Published in final edited form as:

Biol Psychiatry. 2018 July 15; 84(2): 129–137. doi:10.1016/j.biopsych.2017.10.022.

Prefrontal cortex stimulation enhances fear extinction memory in humans

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Abstract

Background—Animal fear conditioning studies have illuminated neuronal mechanisms of learned associations between sensory stimuli and fear responses. In rats, brief electrical stimulation of the infralimbic (IL) cortex has been shown to reduce conditioned freezing during recall of extinction memory. Here, we translate this finding to humans with MRI-navigated transcranial magnetic stimulation (TMS).

Methods—Subjects (N=28) were aversively conditioned to two different cues (day 1). During extinction learning (day 2), TMS was paired with one of the conditioned cues but not the other. TMS parameters were similar to those used in rat IL: brief pulse trains (300 ms at 20 Hz) starting 100ms after cue onset, total of 4 trains (28 TMS pulses). TMS was applied to one of two targets in the left frontal cortex, one functionally connected (Target 1) and the other unconnected (Target 2, control) with a human homologue of IL in the ventromedial prefrontal cortex (vmPFC). Skin conductance responses (SCRs) were used as an index of conditioned fear.

Results—During extinction recall (day 3), the cue paired with TMS to Target 1 showed significantly reduced SCRs, whereas TMS to Target 2 had no effect. Further, we built group-level maps that weighted TMS-induced electric fields and diffusion MRI connectivity estimates with fear level. These maps revealed distinct cortical regions and large-scale networks associated with reduced vs. increased fear.

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Financial Disclosures

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. The authors report no biomedical financial interests or potential conflicts of interest.

Conclusions—The results show that spatiotemporally focused TMS may enhance extinction learning and/or consolidation of extinction memory, and suggest novel cortical areas and large-scale networks for targeting in future studies.

Keywords

Fear conditioning; fear extinction; transcranial magnetic stimulation; TMS; cortical map; diffusion tensor imaging; connectivity

Introduction

The neural mechanisms of conditioned fear extinction have been extensively studied across species (*e.g.*, (1, 2)). Fear extinction is relevant in the treatment of anxiety and fear-based disorders, such as posttraumatic stress disorder (PTSD) (3–5), and for example exposure therapy relies on extinction-based mechanisms (6–8). Understanding how the brain acquires and consolidates conditioned fear extinction memories is therefore a clinical priority. Interactions between the amygdala and sub-regions of the ventromedial prefrontal cortex (vmPFC) are key in encoding neural plasticity induced by new learning occurring during fear extinction (1, 9, 10). In animal models, fear and extinction memories can be manipulated by interfering with prefrontal or amygdala activity (11–15). The direction of the effect (*i.e.*, increasing or decreasing fear) depends on the exact location (within the amygdala and/or the vmPFC) and timing of such interference (16, 17).

In rats, we have shown that brief (~300-ms high-frequency) electrical stimulation within the infralimbic cortex (IL) 100ms after onset of a fear conditioned tone significantly reduced freezing tested during extinction recall, a finding that has been replicated (11–13). These latencies appear to translate well to humans, as shown by invasive patient recordings in the vmPFC (18). The human vmPFC (Figure 1) is considered to be a functional homologue of the rat IL, and its activation is associated with expression of extinction memory in healthy subjects (1). vmPFC dysfunction is also associated with impaired fear extinction in a number of psychopathologies including PTSD, anxiety disorders, obsessive-compulsive disorder (OCD), and schizophrenia (19–24). These findings, together with those obtained in rodents, have raised the interesting possibility that manipulations targeting vmPFC or other parts of the network such as amygdala may facilitate extinction memory consolidation.

The goal of the present study was to translate the IL fear reduction findings from rats to humans. To this aim, we designed an experiment to examine the effects of time-locked stimulation of vmPFC on the expression of fear extinction memory in humans. For brain stimulation, we used transcranial magnetic stimulation (TMS) (25, 26). Brief high-frequency repetitive TMS (rTMS) trains were delivered on-line, during extinction learning, time-locked to the cues. The timing and parameters were similar to those previously used in the rat (20-Hz rTMS trains starting 100 ms after cue onset, duration 300 ms). Consequently, only a very small number of TMS pulses were delivered (total of 28 pulses). For experimental control, we fear-conditioned each subject to two cues on day 1, but only one of the cues was paired with TMS during extinction training on day 2. We predicted that conditioned responses measured on day 3 (extinction recall) to the cue paired with TMS would be reduced. We

further hypothesized that the effect of brain stimulation would be site-specific, in that it would be observed only when TMS was delivered to a small area in the frontal cortex functionally connected with vmPFC (Target 1) but not when given to a nearby control site (Target 2) unconnected with vmPFC (Figure 1).

Methods and Materials

Subjects

A total of 43 healthy subjects were enrolled in the study. Due to technical difficulties (SCR recording device malfunction, N=1; poor electrode impedances, N=8) or absence of fear conditioning (N=6) data from 15 subjects were excluded. Thus, data from 28 subjects were included in the analyses.

Stimuli and Tasks

The participants underwent a modified version of our 3-day fear conditioning protocol (24, 27, 28) (Figure 2; Supplement 1 Figure S1). Fear conditioning was achieved by presenting visual stimuli paired with electric finger shocks and estimated using skin conductance responses (SCRs, Coulbourn Instruments, Whitehall, PA). On day 1 (Conditioning), subjects received electric finger shocks paired with visual stimuli of two different colors (red or blue lights, CS+^{TMS} or CS+^{noTMS} presented in different blocks) but not with a third color (yellow light, CS− present in both blocks). The finger shocks were presented at the end of a 12-second CS+ duration (in contrast to the 6-second CS duration used in many studies, we here increased CS length to 12 seconds to capture any delayed effects of TMS on SCR within the cue itself). The intensity of the shock was selected by each subject to be highly annoying but not painful (19, 24, 27–30). On day 2 Extinction Learning took place. Subjects were presented with 2 blocks with the same three colors as before (4 trials for each CS+, and a total of 4 CS−). One of the cues was paired with brief rTMS trains (CS+^{TMS}) delivered to the left PFC starting 100 ms after each CS+ onset; no TMS was given with the other CS+ (CS+^{noTMS}) or CS−. Thus, this design allowed us to use a within-subject control (CS+^{TMS} vs. CS+^{noTMS}). To avoid ‘floor’ effects, the number of extinction trials on day 2 was kept intentionally relatively small (4 trials), similar to a previous study (11) in which subjects were given only 4 trials of extinction training. On day 3 (Extinction Recall) subjects were again presented with the same three CS in 2 blocks. The virtual context was a picture of an office on day 1 and of a library on days 2–3 (Figure 2). The order of CS+^{TMS} and CS+^{noTMS} blocks was randomized across subjects on days 2 and 3, as was the color for CS+^{TMS} and CS+^{noTMS}. SCRs were recorded throughout the experiment and used as an index of fear level. The SCR time series were analyzed using the MNE software (31). For further details on experimental design and SCR data analyses see Supplement 1.

MRI Data and TMS navigation

To navigate TMS to the intended targets, each subject underwent structural 3 Tesla MRI (Siemens Tim Trio, Erlangen, Germany), and the individual MRIs were used in a TMS navigation system (Nexstim NBS, Helsinki, Finland). To estimate the network-level spread of TMS-evoked activations from the primary activation site to distant secondary areas, each

subject was also scanned with simultaneous multi-slice high angular resolution diffusion (HARDI) MRI tractography (32) (Supplement 1 for details).

TMS Protocol and Target Selection

Brain stimulation was applied during extinction learning (day 2) non-invasively with TMS (MagPro X100 MagOption and MC-B70 coil, MagVenture, Farum, Denmark). The rTMS frequency was 20 Hz, duration of each train 300 ms (7 pulses per train), and intensity 100% of resting motor threshold (rMT). Each rTMS train started 100 ms after the cue (CS+^{TMS}) onset. The number of rTMS trains was four (total of 28 TMS pulses). The short rTMS train duration was chosen to allow focusing of the effects of each train on the CS+^{TMS} trial with which it was paired, and to avoid cross-contamination of other trials within and across blocks.

Our target (vmPFC in Figure 1A) is a deep structure, whereas TMS is an inherently superficial stimulation technique. We therefore identified surface areas readily accessible with TMS that would lead to connectivity-based secondary activation of the vmPFC. Specifically, we conducted psychophysiological interaction (PPI) analyses (33) of our previously reported functional magnetic resonance imaging (fMRI) data in a different cohort of healthy subjects (19, 34) (Supplement 1 for details). A location in the left posterior PFC (Figure 1B) showed robust connectivity with the vmPFC (PPI $T=4.14$ $df=16$ $p=0.001$ FDR-corrected) and was thus selected as our TMS Target 1, with the goal that its stimulation would result in secondary activation of the vmPFC. For experimental control, we selected Target 2 (Supplement 1 Figure S2), which was a nearby PFC area situated 23 mm anteromedially, with poor functional connectivity with the vmPFC (PPI $T=0.69$ $df=16$ $p=0.54$ FDR-corrected). TMS was delivered in 16 subjects to Target 1 and in 12 subjects to Target 2. The MNI coordinates for Target 1 were $[-56, 2, 40]$ and for Target 2 $[-46, 23, 43]$ (Supplement 1 Figure S2).

TMS E-field estimates

We employed our published methods to compute individual cortical TMS electric field (E-field) distributions utilizing electromagnetic forward modeling in subject-specific realistically shaped volume conductor models (35, 36). The TMS coil locations relative to the head were extracted from the TMS navigator data. For group-level analyses, we performed FreeSurfer non-linear transforms of the cortical surfaces from the individual brains to an average brain (37) and averaged the distributions across subjects on the surface of an average brain. Note that 3D translation of MNI coordinates (the intended targets) from an average to individual brains necessarily introduces variability in which different gyri/sulci are stimulated across subjects, and TMS induced E-fields are not point-like but have a spatial extent. The computation of E-field distribution maps and these morphing techniques have proven previously informative as they reveal the spatial extent of the stimulated cortical areas both at the individual and group levels (38).

SCR computations and data analysis

Similar to our earlier studies (*e.g.*, (27)) we used pre-stimulus baseline correction to protect against instrument drifts. The responses were averaged in 3-second windows. The analysis

time window included both the 12-sec CS and the 6-sec time window immediately thereafter. During conditioning, the latter time window is often termed the unconditioned response (UCR). For simplicity, we here call this time window UCR during the extinction recall as well (though no US was delivered on day 3). For further details see Supplement 1.

Results

TMS targeting

We first confirmed that our TMS targeting had been successful. Figure 1C shows the group-level TMS-induced E-fields. As expected, these agreed well with the planned targets (Supplement 1 Figure S2).

SCR results

Figure 3 shows the SCRs during Conditioning and Extinction Recall (day 3) separately for Target 1 (N=16) and Target 2 (N=12). The data are shown separately for the two time windows: CR (0–12 seconds) and UCR (12–18 seconds). Figure 3 time courses (top panels) show the CRs in 3-second bins. Figure 3 bar graphs (bottom panels) show the data for the UCRs averaged across their full span (12–18 seconds). Table 1 lists the statistical results related to Figure 3. The rTMS trains during Extinction Learning (day 2) caused a strong artifact in the SCR data at the beginning of each trial, rendering these data uninterpretable. Therefore, day 2 data were not analyzed.

CR time window/Conditioning (day 1)—We first confirmed that Conditioning was similar for CS^{+TMS} and CS^{+noTMS} for both Targets 1 and 2. No differences in conditioning were observed between $CS^{+TMS/noTMS}$ for Target 1 (Figure 3A) or Target 2 (Figure 3B). Specifically, in 4×2 ANOVAs (within-subjects repeated-measures factors $Time^{0s/3s/6s/9s} \times CS^{+TMS/noTMS}$) the only significant effect was for Time, and the main effects for TMS and interaction $Time \times TMS$ were not significant (Table 1). Thus, any differences during Conditioning that could have resulted in carry-over effects during Extinction Recall were unlikely.

CR time window/Extinction Recall (day 3)—During Extinction Recall, for Target 1 the within-subjects comparisons showed that the cue paired with TMS evoked significantly weaker SCRs than the cue not paired with TMS (Figure 3A top). In contrast, for Target 2 no significant differences between CS^{+TMS} and CS^{+noTMS} were observed (Figure 3B top). ANOVAs for the CR time window (4×2 within-subjects repeated-measures factors $Time^{0s/3s/6s/9s} \times CS^{+TMS/noTMS}$) indicated that TMS had significantly modulated fear in Target 1 but not in Target 2 (Table 1). The Target 1 effect size for the CS^{+TMS} vs. CS^{+noTMS} comparison was strong (Partial Eta Squared = 0.25). A *post hoc* test for Target 1 CS^{+TMS} vs. CS^{+noTMS} was significant in the time window 9–12 seconds ($Z = -3.154$, $p = 0.002$; Wilcoxon Signed-Ranks Test, 2-tailed).

Time window UCR/Conditioning and Extinction Recall—We next tested if the TMS effects on fear reduction were specific to the CR time window or if they extended to the UCR (at 12–18s) when the finger shock was anticipated. As before, we first tested if there

were differences during Conditioning between the UCRs to either Cue (CS^{+TMS} vs. CS^{+noTMS}) separately for Target 1 (Figure 3A bottom) and Target 2 (Figure 3B bottom). No differences were observed (Table 1). During Extinction Recall, Target 1 CS^{+TMS} showed significantly weaker SCRs than CS^{+noTMS} (Figure 3A bottom, Table 1). Again, the effect size for TMS in Target 1 was robust ($\eta^2 = 0.53$). TMS delivered to Target 2 did not result in any significant differences between the cues (Figure 3B bottom, Table 1). Thus, the overall conclusion for both the CR and the UCR time windows was that TMS reduced the SCRs when delivered to Target 1 but had no effect in Target 2.

Cortical map for reduced vs. increased fear

To further examine the hypothesis that the differences in the stimulated brain areas (Target 1 vs. Target 2) were responsible for the SCR changes, we conducted two analyses.

First, we examined in more detail which cortical areas were responsible for different fear memory outcomes. Figure 4A shows the stimulated cortical locations averaged across all subjects and both TMS targets ($n=28$), which is informative as any derivative maps are only valid in areas that were stimulated. Figure 4B shows a *cortical map* where yellow indicates locations where stimulation was associated with reduced fear, and blue with increased fear. This map was computed by weighting each subject's TMS-induced E-fields with his/her fear level. Specifically, the individual level E-field distributions were multiplied with the subject's SCR differential index (CS^{+noTMS} minus CS^{+TMS}) and the individual cortical surfaces were morphed into a template brain (39) where the SCR-weighted E-field results were averaged across subjects.

Second, to obtain statistical proof that the stimulated location mattered, we tested if the variability in the spatial E-field distributions across subjects (and TMS targets) can be used to explain the variability in the observed SCR results. Thus, we conducted a principal component regression analysis (PCA regression) including all 28 subjects that correlated the 1st variance component of the TMS induced E-field distributions on the cortex with the individual's SCR index (CS^{+noTMS} minus CS^{+TMS}). This correlation, computed with linear regression (Supplement 1 for details) was significant ($F(1,26) = 5.1$, $p=0.023$), suggesting that stimulation of different cortical targets as reflected by the variance of the E-field distributions may predict different fear recall outcomes (Figures 4C–D).

Network structural connectivity map for reduced vs. increased fear

Figure 5 shows the network-level fear retention index -weighted diffusion MRI tractography results. Here, blue indicates white matter pathways that were associated with increased fear and red those with reduced fear. These results suggest that reduced fear is associated with a more posterior network. To prepare these maps, individual level diffusion MRI data were analyzed using FSL probabilistic tractography techniques (40). The tracts were seeded where each individual's cortical E-field was maximal, and thus reflect the spread of TMS from the primary target to remote connected areas. This map was then multiplied with the individual's SCR index (CS^{+noTMS} minus CS^{+TMS}). At the group level, the maps were co-registered between the individual and average brains using a robust 3D morphing technique in FreeSurfer (37) and averaged across subjects. The main result here is that the large-scale

networks were mostly non-overlapping despite that the cortical Targets 1 and 2 were close to each other. This analysis also shows connectivity patterns to deep brain structures that may serve as hypotheses in future studies.

Discussion

In the present study, we used TMS to reduce the expression of conditioned fear responses in healthy humans. Subjects had been fear conditioned to two cues. During extinction learning, brief TMS trains were paired with sub-optimal extinction (only 4 extinction trials) of one of the cues but not the other. We found that the expression of fear during extinction recall was significantly reduced only to the cue paired with TMS, suggesting that TMS enhanced extinction learning and/or extinction memory consolidation. The effect on fear responses was site-specific, as TMS to the nearby control site (Target 2) did not modulate the fear responses. The main stimulation site (Target 1) was selected based on network-level functional connectivity using fMRI PPI analyses. The timing (start 100 ms after cue onset), duration (300 ms), and frequency (20 Hz) of rTMS used in the present study mimicked those in prior rodent studies. This is a specific and direct translation of findings previously reported with infralimbic stimulation in the rat (11), thus highlighting the parallels between rodent and human findings.

The current study found a previously unreported target in the left posterior PFC for manipulating fear extinction. Moreover, delivering brief rTMS trains on-line time-locked to the cues allowed reducing conditioned fear to a given cue and not for others, and with a minimal amount of brain stimulation. This design benefited from the ability of TMS to deliver spatially and temporally focused stimulation; tDCS is less specific in both aspects. These data raise the exciting possibility of using TMS in a minimal but effective way to alter human responses to specific aversive cues.

The behaviorally weighted E-field maps on the cortical surface suggested that there may be a mosaic of areas where neighboring targets have opposing effects on fear/reward circuitry and behavior. This highlights the importance of finding the correct targets and using accurate individual MRI-based neuronavigation systems for delivering rTMS. These maps could also be used to improve TMS targeting in future fear conditioning studies. The idea of weighting TMS E-fields with behavioral outcomes may motivate future studies where similar maps could be computed for clinical applications such as depression, PTSD, and addiction.

The present rTMS experimental design and effects are quite different from most previous rTMS research and clinical use. rTMS is typically delivered off-line (*i.e.*, not time-locked to sensory stimuli) in long trains (~600–2000 pulses) at low (~1 Hz) or high (~5–25 Hz) frequencies, having suppressive *vs.* facilitatory effects on the primary target, respectively (41, 42). The experimental effects of such trains typically outlast the brain stimulation by only 15–30 minutes, though one previous off-line rTMS (1560 pulses at 10 Hz) fear conditioning study has reported changes that could be observed 24 hours later (*e.g.*, (43)). These findings are in stark contrast with the present study, where a mere 4 trains of 7 (total of 28) pulses were delivered, yet resulting in effects that persisted for at least 24 hours. By itself, this amount of rTMS should not result in long-term excitability changes. This

surprising efficacy is likely based on the on-line event-related design where each rTMS train was time-locked relative to a cue. A similar difference also exists between fear conditioning studies in the rat that have delivered continuous stimulation for prolonged periods of time (*e.g.*, (44)) *vs.* short stimulus bursts time-locked relative to the conditioned cues, where timing has been shown to be critical for modulating fear memories (11–13, 45). Note, however, that while the time windows appears similar between rodents and humans (18), in the current study we did not parametrically manipulate the timing of TMS, and we thus cannot make conclusive remarks about the timing-specificity of rTMS in humans.

Previous animal studies also point to a possible mechanism how vmPFC stimulation may reduce fear expression. In both rodents and cats, medial PFC stimulation has been shown to block fear-related information flow from basolateral to the central nucleus of the amygdala (16); the latter is a primary key output of the amygdala. Thus, when applied at the right location and time window, the rTMS train may be serving as an interrupt signal to block the output of the amygdala responses that mediate conditioned fear, which may result in long-lasting changes in fear expression (46).

While our PPI fMRI connectivity data suggested that Target 1 should activate vmPFC, the diffusion MRI connectivity estimates (Figure 5) did not find direct connections between the targeted PFC regions and vmPFC. The most likely explanation for this apparent discrepancy is that diffusion MRI tends to underestimate interregional functional connectivity, because the technique cannot readily follow polysynaptic connections. Thus, TMS to Target 1 likely activated the vmPFC, but did so indirectly, *via* other cortical or subcortical structures. An alternative explanation is that vmPFC was not activated, in which case its activation was not necessary for the observed SCR changes. In accord with this idea, a recent rodent study suggested that IL may not be necessary for retrieval of extinction memory (15), which would emphasize the roles of other related regions, including the amygdala, hippocampus, and striatum (15, 47, 48). To address this question, future studies (*e.g.*, TMS-fMRI) are needed.

The network-level tractography results are also interesting as they suggested that there were anatomically non-overlapping large-scale networks underlying reduced *vs.* increased fear, despite that the two TMS targets were very close to each other on the cortical surface. Network-level aspects are relevant, because clinical rTMS effects are likely to be mediated *via* network-level activations including the secondarily stimulated deep areas (see, *e.g.*, (49) for a review in depression). Correspondingly, our target selection was based on network-level connectivity analysis of fMRI PPI data, a technique which has been previously used to analyze TMS network-level effects (50, 51). Such maps may also suggest alternative entry points at the brain surface that allow TMS to reach the desired large-scale networks. Future advances revealing large-scale networks are likely to benefit from recent developments in diffusion MRI techniques and their combinations with TMS (32, 52).

Limitations of the study included a relatively small sample size. Furthermore, Targets 1 and 2 were selected based on group-level fMRI data from a different cohort of healthy subjects, whereas both cognitive neuroscience (53) and clinical (54) rTMS studies have suggested that targeting based on individual level fMRI data could be even more accurate. Strengths of the study included robust experimental controls. Each subject received trials with and without

rTMS allowing within-subjects comparisons, and as a between-subjects control we stimulated two different cortical targets that were separated by mere 23 mm but resulted in different behavioral outcomes. Additional strengths included using PPI functional connectivity for TMS target selection and *post hoc* verification with TMS E-field computations. Further, we used between-subject alignment of brains using FreeSurfer morphing techniques that mitigate inaccuracies of standard 3D coregistration techniques which overlap different gyri/sulci across individuals (37, 39). Finally, the behaviorally weighted TMS E-fields and network-level estimates were novel.

The present findings are consistent with previous neuromodulation studies in humans that have manipulated the learning, consolidation, and/or expression of fear extinction memory ((1, 55) for reviews). For example, some transcranial direct current stimulation (tDCS) studies have suggested that conditioned fear expression during extinction memory recall, as measured by SCRs, could be reduced in healthy humans and in PTSD patients (56–58). However, other tDCS studies have found no effect of stimulation (59) or an opposite effect (increased fear) (60), possibly reflecting different stimulation polarities, electrode locations, and outcome measures across the studies. Some rTMS studies, using off-line paradigms with over 1500 pulses, have found reduced fear when stimulating large areas around the frontal midline (43, 61). Together with the present results, these findings are encouraging and highlight the potential of using neuromodulation techniques for manipulating fear.

In the therapeutic domain, rTMS has been explored as a potential treatment for PTSD patients with some success (62–66). Some studies have also found that the combination of exposure therapy - which reactivates the traumatic memory, potentially bringing it to a labile state - and rTMS may be effective (67, 68). Similar results have been found in patients diagnosed with acrophobia (61). Most PTSD studies have targeted the right DLPFC with either high (20 Hz) or low (1 Hz) frequency stimulation ((69) for a review), but the left PFC location in the present study has not been previously explored. However, note that our targets were customized for an experimental fear memory in healthy subjects, whereas neuronal representations of traumatic memories may shift over time to engage more neuronal structures (70). Regardless, the present results could pave way to the development of clinical paradigms aimed at disrupting persistent fearful memories or behavioral patterns. If the time window between reactivation of the memory and delivering rTMS in PTSD is as tight as fear conditioning studies suggests (11–13), it might be reasonable to adapt the present paradigm to a clinical setting, with brief on-line rTMS trains (instead of continuous off-line rTMS) time-locked with each memory reactivation trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by awards DM 102304 from the Department of Defense (DOD) (TR, M-FM, DP, SF, and MM) and R00EB015445 (AN)/R00EB012107 (KS) from the National Institutes of Health (NIH). The research environment was supported by the NIH/National Institute of Biomedical Imaging and Bioengineering (NIBIB) grant P41EB015896 (Center for Functional Neuroimaging Techniques, CFNT), NIH Shared Instrumentation Grants S10RR024694 and S10RR023043, and the Harvard Clinical and Translational Science Center (Harvard Catalyst;

NCRR-NIH UL1 RR025758; NCRRNIH UL1 TR000170). We thank Dr. Anastasia Yendiki for expert advice in probabilistic tractography.

References

1. Milad M, Quirk G. 2012; Fear extinction as a model for translational neuroscience: ten years of progress. *Annu Rev Psychol.* 63:129–151. [PubMed: 22129456]
2. Milad M, Rauch S, Pitman R, Quirk G. 2006; Fear extinction in rats: Implications for human brain imaging and anxiety disorders. *Biol Psychol.* 73:61–71. [PubMed: 16476517]
3. Graham B, Milad M. 2011; The study of fear extinction: implications for anxiety disorders. *Am J Psychiatry.* 168:1255–1265. [PubMed: 21865528]
4. Smith N, Doran J, Sippel L, Harpaz-Rotem I. 2017; Fear extinction and memory reconsolidation as critical components in behavioral treatment for posttraumatic stress disorder and potential augmentation of these processes. *Neurosci Lett.* 649:170–175. [PubMed: 28065842]
5. Bowers M, Ressler K. 2015; An overview of translationally informed treatments for posttraumatic stress disorder: Animal models of Pavlovian fear conditioning to human clinical trials. *Biol Psychiatry.* 78:E15–27. [PubMed: 26238379]
6. Vervliet B, Craske M, Hermans D. 2013; Fear extinction and relapse: state of the art. *Annu Rev Clin Psychol.* 9:215–248. [PubMed: 23537484]
7. Foa E. 2011; Prolonged exposure therapy: past, present, and future. *Depress Anxiety.* 28:1043–1047. [PubMed: 22134957]
8. Foa E, McLean C. 2016; The efficacy of exposure therapy for anxiety-related disorders and its underlying mechanisms: The case of OCD and PTSD. *Annu Rev Clin Psychol.* 12:1–28.
9. Dejean C, Courtin J, Rozeske R, Bonnet M, Dousset V, Michelet T, et al. 2015; Neuronal circuits for fear expression and recovery: Recent advances and potential therapeutic strategies. *Biol Psychiatry.* 78:298–306. [PubMed: 25908496]
10. Quirk G, Mueller D. 2008; Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology.* 33:56–72. [PubMed: 17882236]
11. Milad M, Quirk G. 2002; Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature.* 420:70–74. [PubMed: 12422216]
12. Milad M, Vidal-Gonzalez I, Quirk G. 2004; Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behav Neurosci.* 118:389–394. [PubMed: 15113265]
13. Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch S, Quirk G. 2006; Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learn Mem.* 13:728–733. [PubMed: 17142302]
14. Kim H, Cho H, Augustine G, Han J. 2016; Selective control of fear expression by optogenetic manipulation of infralimbic cortex after extinction. *Neuropsychopharmacology.* 41:1261–1273. [PubMed: 26354044]
15. Do-Monte F, Manzano-Nieves G, Quiñones-Laracuente K, Ramos-Medina L, Quirk G. 2015; Revisiting the role of infralimbic cortex in fear extinction with optogenetics. *J Neurosci.* 35:3607–3615. [PubMed: 25716859]
16. Quirk G, Likhtik E, Pelletier J, Pare D. 2003; Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *J Neurosci.* 23:8800–8807. [PubMed: 14507980]
17. Herry C, Ciocchi S, Senn V, Demmou L, Muller C, Luthi A. 2008; Switching on and off fear by distinct neuronal circuits. *Nature.* 454:600–606. [PubMed: 18615015]
18. Kawasaki H, Kaufman O, Damasio H, Damasio A, Granner M, Bakken H, et al. 2001; Single-neuron responses to emotional visual stimuli recorded in human ventral prefrontal cortex. *Nat Neurosci.* 4:15–16. [PubMed: 11135639]
19. Milad M, Pitman R, Ellis C, Gold A, Shin L, Lasko N, et al. 2009; Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry.* 66:1075–1082. [PubMed: 19748076]

20. Milad M, Furtak S, Greenberg J, Keshaviah A, Im J, Falkenstein M, et al. 2013; Deficits in conditioned fear extinction in obsessive-compulsive disorder and neurobiological changes in the fear circuit. *JAMA Psychiatry*. 70:608–618. [PubMed: 23740049]
21. Holt D, Coombs G, Zeidan M, Goff D, Milad M. 2012; Failure of neural responses to safety cues in schizophrenia. *Arch Gen Psychiatry*. 69:893–903. [PubMed: 22945619]
22. Garfinkel S, Abelson J, King A, Sripada R, Wang X, Gaines L, et al. 2014; Impaired contextual modulation of memories in PTSD: an fMRI and psychophysiological study of extinction retention and fear renewal. *J Neurosci*. 34:13435–13443. [PubMed: 25274821]
23. Marin M, Song H, VanElzakker M, Staples-Bradley L, Linnman C, Pace-Schott E, et al. 2016; Association of resting metabolism in the fear neural network with extinction recall activations and clinical measures in trauma-exposed individuals. *Am J Psychiatry*. 173:930–938. [PubMed: 26917165]
24. Marin M, Zsido R, Song H, Lasko N, Killgore W, Rauch S, et al. 2017; Skin conductance responses and neural activations during fear conditioning and extinction recall across anxiety disorders. *JAMA Psychiatry*. 74:622–631. [PubMed: 28403387]
25. Barker A, Jalinous R, Freeston I. 1985; Non-invasive magnetic stimulation of human motor cortex. *Lancet*. 1:1106–1107. [PubMed: 2860322]
26. Diana M, Raij T, Melis M, Nummenmaa A, Leggio L, Bonci A. 2017; Rehabilitating the addicted brain with transcranial magnetic stimulation. *Nat Rev Neurosci*.
27. Milad M, Orr S, Pitman R, Rauch S. 2005; Context modulation of memory for fear extinction in humans. *Psychophysiology*. 42:456–464. [PubMed: 16008774]
28. Milad M, Quirk G, Pitman R, Orr S, Fischl B, Rauch S. 2007; A role for the human dorsal anterior cingulate cortex in fear expression. *Biol Psychiatry*. 62:1191–1194. [PubMed: 17707349]
29. Linnman C, Zeidan M, Furtak S, Pitman R, Quirk G, Milad M. 2012; Resting amygdala and medial prefrontal metabolism predicts functional activation of the fear extinction circuit. *Am J Psychiatry*. 169:415–423. [PubMed: 22318762]
30. Zeidan M, Lebron-Milad K, Thompson-Hollands J, Im J, Dougherty D, Holt D, et al. 2011; Test-retest reliability during fear acquisition and fear extinction in humans. *CNS Neurosci Ther*. 18:313–317.
31. Gramfort A, Luessi M, Larson E, Engemann D, Strohmeier D, Brodbeck C, et al. 2014; MNE software for processing MEG and EEG data. *Neuroimage*. 86:446–460. [PubMed: 24161808]
32. Setsompop K, Cohen-Adad J, Gagoski B, Raij T, Yendiki A, Keil B, et al. 2012; Improving diffusion MRI using simultaneous multi-slice echo planar imaging. *Neuroimage*. 63:569–580. [PubMed: 22732564]
33. Friston K, Buechel C, Fink G, Morris J, Rolls E, Dolan R. 1997; Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage*. 6:218–229. [PubMed: 9344826]
34. Milad M, Wright C, Orr S, Pitman R, Quirk G, Rauch S. 2007; Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol Psychiatry*. 62:446–454. [PubMed: 17217927]
35. Nummenmaa A, Stenroos M, Ilmoniemi R, Okada Y, Hämäläinen M, Raij T. 2013; Comparison of spherical and anatomically realistic boundary element head models for transcranial magnetic stimulation navigation. *Clin Neurophysiol*. 124:1995–2007. [PubMed: 23890512]
36. Stenroos M, Mäntynen V, Nenonen J. 2007; A Matlab library for solving quasi-static volume conduction problems using the boundary element method. *Comput Methods Programs Biomed*. 88:256–263. [PubMed: 18022274]
37. Postelnicu G, Zollei L, Fischl B. 2009; Combined volumetric and surface registration. *IEEE Trans Med Imaging*. 28:508–522. [PubMed: 19273000]
38. Ahveninen J, Huang S, Nummenmaa A, Belliveau J, Hung A, Jaaskelainen I, et al. 2013; Evidence for distinct human auditory cortex regions for sound location versus identity processing. *Nature Comm*. 4:2585.
39. Fischl B, Sereno M, Tootell R, Dale A. 1999; High-resolution inter-subject averaging and a coordinate system for the cortical surface. *Hum Brain Mapp*. 8:272–284. [PubMed: 10619420]

40. Behrens T, Woolrich M, Jenkinson M, Johansen-Berg H, Nunes R, Clare S, et al. 2003; Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magn Reson Med*. 50:1077–1088. [PubMed: 14587019]
41. Chen R, Classen J, Gerloff C, Celnik P, Wassermann E, Hallett M, et al. 1997; Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*. 48:1398–1403. [PubMed: 9153480]
42. Pascual-Leone A, Valls-Sole J, Wassermann E, Hallett M. 1994; Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain*. 117:847–858. [PubMed: 7922470]
43. Guhn A, Dresler T, Andreatta M, Müller L, Hahn T, Tupak S, et al. 2014; Medial prefrontal cortex stimulation modulates the processing of conditioned fear. *Front Behav Neurosci*. 8:44. [PubMed: 24600362]
44. Stehberg J, Levy D, Zangen A. 2009; Impairment of aversive memory reconsolidation by localized intracranial electrical stimulation. *Eur J Neurosci*. 29:964–969. [PubMed: 19200060]
45. Baek K, Chae J, Jeong J. 2012; The effect of repetitive transcranial magnetic stimulation on fear extinction in rats. *Neuroscience*. 200:159–165. [PubMed: 21989475]
46. Schiller D, Monfils M, Raio C, Johnson D, Ledoux J, Phelps E. 2010; Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature*. 463:49–53. [PubMed: 20010606]
47. Rodriguez-Romaguera J, Do-Monte F, Tanimura Y, Quirk G, Haber S. 2015; Enhancement of fear extinction with deep brain stimulation: evidence for medial orbitofrontal involvement. *Neuropsychopharmacology*. 40:1726–1733. [PubMed: 25601229]
48. Burgos-Robles A, Kimchi E, Izadmehr E, Porzenheim M, Ramos-Guasp W, Nieh E, et al. 2017; Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. *Nat Neurosci*. 824–835:824–835.
49. Baeken C, De Raedt R. 2011; Neurobiological mechanisms of repetitive transcranial magnetic stimulation on the underlying neurocircuitry in unipolar depression. *Dialogues Clin Neurosci*. 13:139–145. [PubMed: 21485753]
50. O'Shea J, Johansen-Berg H, Trief D, Göbel S, Rushworth M. 2007; Functionally specific reorganization in human premotor cortex. *Neuron*. 54:479–490. [PubMed: 17481399]
51. Chen A, Oathes D, Chang C, Bradley T, Zhou Z, Williams L, et al. 2013; Causal interactions between fronto-parietal central executive and default-mode networks in humans. *Proc Natl Acad Sci U S A*. 110(49):19944–9. [PubMed: 24248372]
52. Nummenmaa A, McNab J, Savadjiev P, Okada Y, Hämäläinen M, Wang R, et al. 2014; Targeting of white matter tracts with transcranial magnetic stimulation. *Brain Stimul*. 7:80–84. [PubMed: 24220599]
53. Sack A, Kadosh R, Schuhmann T, Moerel M, Walsh V, Goebel R. 2009; Optimizing functional accuracy of TMS in cognitive studies: A comparison of methods. *J Cogn Neurosci*. 21:207–221. [PubMed: 18823235]
54. Fox M, Liu H, Pascual-Leone A. 2013; Identification of reproducible individualized targets for treatment of depression with TMS based on intrinsic connectivity. *Neuroimage*. 66:151–160. [PubMed: 23142067]
55. Herry C, Johansen J. 2014; Encoding of fear learning and memory in distributed neuronal circuits. *Nat Neurosci*. 17:1644–1654. [PubMed: 25413091]
56. Asthana M, Nueckel K, Mühlberger A, Neueder D, Polak T, Domschke K, et al. 2013; Effects of transcranial direct current stimulation on consolidation of fear memory. *Front Psychiatry*. 4:107. [PubMed: 24069003]
57. van't Wout M, Mariano T, Garnaat S, Reddy M, Rasmussen S, Greenberg B. 2016; Can transcranial direct current stimulation augment extinction of conditioned fear? *Brain Stimul*. 529–36:529–536.
58. van't Wout M, Longo S, Reddy M, Philip N, Bowker M, Greenberg B. 2017; Transcranial direct current stimulation may modulate extinction memory in posttraumatic stress disorder. *Brain Behav*. 7:e00681. [PubMed: 28523223]
59. Mungee A, Burger M, Bajbouj M. 2016; No effect of cathodal transcranial direct current stimulation on fear memory in healthy human subjects. *Brain Sci*. 6:pii: E55.

60. Mungee A, Kazzer P, Feeser M, Nitsche M, Schiller D, Bajbouj M. 2014; Transcranial direct current stimulation of the prefrontal cortex: a means to modulate fear memories. *Neuroreport*. 25:480–484. [PubMed: 24384505]
61. Herrmann M, Katzorce A, Busch Y, Gromer D, Polak T, Pauli P, et al. 2017; Medial prefrontal cortex stimulation accelerates therapy response of exposure therapy in acrophobia. *Brain Stimul*. 10:291–297. [PubMed: 27931887]
62. Grisaru N, Amir M, Cohen H, Kaplan Z. 1998; Effect of transcranial magnetic stimulation in posttraumatic stress disorder: a preliminary study. *Biol Psychiatry*. 44:52–55. [PubMed: 9646883]
63. Cohen H, Kaplan Z, Kotler M, Kouperman I, Moisa R, Grisaru N. 2004; Repetitive transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in posttraumatic stress disorder: a double-blind, placebo-controlled study. *Am J Psychiatry*. 161:515–524. [PubMed: 14992978]
64. Boggio P, Rocha M, Oliveira M, Fecteau S, Cohen R, Campanhã C, et al. 2010; Noninvasive brain stimulation with high-frequency and low-intensity repetitive transcranial magnetic stimulation treatment for posttraumatic stress disorder. *J Clin Psychiatry*. 71:992–999. [PubMed: 20051219]
65. Watts B, Landon B, Groft A, Young-Xu Y. 2012; A sham controlled study of repetitive transcranial magnetic stimulation for posttraumatic stress disorder. *Brain Stimul*. 5:38–43. [PubMed: 22264669]
66. Nam D, Pae C, Chae J. 2013; Low-frequency, repetitive transcranial magnetic stimulation for the treatment of patients with posttraumatic stress disorder: a double-blind, sham-controlled study. *Clin Psychopharmacol Neurosci*. 11:96–102. [PubMed: 24023554]
67. Osuch E, Benson B, Luckenbaugh D, Geraci M, Post R, McCann U. 2009; Repetitive TMS combined with exposure therapy for PTSD: a preliminary study. *J Anxiety Disord*. 23:54–59. [PubMed: 18455908]
68. Isserles M, Shalev A, Roth Y, Peri T, Kutz I, Zlotnick E, et al. 2013; Effectiveness of deep transcranial magnetic stimulation combined with a brief exposure procedure in post-traumatic stress disorder - a pilot study. *Brain Stimul*. 6:377–383. [PubMed: 22921765]
69. Clark C, Cole J, Winter C, Williams K, Grammer G. 2015; A review of transcranial magnetic stimulation as a treatment for post-traumatic stress disorder. *Curr Psychiatry Rep*. 17:83. [PubMed: 26324821]
70. Do-Monte F, Quiñones-Laracuente K, Quirk G. 2015; A temporal shift in the circuits mediating retrieval of fear memory. *Nature*. 519:460–463. [PubMed: 25600268]

Figure 1.

(A) Translational targeting. Left. Rodent studies have shown that stimulation of the infralimbic (IL, red dot) cortex during extinction reduces fear behaviors during recall. Right. The human homologue of IL has been suggested to be situated in ventromedial prefrontal cortex (vmPFC, red dot). (B) Connectivity-based targeting. Since vmPFC cannot be reached with TMS directly, we conducted a psychophysiological interaction (PPI) analysis of fMRI data recorded during fear conditioning to reveal surface candidate areas connected to vmPFC that can be directly stimulated by TMS. An area in the left lateral prefrontal cortex (PFC) exhibited strong functional connectivity with vmPFC (z-scores shown on the cortical

surface) and was therefore chosen as our main TMS target. A nearby location situated anteromedially, having weak functional connectivity with vmPFC, was chosen as the control TMS target (Supplement 1 Figure S2). (C) Post-hoc target verification. Following rTMS, the induced E-fields were computed on the cortical anatomy for each subject individually and morphed into a template brain cortical surface where they were averaged across subjects separately for Target 1 (left) and Target 2 (right).

Figure 2.
Visual stimuli and experimental paradigm. See text for details.

Figure 3.

SCRs for Target 1 (A) and Target 2 (B). The results are shown separately for Conditioning (day 1) and Recall (day 3), and separately for the time windows 0 to 12 s (Conditioned response, CR, time courses in upper panels) and 12 to 18 s (Unconditioned response, UCR, bar graphs in lower panels - note that the term UCR refers to the time window, and there were no US events during day 3). Extinction (day 2) data are not shown as the SCRs were corrupted by the TMS pulses. For CR time courses, the data are binned at 3-second steps from the onset of context (Cx, -3 seconds), CS onset is at $t=0$ seconds, and the time course ends at the offset of the CS (12 seconds) which is when the electric shock was delivered on day 1. Note that data averaged from -3 to 0 seconds is shown at the -3 second mark, *etc.* During Conditioning (day 1), responses to stimuli that did not receive TMS (black) did not significantly differ from those that received TMS (red) for either Target 1 or 2. During Recall (day 3), fear responses for CS+ that did not receive TMS (black) were significantly stronger (*) than for those that received TMS (red) for Target 1 but not for Target 2. This Target 1 difference extended to both the CR and UCR time windows and was maximal at 9–15 seconds, *i.e.*, at the junction between CR and UCR. Error bars show SEM. See also Supplemental Figure S3.

Figure 4.

Cortical map: Finding the areas responsible for the effects. (A) The cortical areas that were stimulated ($n=28$, left hemisphere lateral view). The individual level E-field estimates were thresholded at 80% maximum, binarized, morphed to a standard brain surface, and averaged across subjects. This map thus depicts the number of subjects where each cortical location received TMS exceeding the threshold. (B) Cortical areas associated with reduced *vs.* increased fear ($n=28$). The TMS-induced E-field distribution of each subject was multiplied with the individual SCR index, which could be either positive (reduced fear) or negative (increased fear). Thereafter, the maps were morphed to a standard brain and averaged across subjects. Yellow/red shows areas where stimulation reduced fear, whereas blue indicates regions associated with increased fear (green = overlap). The inset shows the area of interest magnified. To aid visualization, averaging was done separately for subjects where fear was reduced *vs.* increased. (C) PCA regression analysis: Spatial distribution of the 1st PCA component. (D) Linear model of each subject's SCR index regressed against the PCA scores from panel C. The correlation was significant, suggesting that the behavioral effects depended on which brain area was stimulated.

Figure 5.

Network-level structural connectivity map. Networks that increased fear (light blue) differed from those that decreased fear (red). These maps are probabilistic MRI tractography results seeded from the cortical area that was maximally activated by TMS in each individual and then multiplied with their fear retention indices. This tractography result did not detect a direct pathway between the PFC Target 1 and vmPFC, whereas the rs-fMRI PPI data suggested that these areas are functionally connected, meaning that the connection was likely to exist but was polysynaptic. Projections from left lateral (left), top (middle), and posterior (right) views. For orientation we also show putamen (dark blue) and amygdala (magenta). A = anterior, P = posterior, L = left, R = right.

Table 1

Statistical results for Figure 3 SCR data.

Conditioning				
Target		Time	TMS	Time × TMS
1	CR	F(3,45)=8.87 p=0.000	F(1,15)=0.03 p=0.862	F(3,45)=0.98 p=0.409
	UCR	---	F(1,15)=0.77 p=0.393	---
2	CR	F(3,33)=3.49 p=0.027	F(1,11)=2.32 p=0.156	F(3,33)=0.31 p=0.818
	UCR	---	F(1,11)=1.38 p=0.265	---

Recall				
Target		Time	TMS	Time × TMS
1	CR	F(3,45)=8.19 p<0.001	F(1,15)=5.02 <u>p=0.041</u>	F(3,45)=3.10 <u>p=0.036</u>
	UCR	---	F(1,15)=16.85 <u>p=0.001</u>	---
2	CR	F(3,33)=9.23 <u>p<0.001</u>	F(1,11)=0.20 p=0.662	F(3,33)=1.59 p=0.211
	UCR	---	F(1,11)=1.78 p=0.210	---