Sleep Deprivation Disrupts Recall of Conditioned Fear Extinction

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Abstract

Background: Learned fear is crucial in the development and maintenance of posttraumatic stress disorder (PTSD) and other anxiety disorders, and extinction of learned fear is necessary for response to exposure-based treatments. In humans, research suggests disrupted sleep impairs consolidation of extinction, though no studies have examined this experimentally using total sleep deprivation.

Methods: Seventy-one healthy controls underwent a paradigm to acquire conditioned fear to a visual cue. Twenty-four hours after fear conditioning, participants underwent extinction learning. Twenty-four hours after extinction learning, participants underwent extinction recall. Participants were randomized to three groups: 1) well-rested throughout testing (“normal sleep”; $n = 21$); 2) 36 hours total sleep deprivation before extinction learning (“pre-extinction deprivation”; $n = 25$); or 3) 36 hours total sleep deprivation after extinction learning and before extinction recall (“post-extinction deprivation”; $n = 25$). The groups were compared on blink EMG reactivity to the condition stimulus during extinction learning and recall.

Results: There were no differences among the three groups during extinction learning. During extinction recall, the pre-extinction deprivation group demonstrated significantly less extinction recall than the normal sleep group. There was no significant difference between the normal sleep and post-extinction deprivation group during extinction recall. Results indicated sleep deprivation prior to extinction training significantly disrupts extinction recall.

Conclusions: These findings suggest that (1) sleep deprivation in the immediate aftermath of trauma could be a potential contributor to PTSD development and maintenance via interference with natural extinction processes and (2) management of sleep symptoms should be considered during extinction-based therapy.

Introduction

Fear processes play a critical role in the development and maintenance of PTSD and other anxiety disorders. For example, patients with PTSD experience intense fear reactions to cues associated with a traumatic event, which provokes strong avoidance of these cues long after the trauma (1). During natural recovery from trauma, extinction learning reduces fear reactions to such cues (2). In behavioral interventions, repeated exposure to these cues in safe settings should lead to extinction, whereby the cues lose their predictive quality for danger (3, 4). However, strong evidence suggests that patients with PTSD have impaired extinction learning and recall, as demonstrated in psychophysiology studies (5-7). To support better prevention and intervention strategies, it is critical to delineate potential mechanisms that interfere with extinction learning and recall. Such factors could support both the development and maintenance of PTSD and interfere with response to the gold standard treatment for PTSD, exposure-based therapy.

A growing body of research suggests sleep disruption is one mechanism interfering with extinction processes (see 8, for a review). In particular, animal studies report sleep disruption may interfere with initial extinction learning (9) and consistently show sleep disruption interferes with extinction recall, the subsequent ability to consolidate extinction learning and the strongest predictor of long-term extinction (10-12). Few studies have translated this animal research to humans, all of which support the hypothesis that sleep, particularly REM sleep, is important in extinction learning and recall. Pace-Schott and colleagues (13) showed sleep, in general, promotes generalization of extinction, though they did not examine specific sleep stages. In another human study (14), safety learning was associated with subsequent REM consolidation,

which in turn predicted fear and safety recall the following day. Spoormaker and colleagues (15) showed REM sleep promotes extinction recall, with selective REM deprivation after extinction learning interfering with consolidation of that extinction learning (16). While these studies suggest a role for sleep in extinction processes, no studies have examined sleep disruption *prior* to extinction learning to examine if the results from animal studies extend to humans. Additionally, no studies have used experimental sleep deprivation to examine if elimination of sleep altogether has the effects on extinction learning predicted by animal models. Such a study would have clinical implications. For example, sleep disruption is very common in PTSD (17, 18). Demonstrating that sleep deprivation interferes with extinction learning and/or recall processes would suggest sleep disruption may interfere both with an individual's ability to experience natural recovery immediately after a trauma and to benefit from exposure-based therapy once PTSD develops (see 19, for model).

Here, we examined the effect of 36 hours total sleep deprivation on extinction learning and recall in healthy human subjects. All subjects ($n = 71$) underwent a laboratory paradigm to acquire conditioned fear to a visual cue. Twenty-four hours later, participants underwent an extinction learning session, and twenty-four hours after that, participants underwent extinction recall. Participants were randomized to three groups: 1) well-rested throughout testing; 2) 36 hours total sleep deprivation before extinction learning; or 3) 36 hours total sleep deprivation after extinction learning and before extinction recall. Consistent with the animal and human studies, we hypothesized total sleep deprivation would not interfere with extinction learning, but would interfere with recall of extinction memories.

Methods and Materials

Participants

Seventy-three (73) healthy young adults were enrolled. Following written informed consent, participants were screened for sleep disorders, drug use, psychiatric, and medical disorders via structured interview and laboratory tests. Inclusion criteria included: 1) age 18–39 years-old; 2) regular sleep-wake schedule that included 7–9 hours time-in-bed with a bed time 2200-0000 and a wake time 0600-0800; 3) no current medical or psychiatric diagnoses; and d) no personal history of any Axis I diagnosis or family history of mood or psychotic disorders. Female participants were studied in the early follicular phase of the menstrual cycle. Those not exhibiting consistent startle responding upon screening (over 75% discernible responses to 12 105-dB 40-ms startle pulses) were excluded. Of the 73 participants who completed the lab portion of the study, two participants had incomplete datasets and were therefore dropped from the final analyses. Overall, 71 participants were included in analyses. See Table 1 for demographic information.

Procedure

Participants maintained a regular sleep-wake schedule, matching their self-reported habitual schedule, at home for 7 days. Adherence was monitored via actigraphy, voicemail calls and diaries. Participants then spent 4 consecutive days and nights in the laboratory (Figure 1), where they underwent a fear potentiated startle (FPS) protocol. The FPS consisted of three sessions: fear acquisition (Day 1); extinction learning (Day 2); and extinction recall (Day 3). All testing took place in the evening, 10-12 hours following participants' habitual wake time. All

testing took place in the same context to prevent context-dependent alterations in fear responding from influencing the results. Participants were randomized to one of three conditions: 1) well-rested throughout testing (“Normal sleep,” $n=21$); 2) 36 hours total sleep deprivation before extinction learning (“Pre-extinction deprivation,” $n=25$); or 3) 36 hours total sleep deprivation after extinction learning and before extinction recall (Post-extinction deprivation,” $n=25$). Participants were not allowed to leave the laboratory during the study or engage in exercise more vigorous than walking short distances and were restricted from alcohol and caffeine or other stimulants beginning 48 hours before entering the laboratory. For each night of normal sleep, polysomnography (PSG), including EEG, EOG, and chin EMG, monitored sleep. To screen for unreported sleep apnea and periodic leg movements, additional monitors were added on the adaptation night. Placement of monitors and PSG scoring was per AASM recommendations (20).

Fear Conditioning and Extinction Procedure

The FPS apparatus and recording procedures have been described in detail elsewhere by our group and others (14, 21, 22), and are provided in the Supplemental Information. Procedures and timeline for the three FPS sessions are illustrated in Figure 2. All sessions were designed similarly to those used in our previous work (14, 21) as well as others (6). Each session began with six startle pulses presented in the absence of any other stimuli in order for the participants to acclimate/habituate startle responses to baseline level. The acquisition session (Day 1) consisted of: a) eight 6-s presentations of a blue circle serving as a reinforced conditioned stimulus (CS+), followed by a 0.5-s electrical shock unconditioned stimulus (US) in 75% contingency co-terminating with the CS+; b) eight 6-s presentations of a red circle serving as the second CS+ followed by a 0.5-s electrical shock US in 75% contingency co-terminating with the CS+; c)

sixteen 6-s presentations of a yellow circle serving as a non-reinforced conditioned stimulus (CS-) never followed by shock (i.e., safety signal); and d) sixteen presentations of the startle pulse in the absence of any stimuli (i.e., blank screen; “Noise-Alone trial”; NA) serving as a measure of baseline startle reactivity across the session. The first half of the acquisition session consisted of presentation of blue CS+ trials only for all participants, and the second half of the sessions consisted of red CS+ trials only for all participants. Startle pulses were presented 4 s following CS+ or CS- onset. Order of stimuli presentation was block randomized within each CS+ acquisition block (blue vs. red) with the constraint of two trials of each type (CS+, CS-, and NA) per block. This approach prevents confounds of uneven habituation effects on any one stimulus type and assures accurate temporal match of NA baseline responses to CS+ and CS- trials. To measure contingency awareness, participants used a number keypad to report at each CS+ or CS- trial whether they expected to receive a shock (“1” key), were unsure (“2” key), or did not expect to receive a shock (“3” key). Following the acquisition session, contingency awareness was further measured via a questionnaire asking participants which stimuli predicted the shock. Self-reported anxiety was measured by asking how aversive participants found the shock (1 – 5 scale) and how anxious they felt in the presence of the blue and yellow circles (CS+ and CS-; 1 – 10 scale).

On Day 2, participants underwent the extinction session, consisting of 16 presentations of each stimulus type (blue CS+, yellow CS-, and NA), in a block-randomized order as in the acquisition session. No shocks were presented during this session. Startle pulses were presented and subjects rated their expectations as in the acquisition session. Following the extinction session, participants again rated their subjective anxiety in the presence of both the CS+ and CS-. Subjects were not presented with the red circle CS+ on Day 2 to allow for examination of

generalization of extinction learning (13). Participants returned for the recall session on Day 3. This session was identical to the acquisition session on Day 1 with the exception that no US was delivered. Startle pulses were presented and expectancy ratings collected as in the previous sessions. Following the recall session, participants again rated their subjective anxiety to the CS+ and CS-.

Fear Conditioning Data Analysis

Startle. Initial data reduction involved averaging responses to CS+ and CS- trials within each session into blocks of 2 trials each. NA trials within a session were averaged to acquire a baseline startle response. This baseline was then subtracted from the respective CS+ and CS- block within each session, creating scores representing potentiated startle above baseline for each CS type within each block. Thus there were 4 blocks for the CS+ and CS- during the Acquisition session; 8 blocks for the CS+ and CS- for the Extinction session; and 4 blocks for the CS+ and CS- during Recall. Note phases of the acquisition and recall sessions that contained blue or red CS+s (first or last half of the session respectively) were analyzed separately. To reduce between-subjects variability created by individual differences in startle magnitude overall, each individual participant's total number of scores (48 total blocks were standardized into Z-scores such that all scores represented departure from each individual's mean level of potentiated startle across the entire experiment. These scores then underwent secondary data reduction and analysis.

Hypothesis testing. To compare acquisition of fear responding across groups, the final 2 z-scores of the acquisition session across each CS type (CS+ and CS-) were averaged to create the operational measure of learned fear responding (5, 23). These data were then analyzed using

a 3 (Group) x 2 (Cue) mixed ANOVA with cue as a repeated-measure. To assess extinction learning of the CS+ stimulus across groups, each 2 consecutive Z-scores for CS+ stimuli were averaged to produce 4 blocks of CS+ responding over the session: early extinction, early-mid extinction, mid-late extinction and late extinction (5, 23, 24). These data were analyzed using a 3 (Group) x 4 (Block) mixed ANOVA with block as a repeated-measure. To compare extinction recall across groups, recall was operationally defined as the initial fear response to the blue CS+ (first 4 CS+ Z-scores averaged). The *a priori* hypotheses concerned the difference between the normal sleep group and each of the other two groups, separately, so two independent samples t-tests were conducted comparing normal sleep to pre-extinction deprivation and then normal sleep to post-extinction deprivation. Self-reported anxiety and expectancy measures were not directly related to our main aims, and, thus, those analyses and results are presented in the Supplemental Information.

REM Sleep Data Analysis

Previous studies have shown REM sleep promotes extinction recall (15), so additional analyses were conducted to examine the relationship between REM sleep consolidation and extinction recall. As in previous studies from our research group (14), three variables served as measures of REM consolidation: REM percent, REM sleep latency, and REM sleep efficiency. Difference scores were created for all three variables (Night 2 – Baseline). Then, these variables were Z-scored across subjects and linearly combined using the same coefficients as previously described for other analyses of the first sleep night of this data set (14) to create the latent variable representing REM consolidation (see Supplemental Information for details). Bivariate

correlations were then used to examine the relationship between REM consolidation and extinction recall for the Normal Sleep and Pre-Extinction Deprivation groups. Two-tailed independent samples t-tests were used to examine differences in REM consolidation between these two groups.

Results

Acquisition Session

Participants responded with greater potentiated startle to blue CS+ vs CS- [$F(1,68) = 12.51, p < .001$]. As expected, this cue effect did not vary by Group ($F_s < 1$, ns, Figure 3A). Participants showed much weaker discrimination learning in the second acquisition session with the red CS+ and yellow CS- [effect of Cue: $F(1,68) = 5.94, p < .02$], with differences in potentiation across groups [effect of Group: $F(1,68) = 4.47, p < .02$; post-extinction deprivation > pre-extinction deprivation ($p < .005$)]. Because of poor conditioning to this secondary CS+ we were unable to examine extinction recall generalization and have chosen not to report further analyses regarding this stimulus (data not shown).

Extinction Session

There was a strong Block effect across extinction. The response to the CS+ decreased across the session [$F(3,204) = 63.73, p < .0001$]. This effect did not vary by Group, nor was there a main effect of group, suggesting no differences in rate or strength of extinction learning ($F_s < 1$, ns, Figure 3B) due to sleep deprivation.

Recall Session

Comparison of Normal sleep to Pre-extinction deprivation showed sleep deprivation prior to initial extinction learning led to greater fear responding during the first half of the recall session ($t(44) = -2.15, p < .04$, Cohen's $d = .65$, Figure 3C). Comparison of Normal sleep to Post-extinction deprivation did not show a significant effect ($t(44) = -0.53$, ns, Cohen's $d = .16$), suggesting total sleep deprivation following extinction learning did not have an effect on extinction memory recall.

REM Sleep Analyses

REM consolidation was negatively correlated with response to the CS+ during the first half of the recall session ($r = -.407$, $p = .007$), indicating that participants with more consolidated REM sleep exhibited enhanced extinction recall (Figure 4). The Pre-Extinction Deprivation group had slightly less consolidated REM than the group that was never sleep deprived, but this difference was nonsignificant when the groups were compared via two-tailed independent samples t-tests ($t = 1.787$, $p = .081$).

Discussion

The current study investigated the effect of total sleep deprivation on extinction of conditioned fear and ability to recall extinction 24 hours later. On Day 1, fear was successfully conditioned to the CS+ vs. CS- across all participants as indexed by fear potentiated startle, self-reported anxiety, and online expectancy ratings. On Day 2, we observed no differences in initial extinction learning between those participants who received normal sleep the night prior and those who were sleep deprived. However, on Day 3, those who were sleep deprived prior to initial fear extinction learning (pre-extinction deprivation) showed significantly greater potentiated startle responses in the presence of the CS+ (i.e., impaired extinction recall). This elevated startle potentiation suggests sleep deprivation prior to extinction learning interfered with subsequent memory for that extinction learning. Potentiated startle responses are often independent of contingency awareness (21, 24), and indeed we found extinction recall as measured by self-report of anxiety or expectancy ratings was not affected by sleep deprivation (Supplemental Information). These results suggest “automatic” fear responses may be more sensitive to deprivation effects on extinction recall compared to explicit contingency learning. This differential sensitivity may have implications for how extinction is measured during extinction-based therapies, and how best to predict treatment response.

This study expands the current literature in humans suggesting sleep disruption interferes with extinction. Previous studies have shown sleep promotes generalization of extinction (13), and that REM sleep disruption after extinction learning interferes with extinction recall (16). Our study demonstrated sleep deprivation prior to extinction learning did not interfere with extinction acquisition, but did result in impaired extinction recall one day following extinction learning.

The pattern of results observed here are consistent with the larger literature on sleep and the mechanisms involved in emotional memory consolidation in humans. Studies suggest sleep, and REM sleep in particular, is important for consolidating memory for emotional material (25, 26). Previous studies also suggest the effect of sleep deprivation prior to emotional learning differs according to valence of emotional stimuli. For example, one study showed sleep deprivation impaired consolidation of positive emotional stimuli more than negative stimuli (cited in 27). The current study investigated extinction learning, which has been conceptualized as positive emotional learning (28), and thus may be more susceptible to prior sleep deprivation. It has also been posited that ventromedial prefrontal cortex, a critical node in the fear extinction network, shows decreased reactivity to emotional stimuli following total sleep deprivation (29), as well as reduced connectivity to the amygdala (30). Thus, it is possible neural changes during extinction learning in the pre-extinction sleep deprivation group, while not interfering with the ability to show extinction in the moment, did interfere with subsequent consolidation of that extinction learning.

In addition, our study suggested more consolidated REM sleep on Night 2 was associated with enhanced extinction recall the following day. This is consistent with previous research showing a relationship between REM sleep and extinction recall (15, 16). REM sleep may dampen emotional activity by causing changes in monoaminergic systems (31), which in turn may facilitate the fear inhibition necessary for extinction processes (4). In the experimental paradigm used here, participants who slept the night before the extinction recall showed this REM-recall connection, regardless of group status. Therefore, participants may have demonstrated impaired extinction recall responses on Day 3 due to reduced REM consolidation the previous night, rather than some direct effect of being sleep deprived during extinction

learning. Animal studies have demonstrated REM sleep deprivation specifically interferes with extinction acquisition (9), and both animal and human studies have demonstrated REM sleep deprivation negatively impacts extinction recall (10, 16). Future research manipulating REM prior to extinction learning (as opposed to prior to extinction recall, as others have done) is needed to test the hypothesis that reduced REM prior to extinction learning is sufficient to disrupt recall.

Though animal studies have showed sleep disruption impairs consolidation of extinction (e.g., 10), this was not evident from our findings; the Post-Extinction Deprivation group did not show impairment compared to the controls. At present, it is unclear as to whether or not the results from these animal studies translate to humans. To our knowledge, only a single human study has examined this question, showing REM deprivation impaired extinction consolidation (16). Nonetheless, our study had considerably more power, used TSD rather than REM deprivation, and used FPS rather than SCR as the outcome measure. Any of those considerations could help explain why we did not find a TSD effect on extinction consolidation. Thus, we believe our result suggests the question of whether the animal findings in this area translate directly to humans remains open.

While not a main focus of this study, it is important to note sleep deprivation following initial fear acquisition did not impact overnight consolidation of that fear (see Early Ext data in Fig 3B). That is, the pre extinction deprivation group showed as strong of a startle response to the initial re-presentation of the fear conditioned stimulus as did the two groups that had slept the night before. This finding directly refutes prior studies suggesting sleep deprivation in the immediate aftermath of a trauma could be a preventative intervention (32, 33).

The results of this study have important clinical implications. Sleep disruption is common to PTSD, with some studies suggesting sleep disruption in 70-91% of PTSD patients (18). Additionally, exposure to trauma increases the risk for sleep disturbance, which often precedes development of PTSD (34). The current study, though conducted in healthy control subjects, sheds light on the potential mechanistic role of sleep disturbance in the pathogenesis of PTSD. Trauma exposure may result in sleep disturbances such as insomnia or nightmares, which in turn interfere with extinction processes necessary for natural recovery from trauma thus making the development of PTSD more likely. Indeed, several studies have supported this hypothesis by demonstrating REM sleep disturbance in the aftermath of trauma predicts the later development of PTSD (35, 36). As discussed in recent reviews (8, 19), sleep disturbance, and REM fragmentation in particular, may activate central stress system processes which interfere with extinction and promote PTSD symptoms. In addition, these results suggest initiating exposure therapy in the context of ongoing sleep disruption may render treatment less effective due to sleep deprivation-related impairments in memory for corrective emotional learning. Further, as sleep deprivation does not appear to affect within-session extinction learning, it may appear to the therapist as though the exposure therapy is effective when anxiety decreases during a given exposure exercise. However, memory for that anxiety reduction will be impaired, thus leaving the patient “back at square one” when returning for the next session. Given exposure-based interventions are common for many anxiety disorders, this treatment implication reaches well beyond PTSD. As a whole, the current results point to the critical importance of attending to sleep disruption when deciding to initiate exposure therapy and throughout the therapy process itself. They further suggest normalizing sleep disruption via behavioral or (in the case of

nightmares) pharmacological methods prior to initiation of exposure therapy should facilitate optimal response to that therapy. Future studies should directly test that hypothesis.

Finally, there are a few limitations worth noting. First, while total sleep deprivation does occur in some operational settings (e.g., military, first responders), its generalizability to other settings has limitations. An increasing number of studies implicate REM as the sleep stage most critically involved in fear extinction and recall. Thus, further studies could manipulate REM (though not necessarily eliminate it altogether) prior to extinction learning in order to replicate the results of the current study. Also, this study was conducted in healthy control subjects. While the results here have strong implications for clinical populations such as PTSD and other anxiety disorders, it will be necessary to conduct further research in clinical populations to determine the extent to which current findings generalize to those populations. Further, the secondary CS+ failed to acquire a conditioned response and thus we were unable to examine sleep deprivation effects on fear extinction generalization. This was likely habituation to the US during the red CS+ conditioning which was always in the last half of the conditioning session. Future studies may benefit from incorporating a secondary US modality (e.g. air puff), that will be less susceptible to habituation effects across the acquisition phase and thus be more likely to support conditioning to the secondary CS+.

In summary, this was the first study to examine the effect of total sleep deprivation on extinction learning and recall in human subjects. Results indicated sleep deprivation prior to extinction acquisition did not interfere with extinction learning, but instead interfered with extinction recall. These findings have clinical implications for the effectiveness of exposure-based therapies in PTSD and other anxiety disorders associated with comorbid sleep disturbances.

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Figure Legends

Figure 1: Study design and participant groups.

Figure 2: Fear potentiated startle procedure. Schematic of the fear potentiated startle procedure detailing the fear acquisition, extinction learning, and extinction recall phases, as well as illustrating a prototypical block and trial.

Figure 3: Standardized potentiated startle magnitudes by sleep group across (A) the last half of the acquisition session; (B) the extinction session, and (C) the recall session. Normal = normal sleep group; Pre-ext Dep = pre-extinction sleep deprivation group, and Post-ext Dep = post-extinction sleep deprivation group. CS+ = blue circle trials corrected for baseline startle, CS- = yellow circle trials corrected for baseline startle. For A, ***= $p < .05$ for CS+ vs CS- comparisons across all groups. For C, *= $p < .05$ for a priori comparison with the normal sleep group.

Figure 4: Relationship between REM sleep consolidation on Night 2 and extinction recall on Day 3, by group. See Methods for a description of the REM sleep consolidation index. Higher scores on REM consolidation on Night 2 corresponded to reduced fear responsiveness to the CS+ on Day 3, indicating enhanced extinction recall.

Table 1. Subject demographics

	Normal (n = 21)	Pre-ext dep (n = 25)	Post-ext dep (n = 25)
Age	24.5 ± 5.5	23.6 ± 4.0	23.6 ± 4.4
Gender, Male / Female (%)	61.9 / 38.1	60 / 40	56.0 / 44
Race, Caucasian / Asian / African-American / Multiple (%)	61.9 / 23.8 / 4.8 / 9.5	44 / 44 / 4 / 8	68 / 20 / 0 / 12
Ethnicity, Non-Hispanic / Hispanic (%)	66.7 / 33.3	84 / 16	60 / 40
Education (yrs)	15.8 ± 2.0	15.4 ± 1.2	15.1 ± 2.4

Note. Ext = extinction. Dep = deprivation.

Prototypical Block:

6 second duration

Blue Circle

Startle Pulse

.5 sec

Fear Acquisition

Habituation: 6 NA Presentations

Acquisition: 4 CS Blocks

Extinction Learning

Habituation: 6 NA Presentations

Extinction: 8 CS+ Blocks without shock

Extinction Recall

Habituation: 6 NA Presentations

Recall: 2 CS+ Blocks without shock

Definitions:

CS+ = Blue circle paired with shock in .75 contingency

CS - = Yellow circle never paired with shock

NA = Startle pulse alone baseline