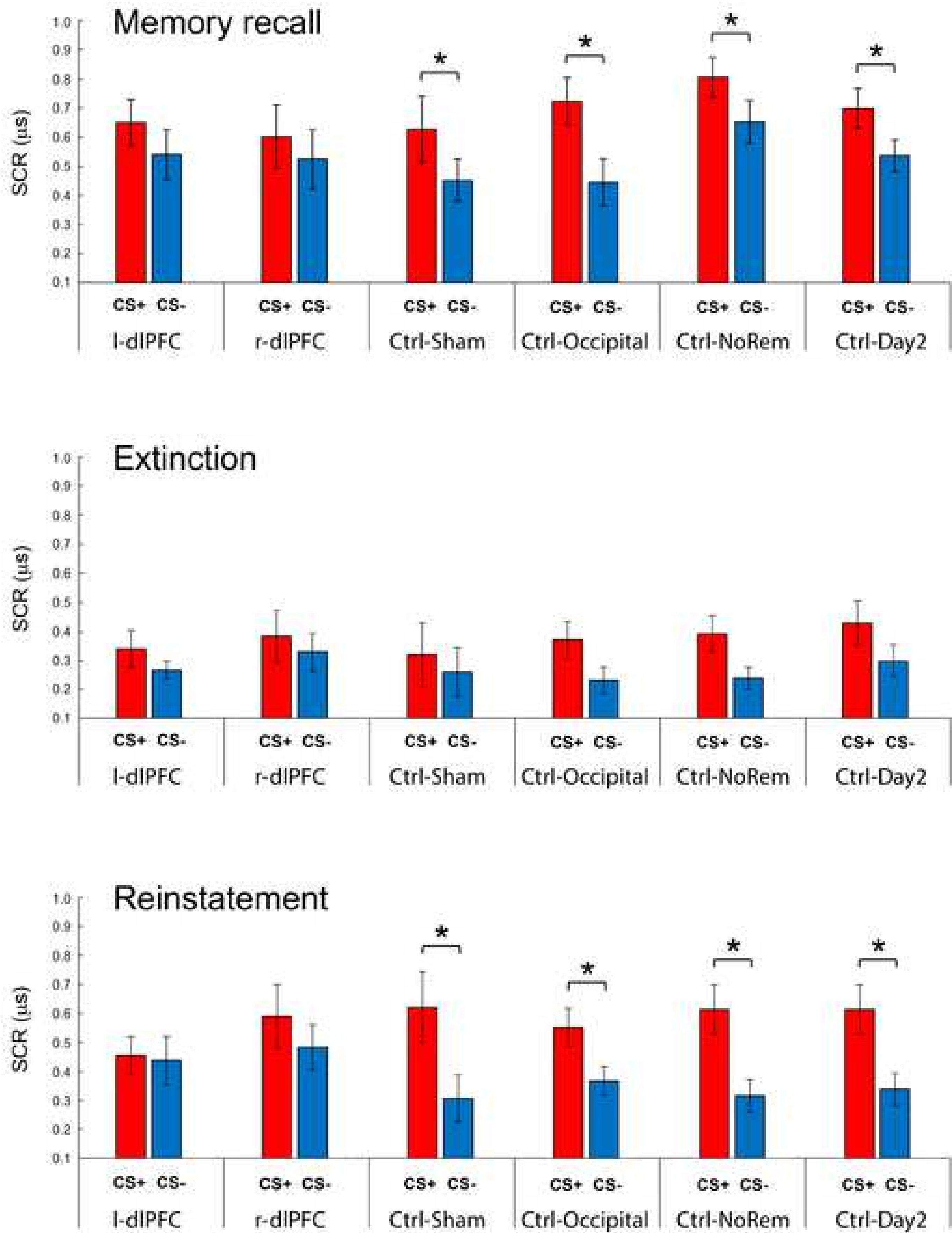


Figure3

Click here to access/download;Figure;Fig3.tif



	Contrast	F	p	Partial eta-squared
DAY 1 ANOVA SCR	Phase	45.428	< 0.0001	0.368
	Stimulus	169.532	< 0.0001	0.684
	Group	1.169	0.331	0.069
	Phase*Group	0.113	0.989	0.007
	Stimulus*Group	0.831	0.531	0.051
	Phase*Stimulus	21.97	< 0.0001	0.221
	Phase*Stimulus*Group	0.212	0.956	0.013
DAY 1 ANOVA VAS	Phase	60.013	< 0.0001	0.434
	Stimulus	56.580	< 0.0001	0.420
	Group	1.583	0.174	0.092
	Phase*Group	0.295	0.914	0.018
	Stimulus*Group	1.535	0.188	0.089
	Phase*Stimulus	88.021	< 0.0001	0.530
	Phase*Stimulus*Group	0.686	0.635	0.042
DAY 3 ANOVA SCR	Phase	58.309	< 0.0001	0.427
	Stimulus	66.684	< 0.0001	0.460
	Group	0.225	0.951	0.014
	Phase*Group	1.062	0.394	0.063
	Stimulus*Group	1.667	0.152	0.096
	Phase*Stimulus	5.620	0.004	0.067
	Phase*Stimulus*Group	1.908	0.048	0.109
DAY 3 ANOVA VAS	Stimulus	25.600	< 0.0001	0.247
	Group	1.955	0.094	0.111
	Stimulus*Group	2.276	0.055	0.127

Table S1. Statistic results obtained at day 1 and 3.