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Causally Probing the Role of the Hippocampus in Fear Discrimination: A Precision Functional Mapping–Guided, Transcranial Magnetic Stimulation Study in Participants With Posttraumatic Stress Symptoms

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ABSTRACT

BACKGROUND: Fear overgeneralization is a promising pathogenic mechanism of clinical anxiety. A dominant model posits that hippocampal pattern separation failures drive overgeneralization. Hippocampal network–targeted transcranial magnetic stimulation (HNT-TMS) has been shown to strengthen hippocampal-dependent learning/memory processes. However, no study has examined whether HNT-TMS can alter fear learning/memory.

METHODS: Continuous theta burst stimulation was delivered to individualized left posterior parietal stimulation sites derived via seed-based connectivity, precision functional mapping, and electric field modeling methods. A vertex control site was also stimulated in a within-participant, randomized controlled design. Continuous theta burst stimulation was delivered prior to 2 visual discrimination tasks (1 fear based, 1 neutral). Multilevel models were used to model and test data. Participants were undergraduates with posttraumatic stress symptoms (final n = 25).

RESULTS: Main analyses did not indicate that HNT-TMS strengthened discrimination. However, multilevel interaction analyses revealed that HNT-TMS strengthened fear discrimination in participants with lower fear sensitization (indexed by responses to a control stimulus with no similarity to the conditioned fear cue) across multiple indices (anxiety ratings: β = 0.10, 95% CI, 0.04 to 0.17, p = .001; risk ratings: β = 0.07, 95% CI, 0.00 to 0.13, p = .037).

CONCLUSIONS: Overgeneralization is an associative process that reflects deficient discrimination of the fear cue from similar cues. In contrast, sensitization reflects nonassociative responding unrelated to fear cue similarity. Our results suggest that HNT-TMS may selectively sharpen fear discrimination when associative response patterns, which putatively implicate the hippocampus, are more strongly engaged.

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Fear generalization involves the spread of fear from a stimulus associated with an aversive outcome to similar stimuli (1,2). This process is generally adaptive because fear cues are rarely re-encountered in precisely the same form. However, the proliferation of fear to safe stimuli with low similarity is maladaptive. It has been theorized that such fear overgeneralization plays a critical role in clinical anxiety (3). Empirical support for this proposition comes from studies that have detected overgeneralization in anxiety-related disorders using systematic laboratory methods (4).

The clinical relevance of overgeneralization has inspired strong interest in its neurobiology (5). A hippocampal schematic matching–based model (6,7) posits that the hippocampus matches cortical representations of stimuli that resemble a conditioned fear cue against stored representations of that cue. Stimuli with sufficient similarity trigger hippocampal-mediated pattern completion that results in fear memory retrieval (5,8); stimuli with insufficient similarity trigger hippocampal-mediated pattern separation and new safety memory encoding. According to this model, overgeneralization occurs when hippocampal pattern separation fails, and safe stimuli with insufficient fear cue similarity trigger fear memory retrieval.

The hippocampal schematic matching model of fear generalization is supported by cross-species findings. Rodent hippocampal facilitation and disruption (particularly of the dentate gyrus subregion) has been shown to strengthen (9,10) and weaken (11,12) fear discrimination (the inverse of generalization), respectively. In human neuroimaging studies, hippocampal activations have been shown to increase as stimuli

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become more unlike the fear cue, consistent with discrimination (5). Posttraumatic stress disorder (PTSD) is associated with more gradual hippocampal activation increases as cues diverge from the fear cue, consistent with deficient discrimination (13). These cross-species findings implicate the hippocampus in fear discrimination and support deficient hippocampal discrimination as a promising mechanism of clinical anxiety. However, neuroimaging results are correlational (14), and cross-species differences (15–17) complicate direct rodent to human translation. Therefore, the hippocampal discrimination function requires causal testing in humans.

Transcranial magnetic stimulation (TMS) is a safe and effective method for manipulating human brain circuits (18). TMS applied to left posterior parietal sites with strong hippocampal connectivity (hippocampal network–targeted TMS [HNT-TMS]) has been shown to modulate hippocampal activation/connectivity and strengthen hippocampal-dependent learning/memory processes (19–25), including encoding (26). HNT-TMS–driven hippocampal activation changes during encoding have been linked to successful memory retrieval (27) and more discriminant neocortical representations of highly similar events (28). These findings support the possibility that HNT-TMS could strengthen fear discrimination by boosting encoding of fear cues and their safe approximates, thereby reducing excessive fear memory retrieval.

The current study represents the first attempt to modulate human fear learning/memory with HNT-TMS. TMS applied at the hippocampus' endogenous firing frequency (i.e., theta) has been shown to rapidly strengthen memory (21,26,28). Therefore, we delivered continuous theta burst stimulation (cTBS) to individualized left posterior parietal sites derived via seed-based connectivity, precision functional mapping, and electric field modeling methods. cTBS was chosen over intermittent TBS because cTBS (21,26,28), not intermittent TBS (21), has been shown to strengthen hippocampal learning/memory. We used the vertex as a control site to minimize unblinding in this within-participant design and recruited individuals with posttraumatic stress symptoms (PTSSs) to increase the study's translational value. We hypothesized that HNT-TMS would strengthen fear and neutral visual stimulus discrimination as measured by systematic laboratory tasks.

METHODS AND MATERIALS

Participants

Data were collected between October 2021 and April 2022 in accordance with the University of Minnesota Institutional Review Board (STUDY00011138). Undergraduates with PTSSs were recruited (mean age of final sample = 19.48 years, SD = 1.19). The Mini-International Neuropsychiatric Interview for DSM-5 was used to screen for exclusionary mood (current major depressive disorder, current bipolar disorder) and psychotic disorders. Current psychotropic medication use and history of epilepsy were additional exclusionary factors. The Clinician-Administered PTSD Scale for DSM-5 (29) and PTSD Checklist for DSM-IV (30) were used to assess for PTSSs, defined by a Clinician-Administered PTSD Scale for DSM-5–confirmed Criterion A-level event and a PTSD Checklist for DSM-IV score exceeding the Veterans Affairs-recommended civilian PTSD cutoff score of 30 (mean = 39.36, SD = 9.03) (31) (see the Supplement). Almost two-thirds of our final sample met Clinician-Administered PTSD Scale for DSM-5 PTSD criteria.

Thirty-two participants met all the criteria and completed all study visits. Data from 7 participants were excluded due to psychophysiological equipment failures (3 participants), an absence of conditioning effects (2 participants) (see the Supplement), poor neuroimaging data (1 participant), and a TMS targeting error (1 participant). The final sample consisted of 25 participants (20 females). Fear acquisition analyses include data from 24 participants due to a storage failure affecting 1 participant's acquisition data; Mnemonic Similarity Task analyses include data from 24 participants due to a task error affecting 1 participant's data.

Protocol Overview

The study protocol is depicted in Figure 1. Session 1 consisted of questionnaires and a ∼1-hour magnetic resonance imaging (MRI) scan. Between sessions 1 and 2, neuroimaging analyses were performed to determine TMS sites. Session 2 took place within 2 weeks of session 1 and included neuronavigated TMS delivered to the active or control site (order randomized) prior to the Farmer Task (32–34) and Mnemonic Similarity Task (35) (task order randomized but kept the same across visits to control for potential order effects). Session 3 took place 1 to 2 weeks after session 2 and included the same procedures with different versions of each task and TMS applied to the other site.

Tasks

Farmer Task. Participants view rings of different sizes (version A) or lines with different angles (version B) (see Figure 2A) across 2 main learning phases: fear conditioning and fear generalization. Stimuli are displayed throughout each trial (16.4 seconds during Pavlovian trials); trials immediately follow one another.

During conditioning, a large ring or an almost vertical line is paired with an unconditioned stimulus (i.e., individually titrated electric shock delivered 12.4 seconds after trial onset on 50% of reinforced trials) (see the Supplement) and becomes a conditioned fear stimulus (CS+), while a small ring or an almost horizonal line is presented without shock, becoming a conditioned safety stimulus (CS−) (see Figure 2A). Another stimulus that is categorically different from the CS+ and CS− (triangle: version A; plus sign: version B) is also shown. This unreinforced stimulus indexes nonassociative fear (i.e., fear unrelated to CS+ similarity). Because this conditioned safety stimulus is outside the conditioned fear continuum, it is labeled CS− outside (CS-o). The CS+, CS−, and CS-o are each shown 6 times during acquisition. Following presentation of each cue, participants are asked to rate their subjective anxiety (0 = no anxiety, 5 = moderate anxiety, 10 = extreme anxiety) or shock expectancy (0 = 0%, 5 = 50%, 10 = 100%) on a continuous 0 to 10 scale using a button box. Fear-potentiated startle is also collected for each trial (see the Supplement).

Following conditioning, there is a generalization phase during which a series of intermediate stimuli that form a continuum of similarity between the CS+ and CS− are shown. This phase includes alternating Pavlovian and instrumental

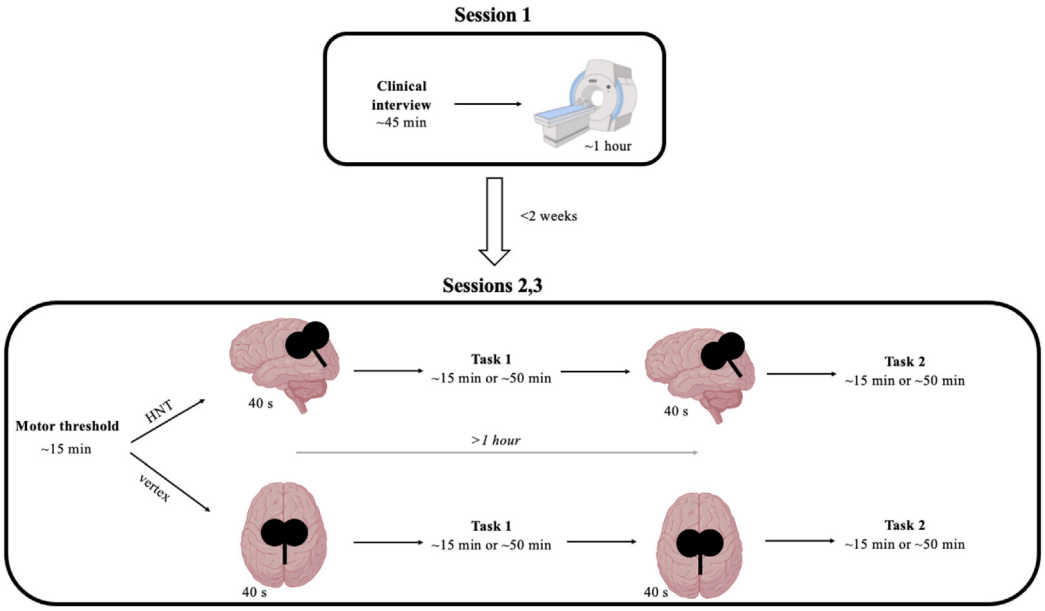

Figure 1. Study protocol. In Session 1, participants underwent a clinical interview to assess study eligibility followed by a 1-hour magnetic resonance imaging session. Less than 2 weeks later, following neuroimaging analyses to determine individualized active and control targets, they returned for session 2. Session 2 included motor threshold determination followed by active or control stimulation (randomized) followed by task 1 (Farmer Task or Mnemonic Similarity Task, order randomized), followed by a break to ensure that 1 hour elapsed after the first round of stimulation, followed by task 2. Session 3 took place within 1 to 2 weeks of session 2 and included the same set of procedures. However, transcranial magnetic stimulation was applied to the other transcranial magnetic stimulation target and alternate task versions were used. HNT, hippocampal network–targeted.

trials. During Pavlovian trials, the CS+ is paired with shock (12.4 seconds after trial onset; 50% reinforcement rate), and no other stimuli are accompanied by shock regardless of their similarity to the danger cue. As in acquisition, anxiety/shock expectancy ratings and fear-potentiated startle are collected. Each stimulus is shown 6 times during Pavlovian trials and 6 times during instrumental trials. During instrumental trials, participants can choose to avoid shock at the cost of reducing reward probability (i.e., successfully harvesting crops) by taking the long road (see Figure 2B). Alternatively, they can choose to confront the risk of shock (delivered 4 seconds after short road choice on CS+ trials; 50% reinforcement rate) but guarantee reward receipt by taking the short road.

Mnemonic Similarity Task. The Mnemonic Similarity Task is designed to measure pattern separation (35). The task includes 2 phases: encoding and retrieval. During a ~5-minute (incidental) encoding phase, participants view 128 neutral object images and are asked to classify them as indoor or outdoor objects. An ~8-minute retrieval phase immediately follows encoding. During retrieval, 192 object images are presented, 64 of which are the same (target), 64 of which are similar to previously presented images (lure), and 64 of which are completely new (foil). Participants are asked to classify these images as new, old, or similar. Two task versions (order counterbalanced) with unique image sets were used to minimize practice effects.

MRI Acquisition/Processing

A comprehensive description of acquisition parameters and processing procedures can be found in the Supplement. Briefly, we used a Siemens MAGNETOM Prisma 3T-A scanner to collect structural (T1-weighted, diffusion MRI) and functional MRI data from each participant. We leveraged a dense sampling approach (8 5-minute runs of open-eyes, resting-state functional MRI) to generate more reliable individualized HNT-TMS sites. A framewise displacement threshold of 0.2 mm was applied (36); 87.82% of frames survived this threshold across participants (mean minutes of usable data per participant = 35.62, SD = 4.78).

TMS Site Selection

Left posterior parietal TMS site selection procedures (see Figure 3A, B) built on previous connectivity-based HNT-TMS approaches (37). Similar to previous studies, we generated a hippocampal seed map using a 3-mm seed in the center of the left hippocampus (22,38) (Montreal Neurological Institute [MNI] −29, −25, −13). Building on these studies, we also generated individualized precision functional network maps using a template matching method (39) (Supplement) to constrain TMS sites to the hippocampus' canonical functional network (i.e., default mode network). This approach was motivated by findings showing that TMS propagates within functional networks (40–42) that are highly variable across individuals (43–45).

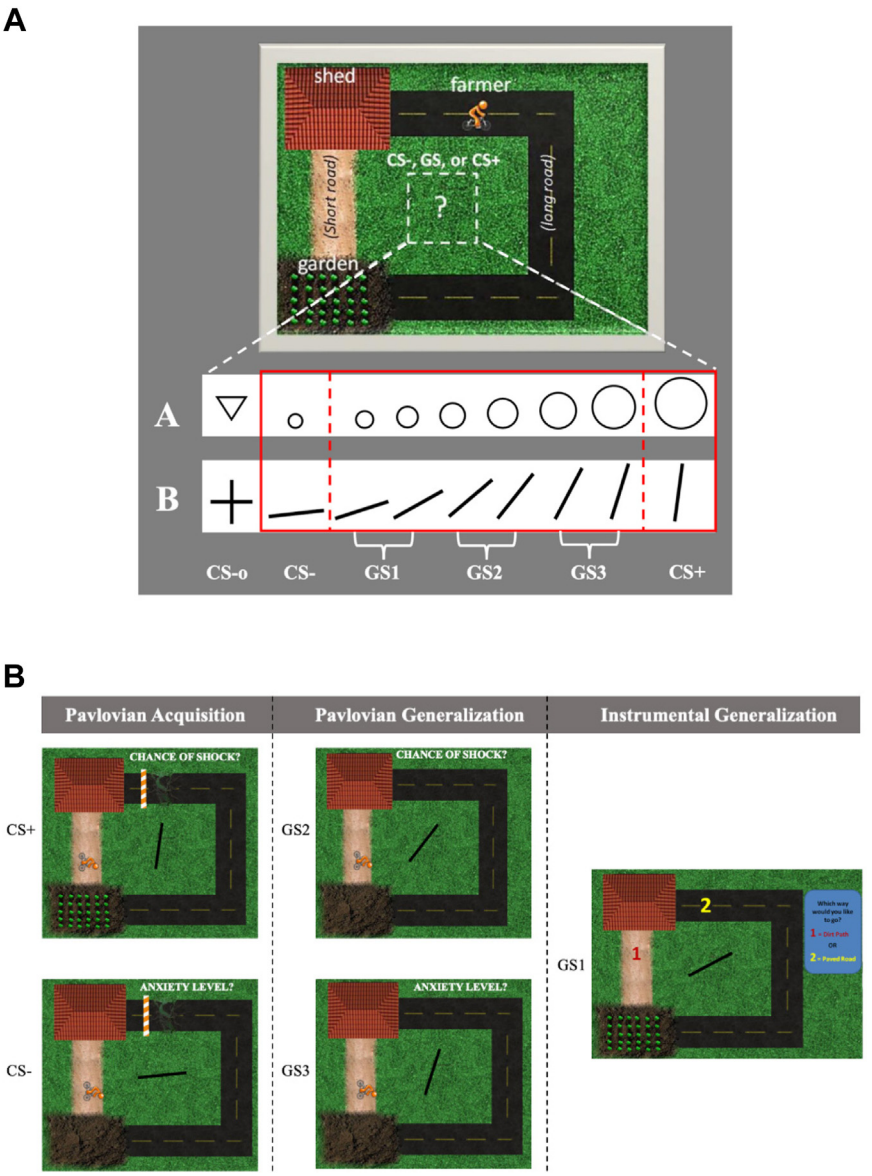

Figure 2. **(A)** Farmer Task stimulus sites. Task stimuli (not to scale) are depicted. Ring sizes were 0.8″, 0.96″, 1.12″, 1.28″, 1.44″, 1.60″, 1.76″, 1.92″ (size increases were established in 20% increments); line angles were: 84°, 73°, 62°, 51°, 40°, 29°, 18°, 8°. **(B)** Farmer Task trial types. Acquisition consisted of Pavlovian trials during which a shape was presented at the center of the screen, and participants were asked to rate chance of shock or anxiety level on a scale of 0 to 10. Generalization trials featured Pavlovian trials and instrumental trials, during which participants were given the choice to approach a reward and risk shock or avoid shock at the cost of potentially relinquishing reward. CS, conditioned stimulus; CS-o, CS− outside; GS, generalization stimulus.

TMS site optimization combined information from each participant's seed map, individualized functional map, and structural topography. First, we used a reference point to orient to the left posterior parietal cortex (MNI −47, −68, 36, mean HNT-TMS site distance from reference point = 14.14 mm, SD = 4.63). Next, we searched for areas within the left posterior parietal default network node that showed strong hippocampal connectivity. Because TMS effects are strongest at the gyral crown (46–48), final optimized TMS sites were gyral crown/gyral crown–proximal locations within the left posterior parietal default network node that showed maximal connectivity to the hippocampal target (mean TMS site correlation to hippocampal seed = 0.31, range = 0.21–0.40, SD = 0.07). We maximized the electric field induced at optimized HNT-TMS sites using electric field modeling [SimNIBS version 3.25 (49)] and oriented the TMS coil according to model specifications. We used the electroencephalogram location Cz for our vertex site (MNI 0, −15, 74) and faced the TMS coil forward at 180°.

TMS Protocol

TMS was delivered using a Magstim Rapid^2 (Magstim Co Ltd) system with a figure-8 coil (D70 Air Film; Magstim Co Ltd). We used a Brainsight neuronavigation system (Rogue Research Inc.) to aid motor threshold determination and to locate the active and control sites for each participant (see the Supplement for additional information). Following motor threshold determination, participants underwent neuronavigated TMS delivered in a cTBS pattern (600 pulses delivered in triplets at 50 Hz every 200 ms for 40 seconds at

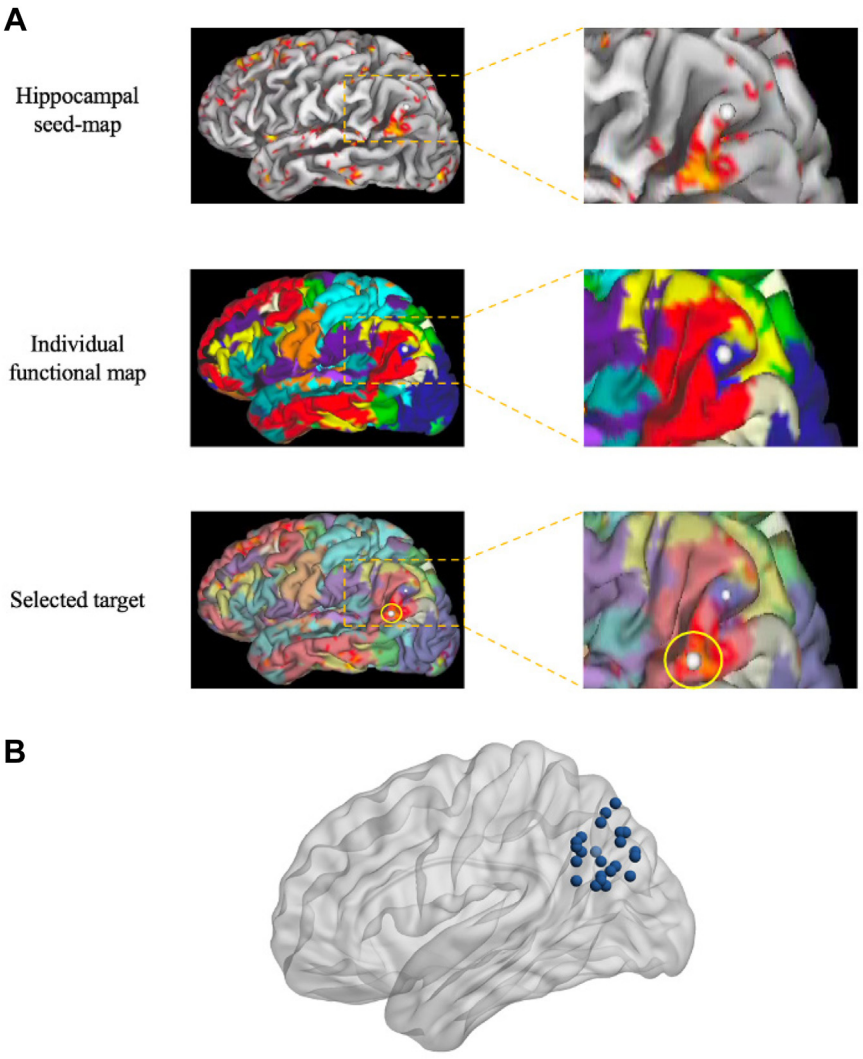

Figure 3. **(A)** Hippocampal network–targeted transcranial magnetic stimulation (TMS) targeting procedure. We generated left hippocampal seed maps to identify cortical locations with strong functional connectivity to the left hippocampal target (top left panel, expanded in top right panel). We also generated individualized functional network maps to constrain TMS sites to the hippocampus' canonical functional network (default mode network, denoted in red) (middle left panel, expanded in middle right panel). As in previous hippocampal network–targeted TMS studies, we started with a left posterior parietal reference location (Montreal Neurological Institute: −47, −68, 36, marked by a white dot in upper seed map and middle functional map). Next, we overlaid the hippocampal seed maps onto the individualized functional maps (bottom left panel, expanded in bottom right panel) to identify areas within the left posterior parietal default network node that showed strong connectivity to the left hippocampal target. The selected TMS site for each participant was the gyral crown/gyral crown adjacent location in the posterior parietal default network node that showed maximal connectivity to the hippocampal target. The selected TMS site for the depicted participant is circled in yellow, adjacent to the smaller, uncircled posterior parietal reference point (bottom left pane and expanded in bottom right pane). **(B)** Hippocampal network–targeted TMS targets for all participants.

80% resting motor threshold). Consistent with data suggesting that cTBS effects on neural activity persist for ∼50 minutes (50), a second round of cTBS was applied >1 hour after the first round (immediately prior to the second task).

Farmer Task Analyses

We used a series of linear or generalized linear mixed models to model and test data at each learning phase for each dependent variable (51) (see the Supplement for additional details). The generalization stimulus (GS) dimension was modeled as a continuous variable. We constructed linear mixed-effects models for anxiety ratings, risk ratings, and fear-potentiated startle data. We constructed binomial (i.e., logistic) mixed-effects models for avoidance ratings because avoidance responding in this task yields binary scores (1 = avoid, 0 = approach). All models were fit using restricted maximum likelihood estimation.

For our main research question (whether hippocampal stimulation strengthens fear discrimination), we modeled a TMS site × fear continuum response (CS+, CS−, GS) interaction, which was our term of interest for hypothesis testing. Although responses to the CS-o were included as a fixed factor, this model did not assess interactions between fear responses to the CS-o or cues within the conditioned fear continuum. Testing for interactions between conditioned fear continuum and CS-o responses is important for theoretical reasons because these factors capture distinct psychological processes (52). Responses to cues within the conditioned fear continuum reflect fear generalization, whereas responses to the cue outside of the conditioned fear continuum (CS-o) reflect nonassociative fear sensitization. HNT-TMS is expected to selectively strengthen associative processes that engage schematic matching between the presented GS and the previously encountered CS+. Thus, HNT-TMS may affect levels of generalization particularly among participants who show lower levels of nonassociative fear responding. Therefore, we created a secondary multilevel interaction model that included TMS site × fear continuum response × (mean) CS-o response interactions.

Similarly, participants who show stronger associative learning as indexed by increased levels of differential conditioning (CS+ > CS−) during acquisition may display stronger effects of HNT-TMS on levels of generalization. To investigate this possibility, we ran a complementary multilevel interaction model that included TMS site × fear continuum response × differential conditioning (CS+ minus CS− during fear conditioning) interactions. This model was only performed for the generalization phase because the differential conditioning factor was drawn from acquisition data.

Mnemonic Similarity Task Analyses

To investigate the effect of HNT-TMS on neutral memory processes, we constructed linear mixed-effects models with a random intercept of participant and fixed effects of TMS site, target order, and task order. In our primary model (model 1), pattern separation was the outcome of interest and was defined by the lure discrimination index, calculated as the percentage of lure items labeled similar minus the percentage of new items labeled similar. Lure items differ with regards to their similarity to target items and represent an aggregate of 5 bins, with bin 1 containing the most similar items and bin 5 containing the least similar items. Therefore, we ran additional models (models 2–6) with bin-specific lure discrimination index (percentage of bin lure items labeled similar minus percentage of new items labeled similar) as the outcome variable. Consistent with previous evidence (35), hippocampal stimulation was expected to modulate pattern separation but not recognition memory. To test this hypothesis, model 7 included recognition memory (percentage of target items labeled old minus the percentage of new items labeled old) as the outcome variable.

RESULTS

Farmer Task: Acquisition Phase

Fear conditioning was evidenced by a significant effect of stimulus (i.e., greater CS+ vs. CS− responses) for all response indices (anxiety ratings: $\beta = 1.25$, 95% CI, 1.14 to 1.35, $p < .001$; shock ratings: $\beta = 0.78$, 95% CI, 0.68 to 0.89, $p < .001$; fear-potentiated startle: $\beta = 0.12$, 95% CI, 0.04 to 0.20, $p = .004$) (see Table S1A–C). Conditioned fear response patterns did not differ by stimulation target, as indicated by the absence of a significant TMS site × stimulus (CS+, CS−) interaction for all response indices. Furthermore, the interaction between TMS site, stimulus, and CS-o responses was not significant, indicating that nonassociative fear responses did not shape TMS effects on conditioned fear responding (see Table S1D–F).

Farmer Task: Generalization Phase

Fear generalization was evidenced by a significant effect of the fear continuum (i.e., CS+, GS, CS− responses) variable for all response indices (anxiety ratings: $\beta = 0.48$, 95% CI, 0.43 to 0.52, $p < .001$; shock ratings: $\beta = 0.67$, 95% CI, 0.62 to 0.72, $p < .001$; fear-potentiated startle: $\beta = 0.23$, 95% CI, 0.16–0.30, $p < .001$; avoidance: $\beta = 5.73$, 95% CI, 4.35 to 7.54, $p < .001$). Fear generalization patterns did not differ as a function of TMS site for anxiety ratings, shock ratings, or fear-potentiated startle (Figure 4). However, the TMS site by fear continuum response interaction predicted avoidance ($\beta = 1.85$, 95% CI, 1.25 to 2.74, $p = .002$) with a significant linear trend coefficient ($\beta = 4.90$, 95% CI, 1.92 to 7.89, $p = .001$), indicating that HNT-TMS avoidance gradients were significantly less linear (i.e., steeper) than vertex avoidance gradients. Quadratic effects were not detected. To investigate this effect more closely, we compared avoidance responses to individual stimuli and assessed CS+ to GS3 and GS3 to GS2 response slopes as a function of TMS site. Although active stimulation yielded numerically lower GS3 and numerically higher GS1 and CS− avoidance rates, no significant differences in individual stimuli or response slopes were detected. The bidirectional and nonsignificant nature of these differences precludes a clear interpretation of HNT-TMS' effects on avoidance generalization (see Table S2A–D).

Although HNT-TMS did not reduce fear generalization as predicted, a significant interaction emerged between TMS site, fear continuum, and CS-o responses for anxiety ($\beta = 0.10$, 95% CI, 0.04 to 0.17, $p = .001$) and risk ratings ($\beta = 0.07$, 95% CI, 0.00 to 0.13, $p = .037$) (not significant for fear-potentiated startle or avoidance) (Figure 5). Specifically, anxiety and risk rating gradients became less linear (steeper) as nonassociative anxiety ($\beta = 1.63$, 95% CI, 0.69 to 2.57, $p \leq .001$) and risk ratings ($\beta = 1.71$, 95% CI, 0.27 to 3.16, $p = .02$) decreased for HNT-TMS. Quadratic effects were not detected. Follow-up analyses revealed that CS+ to GS3 and GS3 to GS2 anxiety rating (CS+ to GS3: $\beta = 0.45$, 95% CI, 0.16 to 0.75, $p = .003$; GS3 to GS2: $\beta = 0.62$, 95% CI, 0.33 to 0.92, $p < .0001$) and risk rating (CS+ to GS3: $\beta = 0.49$, 95% CI, 0.04 to 0.94, $p = .03$; GS3 to GS2: $\beta = 0.53$, 95% CI, 0.07 to 0.98, $p = .02$) slopes became significantly steeper as CS-o responses decreased following active but not vertex stimulation. These results indicate that HNT-TMS sharpened fear discrimination—particularly discrimination of stimuli with high CS+ similarity—to a greater extent in participants with lower fear sensitization (Table S3A–D).

There was no significant interaction between levels of associative learning (i.e., CS+ and CS− differential during acquisition), TMS site, and fear continuum responses for any response index (see Figure 6). However, as with nonassociative response levels, associative response levels shaped CS+ to GS3 anxiety rating ($\beta = -0.26$, 95% CI, −0.47 to −0.05, $p = .02$) and risk rating ($\beta = -0.61$, 95% CI, −0.97 to −0.24, $p = .001$) slopes for HNT but not vertex stimulation (not significant for fear-potentiated startle or avoidance). Specifically, HNT but not vertex stimulation yielded stronger CS+ to GS3 discrimination (steeper response slope) in participants who showed stronger associative learning. Differences for individual stimuli were not detected (see Table S4A–C).

Mnemonic Similarity Task

Lure discrimination index results were similar to those collected from previous healthy control samples (i.e., current study: mean = 36.95, SD = 19.3; Bernstein *et al.*, 2020: mean = 38.68, SD = 15.28) and numerically greater than PTSD samples (Bernstein *et al.*, 2020: mean = 27.48, SD = 21.8) (53). TMS site was not a significant predictor of aggregated lure discrimination index, bin-specific lure discrimination index, or recognition memory (see Table S5A–G).

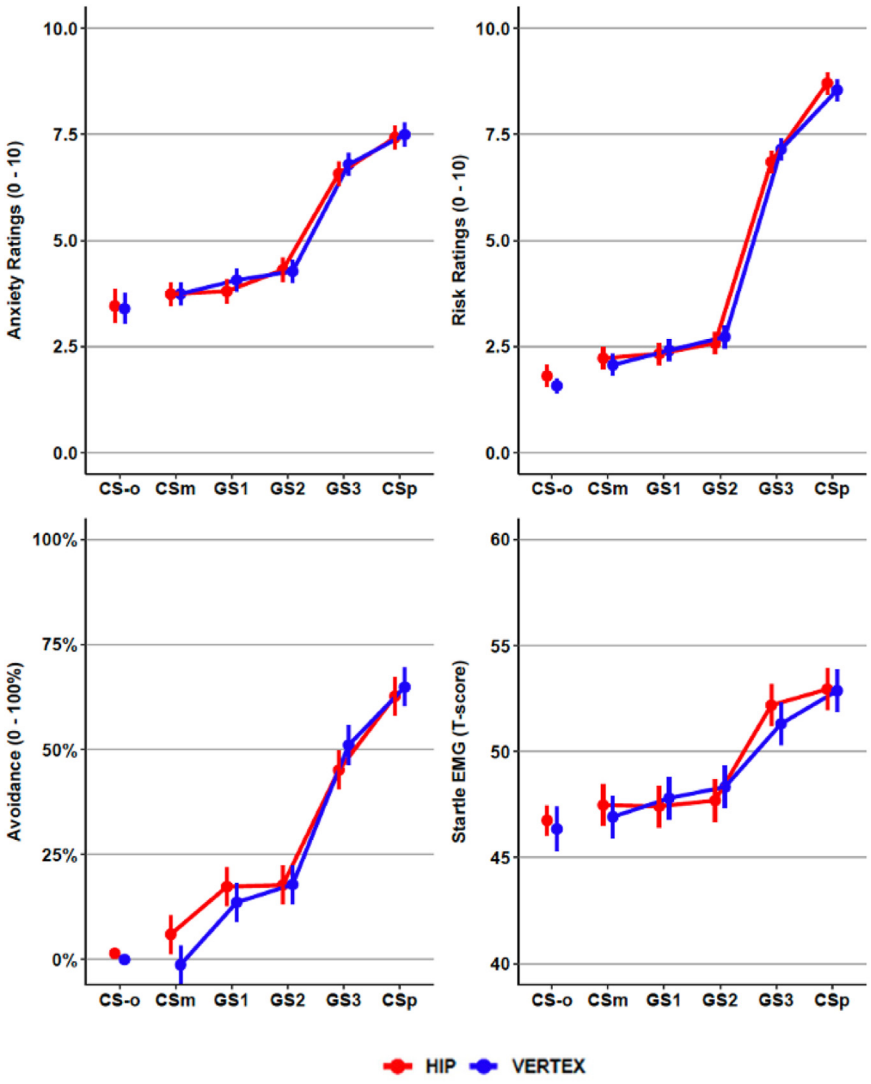

Figure 4. Transcranial magnetic stimulation (TMS) site × fear continuum. Graphs depict threat responses across the stimulus set for each TMS site (red line hippocampal network–targeted, blue line vertex). Top left graph depicts anxiety ratings; top right graph depicts risk ratings; bottom left graph depicts avoidance ratings; bottom right graph depicts fear-potentiated startle ratings. Ratings did not differ significantly between active and control sites. CS, conditioned stimulus; CS-o, CS− outside; CSm, CS−; CSp, CS+; EMG, electromyography; GS, generalization stimulus.

DISCUSSION

Contrary to our hypothesis, main analyses did not indicate that HNT-TMS strengthened fear or neutral stimulus discrimination. However, multilevel interaction analyses revealed that HNT-TMS strengthened fear discrimination in participants with lower fear sensitization. This interaction was detected for both anxiety and risk ratings. HNT-TMS yielded a steeper decline in anxiety and risk ratings from the fear cue to its closest approximation (GS3) and from the GS3 to its closest approximation (GS2) as sensitization decreased. This effect did not emerge for control stimulation. Complementing this finding, HNT-TMS (but not control TMS) promoted a steeper decline in anxiety and risk ratings from the fear cue to its closest approximation in participants who showed stronger associative learning during fear acquisition. Taken together, these findings suggest that HNT-TMS selectively boosted fear discrimination in participants who showed a stronger associative versus nonassociative fear response pattern.

Fear generalization and sensitization are distinct processes that are putatively undergirded by an overlapping but dissociable neural circuitry (52). Generalization is driven by fear cue similarity (i.e., an associative process) and is thought to involve fine-grained stimulus processing by a hippocampal-centered circuitry (5,8). In contrast, sensitization reflects indiscriminate fear responses (i.e., a nonassociative process) to novel stimuli driven by repeated exposure to the unconditioned stimulus, which kindles rapid defense circuits and increases arousal (54,55). Heightened arousal has been linked to reduced hippocampal activation during fear processing in PTSD (56,57), putatively reflective of a bias toward rapid defense circuits and away from the slower hippocampal-centered circuitry. Consistent with this framework, heightened nonassociative fear responses in the current study may reflect decreased engagement of the hippocampal discrimination function that HNT-TMS was hypothesized to strengthen. Therefore, stronger HNT-TMS effects on fear discrimination in participants with

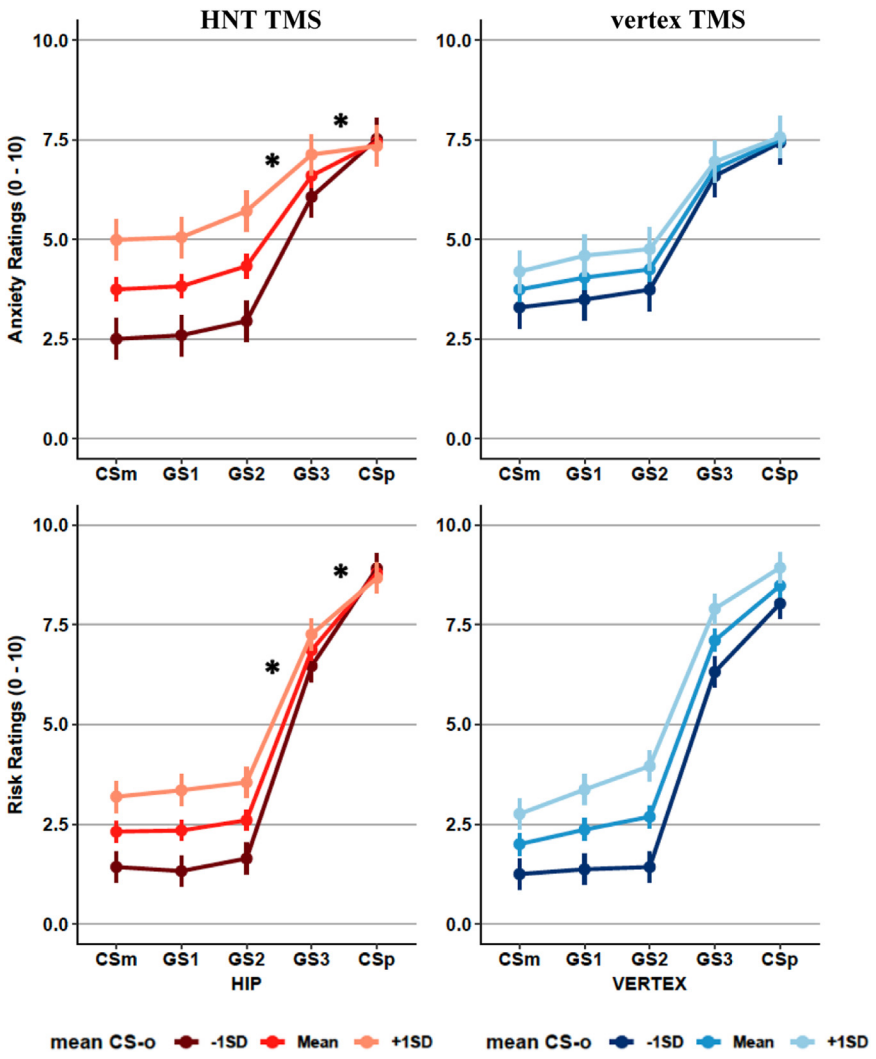

Figure 5. Transcranial magnetic stimulation (TMS) site × fear continuum × CS− outside (CS-o). Graphs depict fear continuum responses as a function of CS-o responses (−1 SD, mean, +1 SD) for active and control TMS. Top left graph depicts hippocampal network–targeted TMS (HNT-TMS) anxiety ratings; top right graph depicts vertex TMS anxiety ratings; bottom left graph depicts HNT-TMS risk ratings; bottom right graph depicts vertex TMS risk ratings. A triple interaction emerged, whereby anxiety and risk ratings declined significantly more steeply across the fear continuum when CS-o responses were lower for HNT vs. vertex TMS. CS+ to GS3 and GS3 to GS2 anxiety and risk rating slopes were significantly steeper (denoted by *) when CS-o responses were lower for HNT but not vertex TMS. CS-o anxiety ratings (−1 SD, mean, +1 SD): 1.88, 3.24, 4.6. CS-o risk ratings (−1 SD, mean, +1 SD): 2.18, 3.24, 4.31. CS, conditioned stimulus; CSm, CS−; CSp, CS+; GS, generalization stimulus.

more associative versus nonassociative response patterns may have been driven by greater engagement of the targeted hippocampal mechanism. Future fear-related HNT-TMS studies that use neuroimaging are necessary to examine links between associative/nonassociative fear levels, hippocampal engagement, and the effects of HNT-TMS.

Despite cross-species evidence implicating the hippocampus in pattern separation (12,58,59), HNT-TMS did not modulate neutral pattern separation as measured by the Mnemonic Similarity Task in the current study. One possibility is that HNT-TMS has a stronger effect on the encoding of associations versus individual stimuli. Previous studies have shown that HNT-TMS strengthens associative memory (i.e., object location/object scene) without affecting memory for individual stimuli (22,26). Associations between stimuli and outcomes (shock/no shock) are encoded during the Farmer Task; individual stimuli are encoded during the Mnemonic Similarity Task. Therefore, differences in the memory processes indexed by these tasks may partially explain why HNT-TMS strengthened fear discrimination in participants with lower sensitization without affecting neutral pattern separation. Another possibility is that neutral pattern separation performance approached a ceiling in the current study. Pattern separation performance in our PTSS sample more closely approximated results from healthy control samples than from PTSD samples (53). This may be related to our sample's subclinical nature or the fact that it consists of young adults, an age cohort that possesses particularly strong pattern separation abilities (60).

The current study has several strengths including its use of relatively dense sampling, which facilitated individualized, precision-targeted TMS. However, the study also has some noteworthy limitations. For example, while PTSSs may have translational relevance, results from our subclinical undergraduate sample cannot be assumed to generalize to real-world PTSD samples. Our sample is also likely less demographically diverse than real-world PTSD samples, although we did not collect race/ethnicity data. We also did not collect data on substance use, which is common in trauma

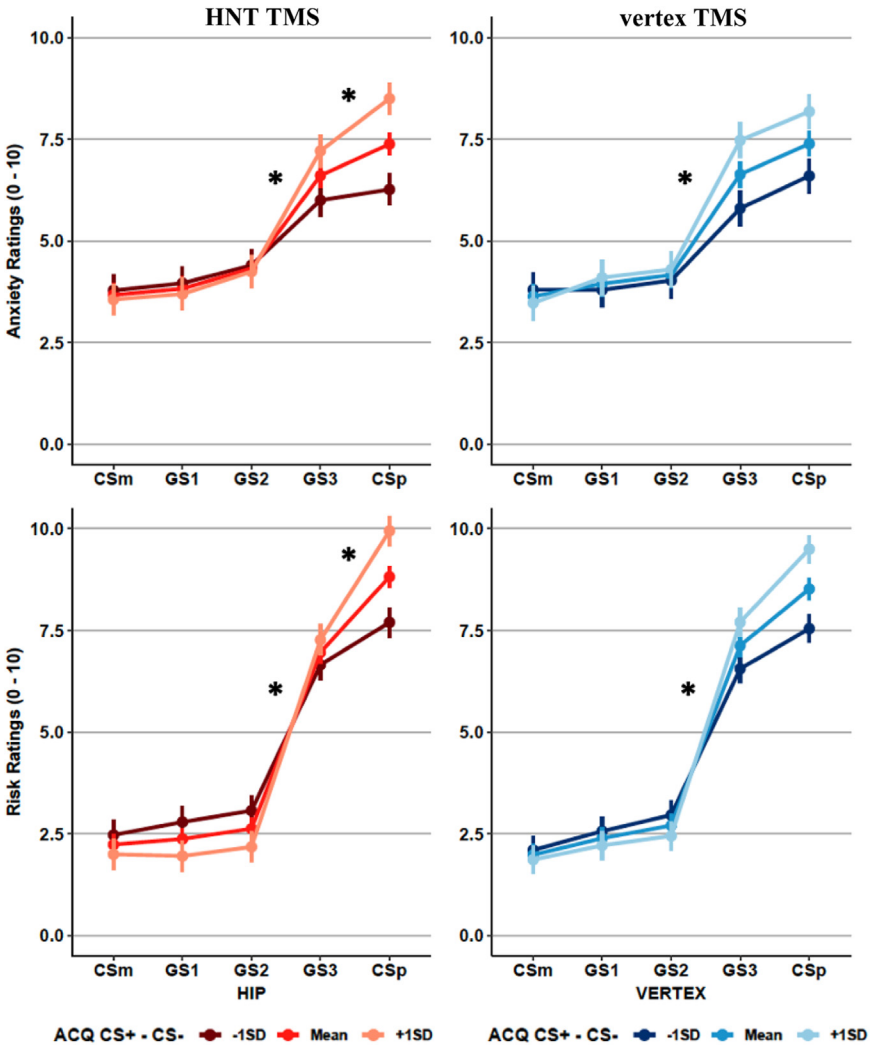

Figure 6. Transcranial magnetic stimulation (TMS) site × fear continuum × acquisition differential. Graphs depict fear continuum responses as a function of acquisition differential (acquisition CS+ minus CS−) responses (−1 SD, mean, +1 SD) for active and control TMS. Top left graph depicts hippocampal network–targeted (HNT) TMS anxiety ratings; top right graph depicts vertex TMS anxiety ratings; bottom left graph depicts HNT-TMS risk ratings; bottom right graph depicts vertex TMS risk ratings. CS+ to GS3 anxiety and risk rating slopes were significantly steeper (denoted by *) when the acquisition differential was greater for HNT but not vertex TMS. GS3 to GS2 anxiety and risk rating slopes were significantly steeper when the acquisition differential was greater for both HNT and vertex TMS. Acquisition differential anxiety ratings (−1 SD, mean, +1 SD): 1.83, 3.21, 4.59. Acquisition differential risk ratings (−1 SD, mean, +1 SD): 2.18, 3.26, 4.33. ACQ, acquisition; CS, conditioned stimulus; CSm, CS−; CSp, CS+; GS, generalization stimulus.

populations and may have shaped TMS effects. Next, although our sample size approximates those of previous HNT-TMS studies (19,22,25–28,61), our secondary results should be interpreted with greater caution due to sample size reductions driven by technical challenges and conditioning failures. The complex interaction effects detected in the current study would benefit from additional power and require replication in larger samples. Our selective use of multiple comparison correction also bears mentioning. Although we used Hochberg (false discovery rate) correction for TMS site–related comparisons of individual stimuli, analyses with clear predictions, most notably the linear/quadratic trend and response slope analyses, were not multiple comparison corrected. This was due to concerns about overcorrection on a small number of tests and because these tests are already conservative due to the use of estimated marginal means (62).

Potential variability introduced by our TMS protocol also merits comment. Although cTBS applied to the hippocampal network has been shown to strengthen hippocampal memory (21,26,28), the neurobehavioral effects of TBS are variable and remain poorly understood (63–65). Moreover, while previous HNT-TMS studies have generated significant memory effects using single-session designs (21,26,28), multisession TMS protocols have generally produced more robust neurobehavioral effects (66–68). Finally, while the vertex active control site may have reduced unblinding, vertex TMS has recently been shown to strengthen visual attention (69), a process that is putatively implicated in the visual discrimination tasks used in the current study. Future studies should leverage advancements in the field's understanding of the TMS parameter space to account for variability introduced by different protocol features.

Tempered by these limitations, our results provide preliminary evidence that HNT-TMS may selectively sharpen putative hippocampal-mediated fear discrimination when associative learning patterns are more strongly engaged.

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