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Sleep to remember, sleep to forget: Rapid eye movement sleep can have inverse effects on recall and generalization of fear memories

Itamar Lerner [a,b,*], Shira M. Lupkin [b,c], Alan Tsai [b], Anosha Khawaja [b], Mark A. Gluck [b]

[a] Department of Psychology, The University of Texas at San Antonio, 1 UTSA Circle, San Antonio, TX 78249, USA
[b] The Center for Molecular and Behavioral Neuroscience, Rutgers University—Newark, 197 University Avenue, Newark, NJ 07102, USA
[c] The Behavioral and Neural Sciences Graduate Program, Rutgers University—Newark, 197 University Avenue, Newark, NJ 07102, USA

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ABSTRACT

Rapid Eye Movement (REM) sleep has been shown to modulate the consolidation of fear memories, a process that may contribute to the development of Post-Traumatic Stress Disorder (PTSD). However, contradictory findings have been reported regarding the direction of this modulation and its differential effects on recall versus generalization. In two complementary experiments, we addressed this by employing sleep deprivation protocols together with a novel fear-conditioning paradigm that required the discrimination between coexisting threat and safety signals. Using skin conductance responses and functional imaging (fMRI), we found two opposing effects of REM sleep: While REM impaired recall of the original threat memories, it improved the ability to generalize these memories to novel situations that emphasized the discrimination between threat and safety signals. These results, as well as previous findings in healthy participants and patients diagnosed with PTSD, could be explained by the degree to which the balance between threat and safety signals for a given stimulus was predictive of threat. We suggest that this account can be integrated with contemporary theories of sleep and fear learning, such as the REM recalibration hypothesis.

1. Introduction

Sleep abnormalities are among the most conspicuous symptoms of Post-Traumatic Stress Disorder (PTSD). Patients often report difficulty falling and staying asleep as well as experiencing debilitating nightmares (Pace-Schott et al., 2015a). On a physiological level, people with PTSD have abnormal sleep architecture (Kobayashi et al., 2007), characterized by increased eye movement density during Rapid-Eye Movement sleep (REM) and a decrease in time spent in slow wave sleep (SWS). While some studies suggest that these sleep abnormalities are the result of the traumatic experiences (Pace-Schott et al., 2015a), others suggest that they may actually precede the trauma and subsequently contribute to the development of the disorder (Gehrman et al., 2013; Lerner et al., 2017; Mellman et al., 1995; Wright et al., 2011).

A common laboratory model for studying PTSD is fear conditioning, in which a neutral stimulus (CS), such as light, is associated with an aversive stimulus (US), such as an electric shock. Following a period of time after conditioning, fear recall can be tested by measuring the fear response to the original CS. Studies examining the effect of sleep on fear conditioning have regularly found a complex relationship between these processes and REM sleep. Specifically, conditioning has been shown to reduce time spent in subsequent REM sleep, which, in turn, affects the ability to recall the original fear association during the next wakeful period (Fu et al., 2007; Pawlyk et al., 2008; Spoormaker et al., 2012). Moreover, REM sleep was shown to modulate three brain regions that have been repeatedly implicated in fear learning and recall: the amygdala, hippocampus, and the ventromedial prefrontal cortex (vmPFC) (Lerner et al., 2017; Pace-Schott et al., 2015b; Walker & van Der Helm, 2009).

While the relationship between sleep and recall of fear memories has been repeatedly tested, only a few studies have examined how sleep affects **fear generalization** — the influence of an existing stimulus-fear association on fear responses to new stimuli that resemble the original one. Fear generalization may be even more relevant as a model of PTSD than fear recall, since overgeneralization of threat signals and undergeneralization of subsequent safety signals are thought to contribute to the initiation and maintenance of the disorder (Anastasiades & Garyfallos, 2015; Jovanovic et al., 2012; Levy-Gigi et al., 2012, 2015; Lissek & van Meurs, 2015). Studies examining sleep and fear generalization have largely indicated that a period of sleep following conditioning can

* Corresponding author at: Department of Psychology, The University of Texas at San Antonio, 1 UTSA Circle, San Antonio, TX 78249, USA.
E-mail address: itamar.lerner@utsa.edu (I. Lerner).

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help generalize the fear memory to related stimuli (e.g., Davidson et al., 2018; Kuriyama et al., 2010; Pace-Schott et al., 2009). However, those prior studies were limited to examining generalization based on the existence of visual or contextual similarities between the conditioned stimulus and the stimuli presented during the generalization test. Such generalization processes are presumed to rely on the ability of the hippocampus to perform pattern completion, in which inferences about novel stimuli are made based on their similarities with a known stimulus (Gluck & Myers, 1993; Yassa & Stark, 2011). Another aspect of fear generalization, which has never been studied in relation to sleep, occurs when subjects are required to learn how to differentiate threat and safety cues based on their ability to predict aversive outcomes. In this case, generalization is tested by the degree to which the particular threat and safety cues elicit fear in new situations. Such process is considered to depend on another well-documented function of the hippocampus, pattern separation: the process of discriminating between similar stimuli due to differences in their associated outcomes (Gluck & Myers, 1993).

To address this knowledge gap, the current study sought to examine how sleep affects fear generalization that is based on separation/discrimination processes. Over two experiments, our results point to opposing effects of REM sleep on fear recall and discrimination-based fear generalization. These results suggest a more complex relation of sleep and fear learning than originally presumed, leading to several new open questions regarding how REM sleep abnormalities should be seen as contributing to PTSD maintenance.

2. Experiment 1

2.1. Materials and methods

2.1.1. Participants

Twenty-four healthy students (n = 11 females) from Rutgers University-Newark and the New Jersey Institute of Technology participated in this study for monetary compensation. Exclusion criteria included any personal or family history of sleep problems, neurological or psychiatric disorders (including clinical depression, anxiety, bipolar, obsessive-compulsive disorder or schizophrenia), drug or alcohol abuse, and/or use of medications that have any effect on sleep. Three additional participants were dismissed from the study despite passing the criteria: two for malfunction of equipment, and one for failure to follow instructions. Throughout the experiment, participants were asked not to increase their daily caffeine intake, to maintain their regular sleep schedule, and to refrain from alcohol consumption and daytime napping (see demographic information and average sleep measures in Table 1). All participants provided informed consent in accordance with the Institutional Review Board of Rutgers University-Newark.

Table 1
Participant demographics and sleep parameters in Experiment 1.

Demographics	SD Group (N = 12)	Sleep Group (N = 12)
Age (years)	23.0 (1.5)	22.4 (2.2)
Education (years)	16.6 (1.2)	16.1 (1.3)
Sleep Measure	Night 1	Night 1 / Night 2
TST (minutes)	355.7 (120.6)	375.4 (69.4) / 359.4 (89.9)
N1 (minutes)	14.2† (10.3)	7.6 (6.1) / 8.8 (6.7)
% N1 out of TST	4.4* (3.3)	1.9 (1.0) / 2.5 (1.8)
N2 (minutes)	216.8 (81.3)	227.7 (80.0) / 235.6 (59.3)
% N2 out of TST	61.0 (10.1)	60.0 (11.4) / 65.2 (8.9)
SWS (minutes)	40.0* (31.0)	66.4 (23.0) / 48.0 (28.2)
% SWS out of TST	11.3* (8.1)	19.3 (10.1) / 13.8 (8.1)
REM (minutes)	84.7 (46.0)	73.9 (45.4) / 67.0 (29.3)
% REM out of TST	23.2 (7.9)	18.8 (9.8) / 18.4 (7.3)

Note: Presented are mean values with standard deviations in parentheses. SD = Sleep Deprivation. Night 1 = Habituation night; Night 2 = experimental night. Values for the habituation night of the SD group which were significantly different than their counterparts in the Sleep group are marked. * $p < 0.05$; † $p < 0.07$.

2.1.2. Experimental design

The study consisted of two consecutive nights of sleep monitoring accompanied by behavioral testing during wake (Fig. 1C). The first night was a habituation night, during which subjects acclimated to the sleep monitoring system to control for first-night effects (Thomas et al., 1981). During the following evening, subjects underwent a fear acquisition session accompanied by measurement of Skin Conductance Response (SCR) as an indicator of their stress response to the presented stimuli, as well as functional brain imaging. They were then randomly divided into two groups. The "Sleep" group (N = 12; 5 females) spent the second night asleep, whereas the "Sleep Deprivation" group (N = 12; 6 females) remained awake (see *2.1.5. Procedure*). Both groups were monitored with polysomnography (PSG) throughout the night. The next morning, subjects returned to the lab for a behavioral testing session where their ability to recall the learned fear memories and generalize them to new stimuli were examined, again with concurrent SCR and functional imaging.

2.1.3. Polysomnographic recording montage

Sleep was monitored using the Somte PSG mobile recording system (Compumedics Inc., Charlotte, NC). The recording montage included 6 EEG channels (F3, F4, C3, C4, O1, O2) referenced to contralateral mastoids (A1, A2), as well as 2 EOG channels (both outer canthi, one above and one below the eye) and 2 channels of submental EMG (referenced to a third submental electrode). Sleep scoring was conducted by a licensed sleep technician (Sleep Scoring Services, LLC) using standard AASM criteria (Iber et al., 2007). Data for one participant in the Sleep group during the second night were corrupted and designated as missing data.

2.1.4. Predictive fear-differentiation paradigm

The behavioral paradigm was composed of an acquisition and a testing session. During both sessions, three equally salient cues, "A", "B", and "C" (CSs) were presented in a multifaceted background context. Specifically, in our task, the cues were three chairs (chair A, B and C) placed within the context of a living room (Fig. 1A, 1B). In each trial of the acquisition session, one of the three possible cue combinations were presented; AB, AC, or BC. The presentation of two of these three combinations were paired with mild electrical stimulation (US) at a partial reinforcement rate of 60% depending on the presence of a "crucial predictor". For example, if Chair B was the crucial predictor, then combinations AB and BC would have been paired with the US, thus constituting the CS+. The third combination, AC, which lacks the crucial predictor, was never paired with stimulation (hence is a CS−). During the testing session, trials consisted of either the original 2-cue combinations stimuli (AB, AC, or BC; 'Recall' condition), or new stimuli with the same context but containing only one of the three cues on its own (A, B, C; 'Generalization' condition). None of the stimuli during the testing session were paired with the US. Fear Acquisition was defined for session 1, as the difference in the average fear response for trials containing chair combinations that include the crucial predictor (e.g., AB, BC) compared to those without the crucial predictor (e.g., AC). Fear Recall was defined for the same contrast, but for trials in session 2. Fear Generalization was defined, for session 2, as the difference in the average fear response for trials containing the single crucial predictor (e.g., B) compared to trials with "safety signals", that is, single chairs that are not the crucial predictor (e.g., A, C).

Each trial, across both sessions, began with the presentation of the cues and context for 3 s. Following this, a light fixture in the picture turned on and remained on for 6 s, followed by the disappearance of the entire scene, which remained off for the duration of the inter-trial interval. The purpose of the light fixture was to establish an event that splits the time between the appearance of the cues and the potential delivery of an aversive stimulus, such that the "safe" period before that event could be used as baseline for SCR computation (see SCR analysis below). During acquisition trials that included the US, an electric shock

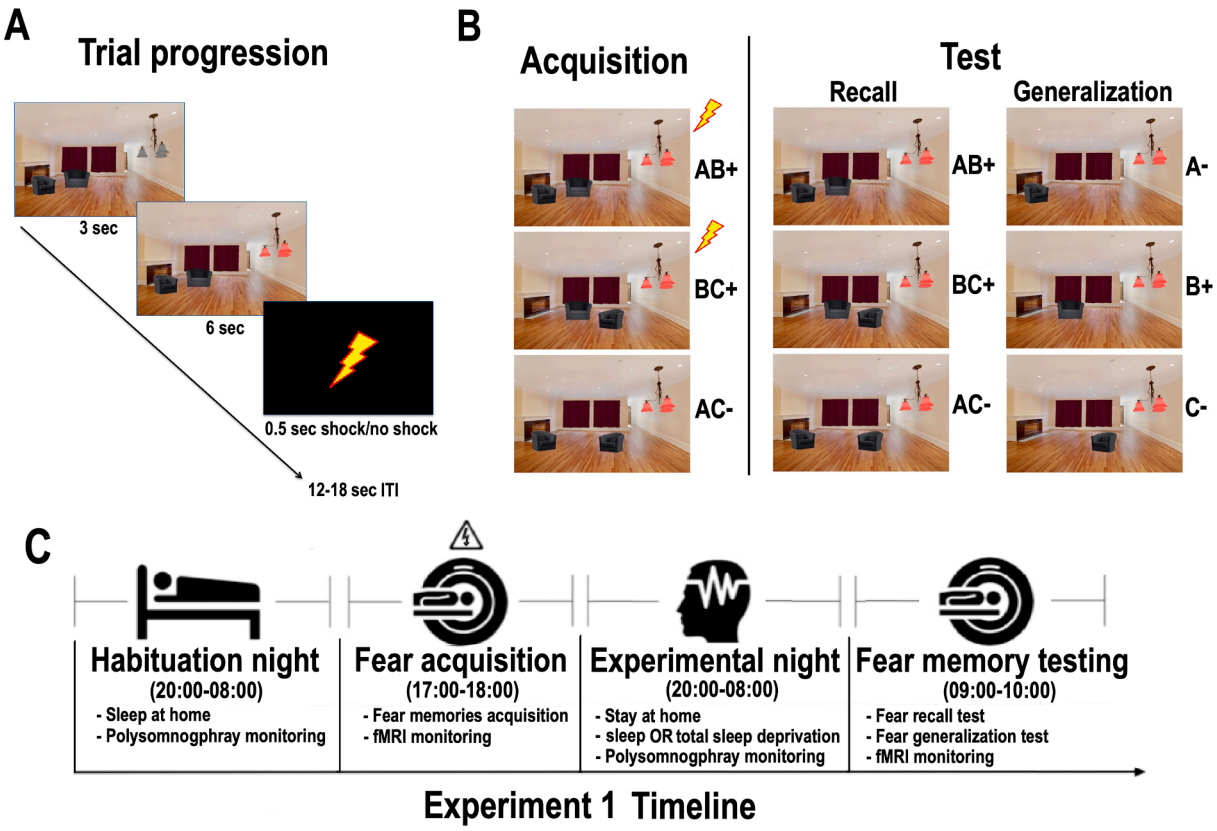

Fig. 1. Schematic of the fear learning paradigm A. Progression of a single trial. ITI: Inter-trial interval. B. Stimuli used in each of the sessions of the predictive fear discrimination task. During acquisition, trials containing two of the three possible combinations of cues (AB and BC in the example presented) were conditioned to shock (CS+), while trials containing the third combination (AC) were never conditioned (CS−). During test, fear recall was examined by comparing responses to the same combination of cues, whereas generalization of fear was examined by presenting new images containing only single cues and measuring the differentiated response to the crucial cue predictor (A) compared to the other two cues (B, C). C. Experimental procedure of Experiment 1.

was applied for 500 ms coincident with the disappearance of the scene. Trials were separated by a 12–18 s inter-trial interval and were presented sequentially in a block design such that in the first half of the session only one of the two stimuli containing the CS+ (e.g., AB) was presented, interspersed with the CS− (e.g., AC), whereas in the second half of the session only the stimulus containing the other CS+ (e.g., BC) was presented, interspersed with the CS−. This type of blocking was shown by Milad et al. (2007) to improve the conditioning response to each of the respective CS+'s. The identity of the crucial predictor (A, B, or C), as well as which of the two cue combinations containing the CS+ appeared first, were counterbalanced across participants. Ten trials were presented for each trial type of the acquisition phase, for a total of 30 trials overall. During the testing session, there were 4 trials for each trial type for a total of 24 trials. Trial order of both sessions was pseudo-randomized across participants.

2.1.5. Procedure

Participants arrived at the lab the evening before the first imaging scan and were fit with the PSG system by a trained research technician. They received detailed instructions on its usage, and were then sent home to spend the night while their sleep was being recorded. Participants removed the PSG apparatus themselves upon awakening the following morning. At 4:30 pm that afternoon, participants returned to begin the experimental phase. They entered the scanner and had the SCR and electrical stimulation electrodes attached to their fingers (for details on skin conductance and fMRI analyses, see *2.1.6. Statistical Analyses*). Next, they underwent an incremental titration procedure in order to establish their individual electrical stimulation threshold. Electric shocks of 500 ms duration were delivered to the middle and index finger of the participant's dominant hand in increasing intensities from 0.2 mA to a maximum of 4 mA, using a Coulbourn Transcutaneous Aversive Finger Stimulator (Coulbourn Instruments, Allentown, PA). The titration procedure continued as long as the shock level was deemed by participants as "highly annoying but not painful". The highest level reached under this definition was chosen as the participant's selected intensity and used throughout the entirety of the experiment. Before beginning the acquisition phase, participants were presented with images of all three salient item combinations in the absence of any aversive shocks (24 in total, 8 repetitions of each), to allow them to habituate to the new stimuli and avoid subsequent stress responses driven by novelty ('Habituation phase'; Milad et al., 2007). Following habituation, participants underwent the conditioning procedure as described above. At the end of the acquisition phase, the PSG system was again applied and the participant was sent home for the evening. Depending on random assignment, participants were either allowed a full night's sleep or were instructed to remain awake until after the second scanning session the following morning. No caffeine or alcohol intake was allowed during this period, and only certain behaviors, such as watching non-stimulating movies on a laptop supplied by the experimenter, were permitted. In order to ensure that the Sleep-Deprivation group remained awake, participants were instructed to remain in contact with our researchers via email in 20-minute increments. All participants then returned in the morning at 8:30am to undergo the testing session. Compliance with the sleep deprivation regimen in the Sleep-Deprivation group was verified offline by sleep staging their PSG data. All subjects were determined to have stayed awake for more than 95% of the interval between acquisition and testing, with only two participants exhibiting more than 5 min of sleep (around 30 min each). Because this sleep occurred in bouts of no more than 10 consecutive minutes and was comprised almost entirely from sleep stage N2, we kept these subjects in

the analysis.

2.1.6. Statistical analyses

2.1.6.1. Skin conductance analyses. SCR was recorded using two 11 mm Ag/AgCl electrodes with isotonic electrolyte gel (BIOPAC Systems Inc., Goleta, CA). The electrodes were attached to the anterior surface of the medial phalanx of the participant's index and middle fingers on their non-dominant hand. SCR was recorded using the GSR100c amplifier on the MP150 data acquisition unit (BIOPAC Systems Inc., Goleta, CA). Pulse event markers were transmitted from the stimulus presentation software (Superlab 5.0, Cedrus Corporation, San Pedro CA) on a PC, via a PCI-DIO24 digital input/output card to the MP150 and AcqKnowledge version 4.4.2 software using a BIOPAC STP100C optical interface (BIOPAC Systems Inc., Goleta, CA). Skin conductance levels were measured in microsiemens at a sampling rate of 2 kHz.

Following previous studies (Lerner et al., 2017; Milad et al., 2007), for each trial in each phase, SCR was calculated by taking the square root of the mean SCR value in the 2 s before stimulus onset (light fixture turns on), subtracted from the peak SCR value in the 6 s following stimulus onset. If this difference was less than zero, the square root of the absolute value was taken and then multiplied by negative one to preserve the direction of the relationship after the initial subtraction. Contrasts were then computed by subtracting the mean of one type of stimulus (i.e., CS−) from another (i.e., CS+), to produce the level of fear response for each condition in the experiment (Acquisition, Recall, Generalization). As previously suggested by Milad et al. (2007), the average CS+ for Acquisition was computed only over the first half of the trials (5 out of 10) of each of the two stimuli containing the crucial predictor, in order to minimize effects of adaptation to the electric stimulation. The average CS− was computed over all 10 trials without the crucial predictor, such that the total amount of trials for computing CS+ and CS− was equal. For Recall (during which no stimulation was given), the average CS+ was calculated over all trials containing cue combinations with the crucial predictor, and the average CS− was calculated over all trials containing the cue combination without the crucial predictor. Finally, for Generalization (during which, again, no stimulation was given), the average CS+ was calculated over all trials containing the crucial predictor as a single cue and the average CS− was calculated over all trials containing a single cue that is not the crucial predictor. Statistical analysis of SCR results was conducted using SPSS 26.0 (IBM SPSS statistics) and Matlab 2019a (MathWorks).

2.1.6.2. Imaging parameters and preprocessing. Functional imaging was conducted at the Rutgers University Brain Imaging Center. Images were obtained using a Siemens Trio 3 T full-body scanner with a 32-channel head coil. Anatomical images for use in spatial normalization were acquired using a T1-weighted protocol (MPRAGE, 176 1 mm isotropic sagittal slices). Next, functional images (i.e., BOLD) were acquired using a single-shot gradient echo EPI sequence (TR, 2000 ms; TE, 23 ms; FOV, 192 cm; flip angle, 90°; bandwidth, 4340 Hz/px; echo spacing, 0.51 ms). In total, 37 contiguous oblique-axial slices (3 mm isotropic voxels) were obtained for all BOLD sequences.

Primary analysis of imaging data was conducted using FSL (FMRIB Software Library; Dégenètais, Thierry, Glowinski, & Gioanni, 2003). Skull stripping was conducted using the FSL brain extraction tool (BET; Smith, 2002) with the center of gravity of each image as a reference point. For each participant, BOLD images were registered to their structural images and then to a standard MNI-152 2 mm template (degrees of freedom, 9; cost function, normalized mutual information; interpolation, sinc function) using FSL's linear inline registration tool (FLIRT, Jenkinson & Smith, 2001; Jenkinson, Bannister, Brady, & Smith, 2002). Individual whole-brain general linear model (GLM) analyses were conducted using the fMRI Expert Analysis Tool (FEAT) utility with motion correction, 5 mm FWHM spatial smoothing, and high-pass temporal filtering. Regressors of each GLM were the waveforms for CS+ and CS− of the relevant contrast (Acquisition, Recall, or Generalization, as defined above for SCR analysis), computed as the convolution between the binary vector representing the timing of each CS and the haemodynamic response function. The timing of each CS of each trial was determined as the 12 s (6 TR) window beginning from trial onset and until 3 s after the scene disappeared (Lerner et al., 2017).

Analysis at the group level was performed on the contrast of parameter estimates (COPE) produced by the individual analysis, using FSL's non-parametric permutation tool *randomise*, recently demonstrated to be the most accurate method of controlling for type-I errors in functional imaging data (Eklund et al., 2016). We used the threshold-free cluster enhancement method (TFCE, Smith & Nichols, 2009), with and without variance smoothing (of 5 mm), and report the better of the two results. One analysis was performed for each of the three CS+>CS− contrasts in each of the three a priori regions of interest (ROIs) chosen based on their established roles in fear learning: hippocampus, amygdala, and vmPFC. ROIs were defined based on probabilistic maps from the Harvard-Oxford Cortical and Subcortical Structural Atlases. The hippocampus and amygdala were defined as the voxels with a minimum of 50% probability (Zuo et al., 2010). For the vmPFC, which is not precisely defined in the Harvard-Oxford atlas (Santos et al., 2011), we took the full probabilistic mask of the frontal medial cortex without any thresholding. As detailed in section *2.2 Results*, the analysis also included Group (Sleep, Sleep Deprivation) as a between-subject factor. Additionally, a permutation test for the sleep group alone was run using the demeaned values of the relevant sleep parameters (i.e., the total time in each of the four sleep stages over the previously monitoring sleep period), entered simultaneously as 4 covariates of interest to assess their correlation with brain activity (one permutation analysis per ROI and contrast, including all four sleep parameters as 4 regressors in addition to the group mean effect). If any of the sleep parameters proved significant, another permutation analysis was run, constrained to the voxels that showed significance, to examine whether these voxels also correlate with the percentage of time spent in that sleep stage out of total sleep time (entered as a single sleep covariate). Finally, an additional analysis was performed for within-group inter-session differences by computing, separately for each group, the difference in contrasts between each session pairing (Acquisition-Recall, Acquisition-Generalization, Recall-Generalization) for each ROI and examining whether any of these differences was significantly different than zero while controlling for 3 multiple comparisons, corresponding to the three pairings. Visualization of the imaging results was performed by overlaying the thresholded statistical maps onto a 3-dimentional surface rendering of the MNI-152 template using the Surf Ice 10.14.6 software (McCausland Center for Brain Imaging, Columbia, SC).

2.2. Results

2.2.1. Skin conductance response

We collected subjects' Skin Conductance Response (SCR) for the CS+ versus CS− conditions during Acquisition, Recall and Generalization, as described in Methods. Average shock intensity was 2.30 ± 1.04 mA. Unfortunately, due to equipment failure, SCR data was available for only 7 subjects (2 Sleep-deprived, 5 Control). Therefore, we did not attempt to analyze these data based on subject group or correlate it to the imaging data. Instead, we simply computed the average SCR effect across all seven subjects, for each experimental phase, to verify that fear learning had occurred as intended. Results are presented in Fig. 2. Across the seven subjects, SCR was significantly larger for CS+ than CS− during the Acquisition phase, ($t(6) = 3.491$, $p = 0.013$, d = 1.32, CI = 0.07–0.40), indicating that the participants acquired the CS−US associations. Similarly, both the Recall and Generalization effects were significant ($t(6) = 2.766$, $p = 0.033$, d = 1.05, CI = 0.02–0.26, and $t(6) = 2.937$, $p = 0.026$, d = 1.11, CI = 0.02–0.19, respectively). Overall, the SCR data indicated that participants were able to acquire the fear

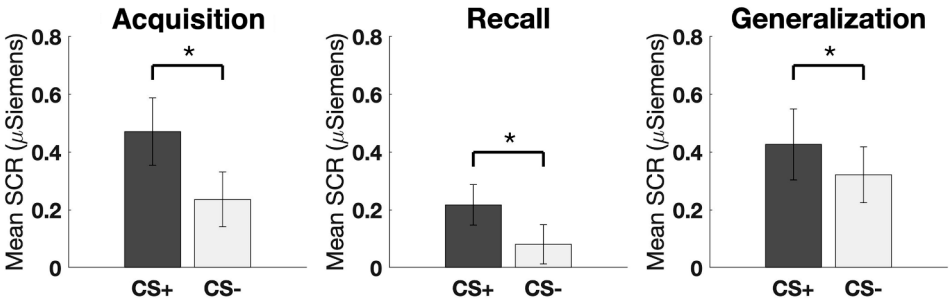

Fig. 2. Skin Conductance Response (SCR) of Experiment 1 (aggregated for the 7 participants with available data). Significant fear acquisition, recall and generalization were achieved for these participants. * $p < 0.05$. Error bars represent the standard error of the mean.

association, recall it the following morning, and differentiate between components of the fear memory that signaled threat and safety.

2.2.2. Sleep analyses

We examined whether the sleep and sleep deprivation groups differed in their baseline sleep parameters during habituation, as well as whether the sleep parameters for the sleep group differed between the habituation and experimental night. Table 1 displays the mean values for each group and night. We found that the sleep group had more SWS and a trend towards less N1 sleep than the sleep deprivation group ($p = 0.027$ and $p = 0.069$, respectively), as well as more % SWS and less % N1 ($p = 0.044$ and $p = 0.018$, respectively). There was no difference between the habituation and the experimental nights in the sleep group.

2.2.3. ROI and sleep analyses

We examined functional activity for the CS+>CS− contrast during each experimental phase in the three regions we expected, *a priori*, to be involved in fear learning; the amygdala, hippocampus, and the vmPFC (Pace-Schott et al., 2015a). For each region, we examined the difference in activation between and within groups and the correlation of the time spent in each sleep stage with the activation level, using non-parametric permutation tests. Significant effects are summarized in Table 2.

We first compared activation between the two experimental groups during fear acquisition, and whether this activation was modulated by the time the subjects spent in each sleep stage (N1, N2, SWS, REM) during the habituation night prior to acquisition. We found that there were no differences in activation between the groups in any of the three *a priori* brain regions (all p's > 0.23). This lack of baseline differences in activation is expected given that subjects were only divided into the experimental groups *after* the acquisition phase. Additionally, none of the sleep stages were predictive of ROI activation (all p's > 0.46). Given that there were no activity differences between the groups, we then collapsed the groups together to examine activation effects during fear acquisition across subjects, but, again, no effects reached statistical significance (all p's > 0.31).

Next, we compared activation between the two groups for the Recall contrast. We found that the sleep group had significantly less activation in the left hippocampus compared to the sleep deprivation group ($p < 0.04$; Fig. 3A, left). A similar effect was found for the left amygdala, at a marginally significant level ($p < 0.06$). Follow-up t-tests for the voxel with the peak difference between the groups in each region showed that for the sleep deprivation group, the hippocampal activity was significantly higher than 0 ($t(11) = 3.153$, $p < 0.01$, d = 0.91, CI = 10.43 to 58.63), with amygdala activity showing a similar trend ($t(11) = 2.158$, $p = 0.0539$, d = 0.62, CI = −1.09 to 111.15). In contrast, in the sleep group, hippocampal activity was trending towards negative activation (i.e., CS− higher than CS+; t(11) = 1.972, $p = 0.074$, d = −0.57, CI = −25.78 to 1.42), and the amygdala activity did not show any significant differences between the CS+ and CS− ($p = 0.4056$).

In order to evaluate the effects of particular sleep stages on fear Recall, we next examined whether the time spent in specific sleep stages during the night between acquisition and test was predictive of brain activity during fear recall in the sleep group (including 11 out of the 12 participants with available sleep data). Using a permutation test, we found that REM sleep time was negatively correlated with activity in the vmPFC (Fig. 3B, left; $p < 0.03$[1]), whereas time in N1 sleep was positively correlated with activity in the right hippocampus ($p < 0.02$). Re-

Table 2
Region of Interest Analysis for Experiment 1.

Session/Region/ Effect	MNI Coordinates X	Y	Z	Number of Voxels	*t-Value*	*p-*Value
Recall						
Sleep > SD						
Amygdala Activation	52	60	28	7*	−2.94	0.059
Hippocampus Activation	60	54	29	14	−3.71	0.032
Sleep Group Correlations						
vmPFC + REM	51	86	27	51	−5.39	0.027
Hippocampus + N1	30	52	28	12	8.28	0.016
Hippocampus + % N1	33	52	28	2	2.58	0.039
Generalization						
Sleep > SD						
Hippocampus	29	46	33	16	3.46	0.037
Activation	43	91	32	604	3.63	0.011
vmPFC Activation						
Sleep Group Correlations						
vmPFC + REM	41	88	23	11	13.98	0.007
vmPFC + % REM	44	86	23	1	2.66	0.045
Between-sessions (Sleep Deprivation Group)						
Recall > Generalization						
Hippocampus	62	49	31	4	4.71	0.038
Activation	44	80	25	6*	5.49	0.059
vmPFC Activation						
Acquisition > Generalization	41	88	23	4*	3.64	0.066
Hippocampus Activation						

Note: Results of the nonparametric ROI analysis in Experiment 1. Reported above are the MNI-coordinates of the peak voxel, *t*-values and corrected *p*-values for the peak voxel within the cluster, as well as the size of the cluster. Cluster size is determined based on voxels significant at the $p < 0.05$ level, with the exception of activation effects marked with *, whose peak significance was only at the trend level and therefore the cluster threshold was arbitrarily set to $p < 0.07$. Confidence Interval for all results is 0.0443–0.0564. SD = Sleep Deprivation; REM = Rapid Eye Movement sleep. vmPFC = ventromedial prefrontal cortex.

[1] Fig. 3B (left) might suggest that this effect stems from one outlier subject that had a particularly low vmPFC parameter estimate; however, the effect remains marginally significant (r = −0.625, $p = 0.0556$) even when this subject is excluded.

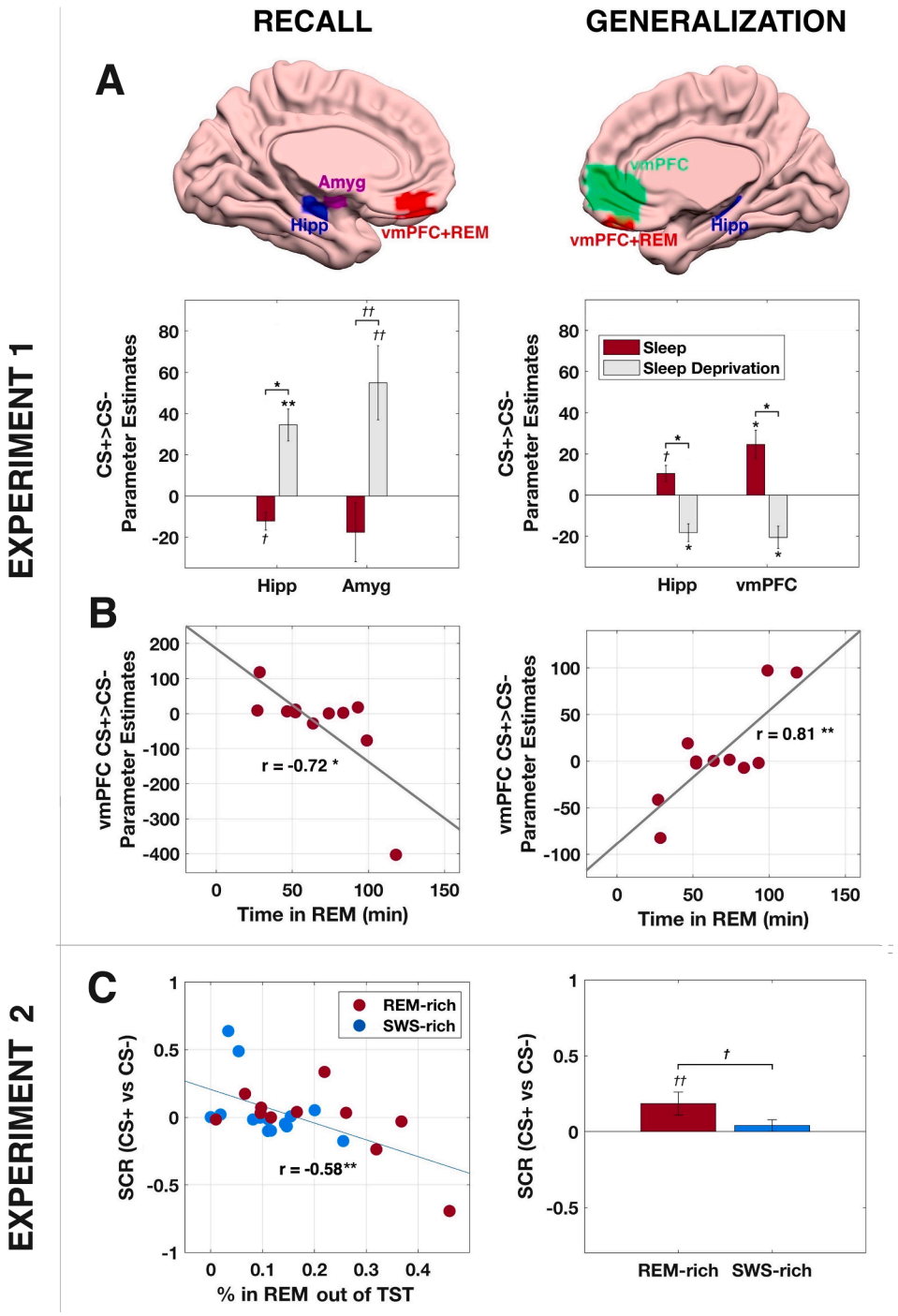

Fig. 3. Main effects of recall and generalization in Experiments 1 and 2. A. ROI effects in Experiment 1 for the sleep and sleep deprivation group in the three brain regions examined. Hipp – hippocampus; Amyg – amygdala; vmPFC – ventromedial prefrontal cortex. B. Correlations of vmPFC activation with the time spent in Rapid Eye Movement sleep in the sleep group of Experiment 1. C. SCR Effects for the SWS-rich and REM-rich groups in Experiment 2. Left: Correlation of % REM (out of total sleep time, TST) with fear recall across both groups. Right: Generalization effects for each group. ** $p < 0.01$; * $p < 0.05$; †† $p < 0.06$; † $p < 0.1$. Error bars represent the standard error of the mean.

computing these two correlations for the voxels that showed the most significant effect but using relative sleep time measures (percent of time spent in the relevant sleep stage out of total sleep time), showed that % N1 remained significantly correlated to hippocampal activation ($p < 0.04$), though the correlation of %REM with vmPFC activation failed to reach statistical significance ($p = 0.102$).

We repeated this analysis for the Generalization condition. We found that the generalization effects were, to a large extent, a mirror image of those found during Recall. Specifically, the sleep group had significantly higher activation than the sleep deprivation group in both the right hippocampus and the vmPFC ($p < 0.04$ and $p < 0.02$, respectively; Fig. 3A, right). Follow-up t-tests for the peak-difference voxels showed that for the sleep group, the activity in the vmPFC was significantly higher for the CS+ compared to the CS− ($t(11) = 2.520$, $p < 0.03$, d = 0.73, CI = 3.12–46.23) with activity in the hippocampus having a similar trend ($t(11) = 1.891$, $p = 0.085$, d = 0.55, CI = −1.72 to 22.78). For the sleep deprivation group, both effects were significantly lower for the CS+ than the CS− ($t(11) = 2.665$, $p < 0.03$, d = −0.77, CI = −37.44 to −3.57, and $t(11) = 2.956$, $p < 0.02$, d = −0.85, CI = −31.75 to −4.65 for the vmPFC and hippocampus, respectively). Within the sleep group, correlating brain activation with the time spent in each sleep stage revealed that activity in the vmPFC was positively correlated with REM sleep (Fig. 3B, right; $p < 0.01$). This effect was maintained when repeated using %REM sleep ($p < 0.05$). Moreover, the mirroring effect between recall and generalization was also evident at the individual level. Fig. 4 presents pairwise correlations across all subjects between

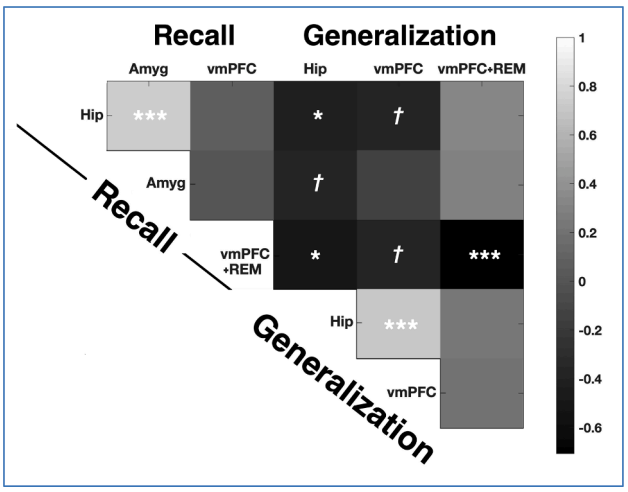

Fig. 4. Pairwise correlations, across subjects, between voxels in regions of interests with peak fear-related activation for Recall and Generalization. Gray-scale levels represent the strength of the correlation, from −1 (black) to 1 (white). Cells with correlational levels reaching significance (or trending) are marked. *** $p < 0.0002$; * $p < 0.05$; † $p < 0.07$ (uncorrected).

the peak-difference voxels in each testing condition. We found that correlations between voxels sensitive to fear recall and voxels sensitive to fear generalization tended to be negative, with many reaching statistical significance or a statistical trend. In contrast, correlations within fear-recall voxels, or within fear generalization voxels, tended to be positive. In other words, the same brain regions that differentiated between the groups tended, on an individual level, to act in concert if encoding for the same fear-related operations, but opposingly if encoding for different operations.

We next examined whether brain activation in the ROIs changed from session to session within each group. To that end, we computed the differences between each pair of sessions (Acquisition-Recall, Acquisition-Generalization, Recall-Generalization) for each ROI in each group, and used a nonparametric test to examine whether it differed from zero (Bonferroni-corrected for 3 multiple comparisons). We found that for the Sleep Deprivation group, activation in the left hippocampus was weaker in the Generalization compared to the Recall session ($p < 0.04$). A similar statistical trend was observed for the right hippocampus ($p < 0.07$), and the vmPFC ($p < 0.06$). Finally, the activation in the right hippocampus was weaker, at a trend level, in the Generalization compared to the Acquisition phase ($p < 0.07$). No significant effects between sessions was found for the Sleep group.

To conclude this analysis, we examined whether the differences in brain activation found between the groups could be explained by the differences in their sleep parameters from the habituation night. To that end, we ran four univariate ANOVAs for each of the brain areas differentiating between the groups (hippocampus and amygdala in Recall, hippocampus and vmPFC in generalization). In each analysis, the dependent variable was the voxel in each region with the maximal difference, Group was the main factor, and the relevant sleep parameters from the habituation night (N1, % N1, SWS, % SWS) were entered as covariates. The analysis showed that no covariate significantly contributed to the differences between the groups in any of the activation areas (all p's > 0.12 with the exception of % N1 for vmPFC in generalization that had a p value of 0.079), indicating that the sleep differences between the groups during the habituation night did not modulate the differences in brain activation.

To summarize, the results of Experiment 1 showed that sleep following the acquisition of fear memories, particularly REM sleep, is associated with reduced fear-related brain activation when trying to recall those memories. However, REM sleep was also associated with increased sensitivity of the same brain regions when comparing threat and safety cues that were implicitly embedded in the acquired fear memories, and the weaker the fear-recall activation was, the stronger was the sensitivity.

3. Experiment 2

Following our first experiment, we sought to determine if a more causal relationship between REM sleep and the recall and generalization of fear in our task can be demonstrated. To that end, we ran a second, ancillary experiment where we administered the same fear-discrimination task while manipulating sleep using the split-night design (Plihal & Born, 1997; see details below in *3.1 Materials and Methods*). This type of sleep manipulation leverages the higher percentages of SWS and REM during the first and second halves of the night, respectively, resulting in one group of subjects with SWS-rich sleep in between fear acquisition and testing ("SWS-rich" group), and a second group with REM-rich sleep ("REM-rich" group). Unlike the first experiment, this experiment was conducted solely with concurrent SCR and not with functional imaging. We compared the degree of fear recall and generalization between the groups, as well as the correlations between these two measures and the time spent in each sleep stage across subjects from both groups.

3.1. Materials and methods

3.1.1. Participants

Twenty-six healthy students (n = 14 females) from Rutgers University-Newark and the New Jersey Institute of Technology participated in this study for monetary compensation (see demographic information and average sleep measures in Table 3). Exclusion criteria were the same as in the first experiment. Three additional participants were disqualified, one for not obtaining a significant amount of sleep throughout the experiment, and two for SCR equipment failure. All participants provided informed consent in accordance with the Institutional Review Board of Rutgers University—Newark.

3.1.2. Experimental design

This study consisted of one night of habituation sleep followed by an overnight session in which participants performed the predictive fear-discrimination task (without concurrent fMRI imaging). Sleep during the overnight session was manipulated using the split-night design, which takes advantage of the fact that sleep at the first half of the night tends to have more SWS than REM sleep, whereas the opposite occurs at the second half of the night (Plihal & Born, 1997). Specifically, one group of participants experienced sleep rich with SWS during the interval between acquisition and testing, whereas the other group

Table 3
Participant demographics and sleep parameters in Experiment 2.

Demographics	SWS-rich group	REM-rich group
Age (years)	21.1 (2.9)	19.5 (7.2)
Education (years)	14.1 (1.6)	13.0 (4.6)
Sleep Measure	Habituation / Experimental	Habituation / Experimental
TST (minutes)	378.8 (81.2) / 182.9 (17.9)	347.4 (134.7) / 174.4 (39.6)
N1 (minutes)	7.3 (5.6) / 3.6 (2.5)	10.4 (8.4) / 5.1 (3.6)
%N1 out of TST	2.2 (1.9) / 2.0 (1.3)	3.3 (2.5) / 3.2 (2.2)
N2 (minutes)	249.5 (64.6) / 110.4 (25.8)	236.7 (99.7) / 122.7 (36.7)
%N2 out of TST	66.5 (11.9) / 60.8 (14.4)	67.2 (7.3) / 69.8 (13.9)
SWS (minutes)	49.8 (37.9) / 48.8** (31.6)	37.8 (25.7) / 13.3 (15.7)
%SWS out of TST	12.8 (10.0) / 26.4** (15.7)	13.4 (11.6) / 7.3 (8.3)
REM (minutes)	72.2 (32.7) / 20.1† (13.3)	62.5 (47.1) / 33.2 (22.3)
%REM out of TST	18.6 (6.0) / 10.9* (7.0)	16.1 (9.8) / 19.8 (14.1)

Note: Presented are mean values with standard deviations in parentheses. Habituation = sleep during habituation night; Experimental = sleep during experimental night between training and testing. Values for the SWS-rich group which were significantly different than their counterparts in the REM-rich group are marked. ** $p < 0.003$; * $p < 0.05$; † $p < 0.08$

experienced sleep rich with REM sleep (see details in *3.1.3 procedure below*). The behavioral paradigm was identical to Experiment 1 with one exception: Both images belonging to the CS+ condition during acquisition were presented in a pseudo-random order as opposed to being blocked. This change was driven by our attempt to equate the timing of the two types of images, though, ultimately, it produced no obvious effects on the results. Sleep monitoring, fear titration, SCR measurement and US administration were all identical to Experiment 1, with the exception that sleep/wake periods during the second night took place in a designated quiet room in the lab, as opposed to at participants' homes. The room was designed to look like a typical bedroom, containing a standard twin sized bed, a desk, a bookcase, and several decorative items including plants and pictures.

3.1.3. Procedure

The procedure for the Habituation night was identical to that described in Experiment 1, with participants acclimating to the sleep monitoring system in their own homes. The following day, participants arrived at the lab at 10:00 pm and were randomly assigned to one of two groups: a SWS-rich group or a REM-rich group (see Fig. 5 for illustration). In the SWS-rich group, fear acquisition took place at 10:30 pm, after which participants were allowed to sleep for three and a half hours from 11:00 pm to 2:30am. They were then awakened, and after a 30-minute delay (to reduce sleep inertia), testing of recall and generalization took place. In the REM-rich group, participants were first allowed to sleep for three and a half hours, from 11:00 pm to 2:30am. They were then awakened, and after a 30-minute delay, the fear-acquisition phase took place. Participants were then allowed to sleep for additional three and a half hours, after which they were awakened for the recall and generalization phases. One participant failed to fall asleep for more than a few minutes during the first part of the night but slept normally the second part of the night and was therefore moved from the REM to the SWS group. Results remain largely unchanged when excluding this participant altogether. The sleep recording of one more participant in the SWS-rich group was corrupted and thus designated as missing data. There were a total of 15 participants in the SWS-rich group (8 females) and 11 participants in the REM-rich group (6 females). Statistical analysis of the results was performed using SPSS 26.0 and Matlab 2019a.

3.2. Results

To evaluate the efficacy of the split-night manipulation, we first compared the percent of time spent in each sleep stage between the two groups. Table 3 portrays these averages. Independent t-tests showed that the SWS-rich group had significantly more % SWS during the intersession interval compare to the REM-rich group ($t(23) = 3.643$, $p < 0.002$, $d = -1.45$; CI = −0.30 to −0.08), whereas the REM-rich had significantly more % REM sleep during the interval compared to the SWS-rich group ($t(23) = 2.080$, $p < 0.05$, $d = 0.84$, CI = 0–0.18), thus confirming the experimental manipulation was successful. There was no difference between the groups in the percent of time spent in sleep stages N1 or N2, nor in total sleep time (all $ps > 0.1$). There were also no differences between the groups in any of the sleep parameters during the habituation night (all $ps > 0.19$).

Next, we analyzed the SCRs of the two groups. Average shock intensity was 2.61 ± 1.02 mA across subjects (2.80 ± 1.08 mA and 2.36 ± 0.90 mA for the SWS-rich and REM-rich groups, respectively; $p = 0.290$). Differences in the SCR responses between CS+ and CS− were entered into a marginal linear model with Group (SWS-Rich, REM-rich) as a between-subject factor and Condition (Acquisition, Recall, Generalization) as a within-subject repeated-measures factor with an unstructured covariance matrix. The analysis showed no significant main effects of Group or Condition (both p's > 0.2), but a marginally significant effect of their interaction ($F(2,24) = 2.73$, $p = 0.086$). We therefore followed the analysis by examining the group effect separately for each condition, and the condition effects separately for each group.

Independent t-tests showed that for Acquisition, there was no difference between the groups ($p = 0.448$; Fig. 6). Collapsing across the groups, the conditioning effect was significantly higher for the CS+ than the CS− ($t(25) = 3.399$, $p < 0.003$, $d = 0.67$, CI = 0.04–0.17).

For the Recall condition, there was, again, no difference between the groups ($p = 0.498$), nor was there significant evidence of recall across subjects of the two groups together ($p = 0.814$). Despite the lack of significance, the small numerical differences between the groups seemed to have matched our brain activation results from Experiment 1, with the REM-rich group having lower (in fact, negative) average recall values compared to the SWS-rich group, which experienced less REM. We therefore computed the correlations between the percent of time spent in each sleep stage and recall performance across subjects (including 25 out of the 26 participants with available sleep data). These are presented in Table 4. We found a highly significant correlation between REM and recall ($r(24) = -0.580$, $p < 0.01$, Bonferroni corrected for 4 multiple comparison; $R^2 = 0.34$, CI = −0.79 to −0.24), such that the more percent of time subjects spent in REM sleep, the lower was fear recall, reminiscent of our results with vmPFC activation in Experiment 1 (Fig. 3C, left). This effect was driven by responses to the CS− recall condition, which showed a strong positive correlation with REM across subjects ($r(24) = 0.712$, $p < 0.0001$, $R^2 = 0.50$, CI = 0.44–0.86), whereas the CS+ recall condition did not correlate with REM ($p = 0.81$). Moreover, this effect was also significant when computed using raw time in REM sleep ($r(24) = 0.533$, $p < 0.007$, $R^2 = 0.28$, CI = 0.17–0.77). This pattern of results suggested that the difference between the groups was driven by a larger fear from the safety signals in the REM-rich group rather than reduced fear from the crucial predictor. No other sleep stage was significantly correlated to fear recall.

In the Generalization condition, we found a trend for higher effects in the REM-rich group compared to the SWS-rich group ($t(24) = 1.755$, $p = 0.092$, $d = 0.70$, CI = −0.03 to 0.32; Fig. 3C, right). Examining each group separately, the REM-rich group had a marginally significant generalization effect ($t(10) = 2.208$, $p = 0.052$, $d = 0.67$, CI = 0–0.37) whereas in the SWS-rich group no effect was found ($p = 0.292$). Across all subjects, there was no correlation between any of the sleep stages and generalization (Table 4), though REM sleep was positively correlated to the CS+ trials at a trend level ($r(24) = 0.351$, $p = 0.085$, $R^2 = 0.12$, CI =

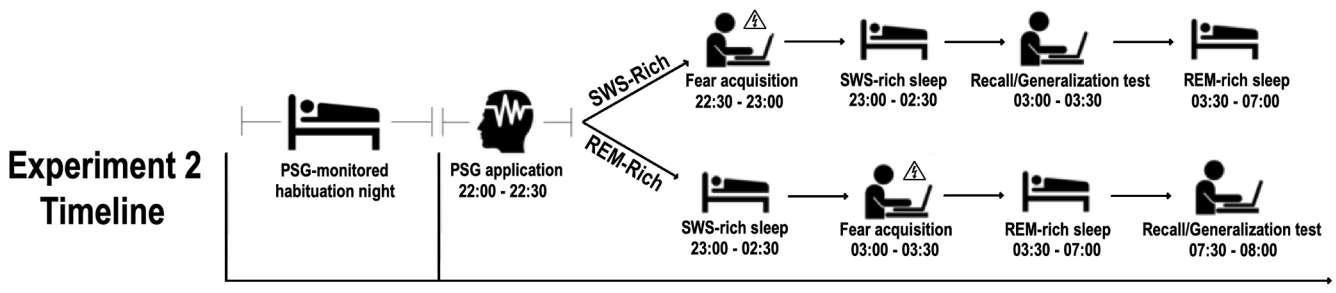

Fig. 5. Experimental procedure of Experiment 2. See text for details.

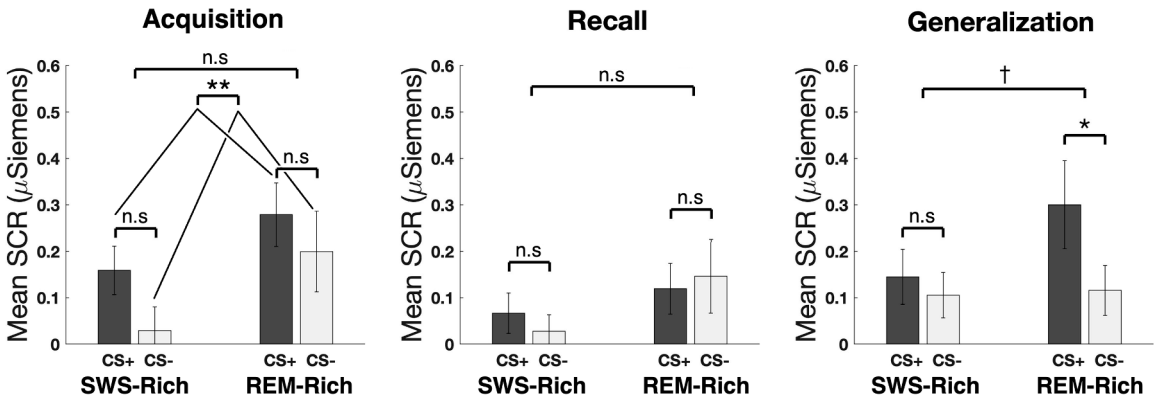

Fig. 6. Skin Conductance Response (SCR) of Experiment 2. Fear acquisition did not differ between the SWS-rich and REM-rich groups, with the effect being significant across groups. No effects were found in the fear recall condition. For generalization, there was a trend towards a stronger effect in the REM-rich group. ** $p < 0.003$; * $p = 0.052$; † $p = 0.092$. Error bars represent the standard error of the mean. n.s – not significant.

Table 4
Correlations of sleep stages and performance across all subjects in Experiment 2.

	Relative Time in Sleep Stage				Raw Time in Sleep Stage			
Sleep Stage Condition	%N1	%N2	%SWS	%REM	N1	N2	SWS	REM
Recall	0.2662	0.2614	0.2753	**0.0024**	0.7335	0.0804	0.2414	0.0658
Generalization	0.0133	0.7956	0.0946	0.1061	0.2542	0.2778	0.6418	0.3274

Note: Uncorrected p-values are presented. Values that are significant after Bonferroni correction for multiple comparisons are bolded.

−0.05 to 0.65), suggesting that the difference between the groups was driven by an increase in fear from the crucial predictor rather than reduction in fear from the safety signals. Overall, these effects were once again consistent with the results obtained for vmPFC activity in Experiment 1 (Fig. 3A, 3B).

Turning to examine whether the SCR differences between CS+ and CS− in each group were modulated by condition, we ran a marginal linear model separately for the SWS-rich and REM-rich group, with Condition as a lone repeated measures factor with unstructured covariance matrix. However, we found no significant effect of Condition for either group (both *p*s > 0.1).

To conclude our analysis, we tried to qualitatively compare between the results of Experiment 1 and 2 to see if any similarities could be drawn despite only having few subjects with valid SCR data from Experiment 1 (due to the equipment malfunction). We concentrated on the 5 subjects from Experiment 1 that were sleep-deprived (out of the 7 with valid datapoints[2]). Since each group had different baseline sensitivity to the aversive stimulation, and since slightly different protocols were used during the Acquisition phase of the two experiments, we normalized the SCR values for each of the 6 stimuli types (CS+ and CS−, in Acquisition, Recall and Generalization) of each subject by the subject's group average for CS+ during Acquisition. CS+ and CS− in Acquisition, in turn, were computed only over the first 5 trials where they appeared (to avoid differences in habituation rates between the two protocols). Results are presented in Fig. 7A. Overall, the three groups displayed remarkably similar patterns of SCR responses across conditions (note that the average value for CS+ in Acquisition is exactly 1 for all groups, due to the normalization procedure). Nevertheless, the similarity was especially notable between the Sleep-Deprived group from Experiment 1 and the SWS-Rich group in Experiment 2, whereas the REM-rich group from Experiment 2 differed in two ways: It had a higher gap between CS+ and CS− for Generalization than the other two groups; and it had a higher CS− response for Recall. These differences mirrored the statistical analysis presented earlier when considering only the two groups in Experiment 2, and implied that the SCR results of the sleep-deprived group in Experiment 1 were most likely due to the lack of REM sleep rather than lack of SWS sleep.

4. Discussion

In two experiments, we demonstrated opposing effects of REM sleep on the ability to recall and generalize fear-related memories. Specifically, REM sleep decreased participants' ability to differentiate between encoded memories that contained a combination of threat and safety signals, but increased participants' ability to differentiate between novel stimuli containing either threat *or* safety signals. These REM sleep effects were accompanied by corresponding changes in fear-related vmPFC activity and opposing effects on hippocampal activity for Recall and Generalization between the sleep and sleep-deprived groups.

One possible account for our findings builds on the REM recalibration hypothesis (Goldstein & Walker, 2014). According to this theory, REM sleep contributes to the daily restoration of low tonic, high phasic mode of norepinephrine activity in the locus coeruleus. During exposure to potentially threatening stimuli, the locus coeruleus projects to the amygdala (both directly, and indirectly through vmPFC mediation) and this impacts how well one can discriminate between threats and safety signals. Without REM, the amygdala is subject to excessive amounts of secreted norepinephrine and a lack of vmPFC top-down control, which prevents it from selectively reacting to only the most threatening stimuli.

Extending the theory to our paradigm, we assume that when the brain encodes stimuli composed of several cues, such as 'AB', it creates representations of the individual components (A, B) as well as representations of their combination 'AB' (cf. Gluck, 1991; Kumaran & McClelland, 2012), and associates each of them to threat based on the conditioning schedule of the fear acquisition phase. Under this assumption, we can describe our stimuli as lying on a continuum of likelihood to predict threat during the test phase. Stimuli containing only the crucial threat predictor (e.g., B) would be most likely to predict

[2] We did not include the SCR data of the sleep group from Experiment 1 in this analysis given that it contained only 2 subjects with usable datapoints, rendering it too unreliable.

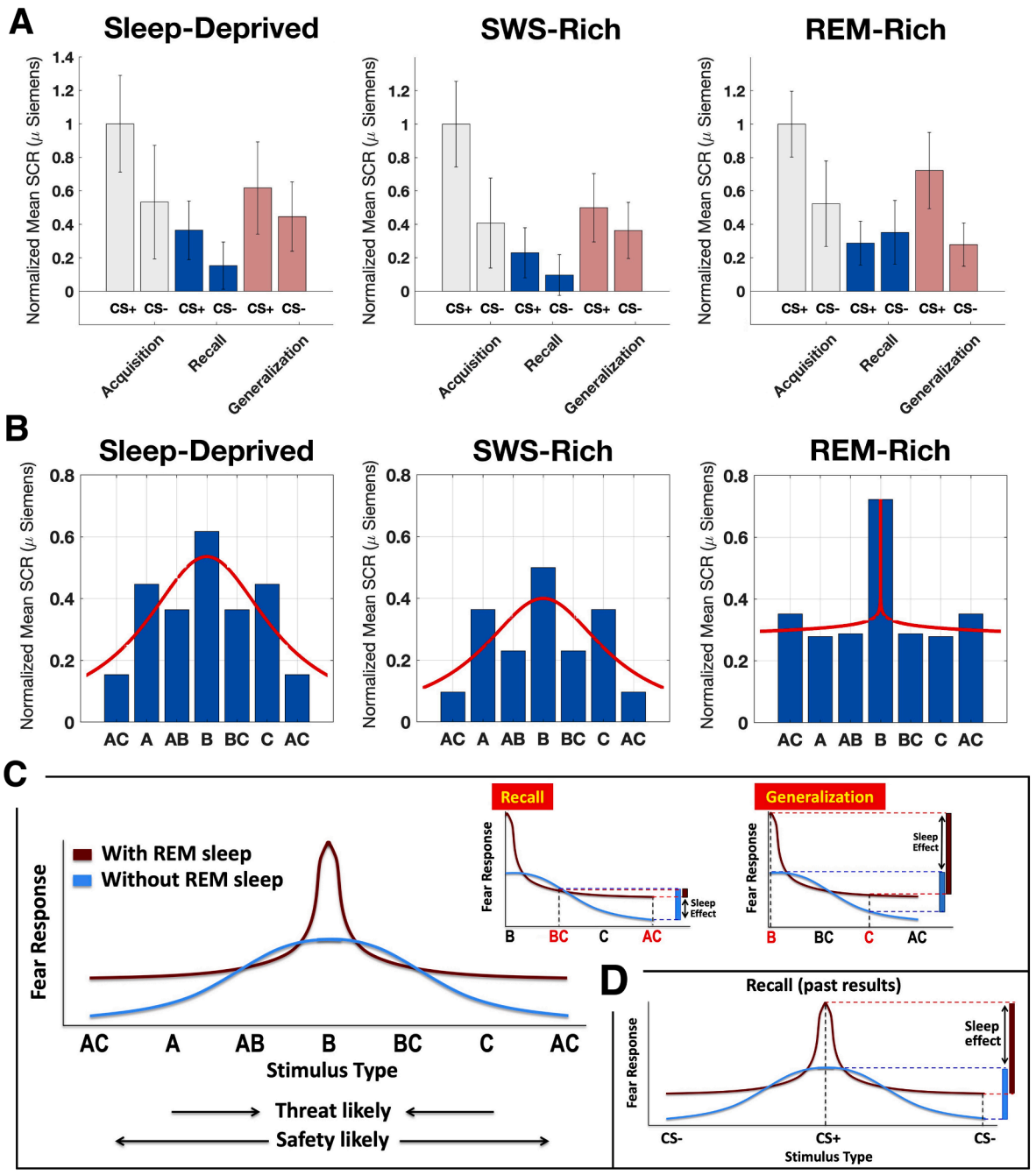

Fig. 7. A: Comparison of normalized SCR effects between Experiment 1 ("sleep-Deprived") and Experiment 2 ("SWS-Rich" and" REM-Rich"). B: Rearrangement of the Recall and Generalization data from panel A according to the likelihood of each stimuli to signal threat (see text for details). For illustration purposes, 'B' is depicted as the crucial threat predictor in the center, stimuli with equal threat value (i.e., 'AB' and 'BC'; 'A' and 'C') are presented in equidistant points from both sides of the center, and 'AC', the only stimulus always signaling safety, is presented twice at the periphery. Best-fit generalized bell-shaped function for each group is displayed in red. C: Interpretation of results from the standpoint of the REM recalibration hypothesis. Without REM sleep, threat discrimination is low, producing fear response that gradually decreases with threat predictability; REM sleep, in contrast, allows for a highly selective threat discrimination where only the most threatening stimulus produces a fear response. Insets: The difference in Fear Recall ('BC' vs. 'AC') and Fear Generalization ('B' vs. 'C') predicted for our stimuli based on the difference in fear response activation with and without REM sleep. Only the right part of the curves is displayed, due to their symmetry. D. Predicted results for fear recall in studies that only employ one CS+ and one CS−.

threat, because they had double the opportunity to be paired with shock (by both AB and BC trials). Stimuli containing a combination of the crucial predictor with a safety signal (e.g., AB) are slightly less likely to predict a threat given that they had the opportunity to be paired with shock only in trials presenting them. Stimuli containing only a safety signal (e.g., A) are lower still in their threat predictability as half of the trials they appeared in could be paired with shock (AB) whereas the other half were never shocked (AC); and stimuli containing a combination of safety signals (e.g., AC) are least likely to predict threat, given that they were never paired with shock.

Following this interpretation, we rearranged the Recall and Generalization data of Fig. 7A according to the continuum of threat

predictability,[3] and fitted it with a generalized bell-shaped function (red line; Fig. 7B). As can be seen, the Sleep-deprived and SWS-rich groups exhibit moderate differentiation of the various levels of threat, whereas the REM-rich group displays a sharp contrast between the most threatening cue and the rest. This result would be predicted by the REM recalibration hypothesis, which suggests that without REM sleep, fear response specificity would be low, diminishing the ability to differentiate salient and less salient threats, whereas with REM sleep, specificity would be high, emphasizing the discrimination of the crucial threat predictor from other signals (Fig. 7C, main), leading to reduced fear recall and enhanced fear generalization following REM sleep (Fig. 7C, insets). Moreover, given evidence suggesting PTSD patients suffer from hypoactivity of the vmPFC (Koenigs & Grafman, 2009), abnormal REM sleep that is signified by excessive locus coeruleus activity (Germain, 2013; Kobayashi et al., 2007), and exhibit less discrimination between fear responses to threatening and non-threatening stimuli (Grillon & Morgan, 1999), our results support the view that REM malfunction might be a leading factor in the initiation and maintenance of PTSD (Goldstein & Walker, 2014).

Note that the empirical results in Fig. 7B slightly differ from the "ideal" model in Fig. 7C. First, breaking away from the smooth bell-shape function suggested in Fig. 7C, SCR responses in both the sleep-deprived and SWS-Rich groups were slightly lower for the AB/BC stimuli compared to A/C stimuli. This seems to go against the interpretation of the stimuli as lying on a decreasing threat-predictability continuum; however, note that the A/C stimuli belonged to the Generalization condition, which subjects saw for the first time only during the testing session, whereas the AB/BC stimuli were already presented during Acquisition. That, by itself, could have increased SCR values for all stimuli belonging to Generalization, given that SCR measurements are known to be affected by novelty (Eisenstein, Eisenstein, & Bonheim, 1991). Second, while the REM-rich group exhibited an SCR pattern that seems to be uniquely tuned to differentiate between the crucial predictor and the rest of the stimuli, it is not clear why the values for the less-threatening stimuli were higher than their counterparts in the other two groups (and not, for example, closer to 0; see Fig. 7B). One possible answer is that this have resulted from especially high baseline SCR values in the REM-rich group (e.g., see the values for CS− during Acquisition in Fig. 6), which our normalization procedure did not correct for. Such a higher baseline could potentially stem from the very same fear consolidation process that REM sleep contributes to – though such a mechanism would seem to go against the REM recalibration hypothesis and its assumption of a diminished amygdala sensitivity due to REM-dependent reduction of tonic norepinephrine. Conversely, it could also be a mere chance occurrence particular to our REM-rich subject group. Future studies would need to investigate this issue further.

Some previous studies have reported REM sleep enhances fear recall rather than diminishing it (Menz et al., 2013; Marshall et al., 2014; but see Spoomaker et al., 2010, for opposite results). These studies, however, did not utilize stimuli that contained multiple levels of threat predictability. It is possible that the consolidation of fear memories during sleep sharpens the differentiation between threat and safety based on the spectrum of learned stimuli presented during acquisition. For example, when a simple CS+ / CS− dichotomy is learned during fear acquisition, with both stimuli being clearly different from each other, sleep may enhance this binary distinction, thus effectively increasing fear recall (Fig. 7D); but when the stimuli represent a more complex differentiation, sleep may selectively sharpen the distinction between specific elements of the stimuli while blurring the differences between the rest, resulting, as described above for our case (Fig. 7C), in reduced fear recall.

A few other studies have demonstrated opposing effects of sleep on the recall and generalization of memories. Using a fear conditioning paradigm, Davidson et al. (2018) presented subjects with visual images of either a small or a large circle, with one of them conditioned to shock. Following an interval in which subjects remained awake or took a nap, they tested fear response to the same circles (fear recall) as well as to novel circles varying in size between the two original ones (fear generalization). They found that the nap group exhibited a lack of discrimination between the various stimuli, reflected as poorer fear recall but a trend towards higher generalization compared to the wake group. Outside the realms of fear conditioning, Alger and Payne (2016) taught participants transitive associations (e.g., A –>B and B –>C), then following sleep tested the ability to recall those associations as well as to generalize them to deduce the novel association B->C. They found that REM sleep was negatively correlated with recall ability, but positively correlated with generalization performance. In another study, Gomez et al. (2006) found that following a nap, infants exhibit poorer recognition of familiar linguistic strings compared to infants who stayed awake, but better recognition of novel strings that follow the same grammatical rule as the previously learned ones, hence demonstrating superior generalization. These studies, as well as ours, are consistent with the theory that sleep serves to extract the 'gist' of encoded memories while diminishing the details of each individual memory (Stickgold & Walker, 2013). By maintaining the common aspects of related experiences ('gist') but removing their idiosyncratic features, sleep might act to preserve the signals that help us generalize from past events to new but partially similar situations in the future. Note, however, that the term 'generalization' itself has been used to describe various types of abstraction processes across studies, some depending on visual similarity, others on linguistic regularities or rule learning, and some – as in our case – on discrimination abilities. While all of those instances of generalization include a crucial common thread – the utilization of learned information to novel but partially resembling circumstances –the mechanisms contributing to each type of generalization, and the way sleep affects them, could potentially be different.

Though less prominent than REM sleep, we also found moderate effects of N1 sleep. Specifically, within the sleep group in Experiment 1, time spent in N1 was positively correlated with fear-recall activity in the right hippocampus. Increased amounts of N1 sleep are often considered to express a less refreshing, more fragmented sleep (Shrivastava et al., 2014). Therefore, one interpretation of our result is that individuals who had lower quality of sleep tended to have higher hippocampal activation during fear recall. Such interpretation fits well with our finding that the sleep group as a whole had lower fear-recall activity in the hippocampus (albeit in the left, rather than the right hippocampus) compared to the sleep-deprived group (Fig. 3A). Interestingly, increased amounts of N1 sleep are also one of the typical findings in PTSD patients (Kobayashi et al., 2007). Our result may therefore suggest, indirectly, that fragmented sleep could contribute to the maintenance of PTSD since this fragmentation could lead to over-activation of the hippocampus when presented with trauma reminders, possibly resulting in the inability to diminish the memory and consolidate it appropriately.

While we interpret the results of Experiment 1 as indicating a detrimental effect of sleep deprivation on fear consolidation, it could be argued that subjects in the sleep deprivation group were simply more tired or less vigilant than subjects in the Sleep group, leading to the observed effects. While this confound cannot be dismissed entirely, we believe that general sleepiness is unlikely to explain our results since we would expect sleepiness to act similarly on both fear recall and generalization. In contrast, our results indicated an inverse effect of sleep deprivation on those two conditions, which was also consistent with correlations between REM sleep and vmPFC activation in the Sleep

[3] To compare with the original presentation of the REM Recalibration hypothesis in Goldstein & Walker (2014), the data is displayed as a symmetric function with the value corresponding to the peak threat probability in the middle and data for the lower threat probabilities replicated to the right and left of it. For illustration purposes, we arbitrarily labeled the peak probability as if B was the crucial predictor (and hence A, C are the safety cues).

group itself. Therefore, our findings are more likely to have resulted from the way fear memories were processed and consolidated following sleep deprivation (an interpretation shared with other studies of sleep deprivation and fear conditioning employing similar methodologies; see Straus et al., 2017; Zenses et al., 2020).

The effects in Experiment 2, while consistent with Experiment 1, were only marginally significant for fear generalization, and were evident only across subjects for fear recall. While a higher number of subjects might have strengthened the effects, this could also have resulted from a limited effectivity of the split-night design in modulating the time spent in REM sleep in the two groups (20 min vs 33 min; see Table 3). Potentially, higher modulation could have led to stronger effects. Given that REM sleep, rather than SWS, seems to be the crucial factor influencing the fear response, future studies may choose to focus on a simpler experimental design, comparing normal to REM-deprived sleep rather than SWS-rich to REM-rich sleep.

5. Conclusion

Results from our two experiments demonstrate that sleep, particularly REM sleep, does not simply enhance fear recall or fear generalization as some previous studies suggest. Rather, we argue that REM sleep exerts a more nuanced influence on fear memories, which takes into account the relationship between encoded threat and safety cues and could result in sharpened fear differentiation for some stimuli, but diminished discrimination for others. To the extent that fear conditioning models some aspect of PTSD, these findings are consistent with the idea that REM sleep may contribute to the initiation and/or maintenance of the disorder in a way that corresponds to the REM recalibration hypothesis. A future line of research may examine whether emphasizing threat-safety discrimination training, incorporated as part of Cognitive Behavioral Therapy and followed by a period of sleep that includes REM, may relieve some PTSD symptomatology.

All work in this study was conducted according to the Rutgers IRB regulations. Informed consent was obtained for experimentation with human subjects.

CRediT authorship contribution statement

Itamar Lerner: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Funding acquisition. **Shira M. Lupkin:** Methodology, Software, Writing - review & editing, Funding acquisition. **Alan Tsai:** Investigation, Formal analysis, Writing - review & editing. **Anosha Khawaja:** Investigation, Formal analysis, Writing - review & editing. **Mark A. Gluck:** Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

None.

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