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Sleep promotes consolidation and generalization of extinction learning in simulated exposure therapy for spider fear

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ABSTRACT

Simulated exposure therapy for spider phobia served as a clinically naturalistic model to study effects of sleep on extinction. Spider-fearing, young adult women ($N = 66$), instrumented for skin conductance response (SCR), heart rate acceleration (HRA) and corrugator electromyography (EMG), viewed 14 identical 1-min videos of a behaving spider before a 12-hr delay containing a normal night's Sleep ($N = 20$) or continuous daytime Wake ($N = 23$), or a 2-hr delay of continuous wake in the Morning ($N = 11$) or Evening ($N = 12$). Following the delay, all groups viewed this same video 6 times followed by six 1-min videos of a novel spider. After each video, participants rated disgust, fearfulness and unpleasantness. In all 4 groups, all measures except corrugator EMG diminished across Session 1 (extinction learning) and, excepting SCR to a sudden noise, increased from the old to novel spider in Session 2. In Wake only, summed subjective ratings and SCR to the old spider significantly increased across the delay (extinction loss) and were greater for the novel vs. the old spider when it was equally novel at the beginning of Session 1 (sensitization). In Sleep only, SCR to a sudden noise decreased across the inter-session delay (extinction augmentation) and, along with HRA, was lower to the novel spider than initially to the old spider in Session 1 (extinction generalization). None of the above differentiated Morning and Evening groups suggesting that intervening sleep, rather than time-of-testing, produced differences between Sleep and Wake. Thus, sleep following exposure therapy may promote retention and generalization of extinction learning.

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1. Introduction

Abnormal expression of fear, as occurs in anxiety disorders such as post-traumatic stress disorder (PTSD) and specific phobia, may result from abnormally strong fear conditioning (Armfield, 2006; Lissek et al., 2005; Mineka and Oehlberg, 2008; Orr et al., 2000), deficiency of inhibitory mechanisms that normally moderate fear expression (Craske et al., 2008; Hofmann, 2008; Milad et al., 2006), or both. Key among such inhibitory processes is extinction — learning that a once-feared object or event is no longer dangerous (Milad et al., 2006). Rather than erasing a fearful memory, extinction forms a new "safety memory" that competes with the fear memory when the once-feared object or event is re-encountered (Hermans et al., 2006; Quirk and Mueller, 2008).

Formation of such therapeutic extinction memories is the neurocognitive basis for the efficacy of exposure therapy, a first-line behavioral treatment for anxiety disorders (Craske et al., 2008; McNally, 2007). In order for exposure therapy to be successful, consolidation and retention of extinction learning acquired during therapy is essential (Craske et al., 2008). In addition, such learning must generalize in order to ensure that the reduction of fearful responding to specific cues in treatment will extend to stimuli encountered outside the therapist's office (Rowe and Craske, 1998; Vansteenwegen et al., 2007).

Using an experimental fear-conditioning paradigm (Milad et al., 2007), normal sleep has been shown to promote the generalization of extinction memories (Pace-Schott et al., 2009). However, unlike such experimentally induced *de-novo* fears, anxiety disorders are associated with long-standing fears that have complex, multifactorial origins and perpetuating factors (Armfield, 2006). Specific phobias, such as spider phobia, are highly prevalent, mild

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anxiety disorders (LeBeau et al., 2010; Lissek et al., 2007) in which treatment strategies, such as exposure therapy, can be studied in a non-clinical setting (e.g., Vansteenwegen et al., 2007).

Sleep enhances consolidation of emotional memory (reviewed in Walker, 2009). Here we characterize the effect of sleep on the retention and generalization of a specific emotional memory– the extinction of spider fear produced by simulated exposure therapy. We hypothesized that sleep following simulated exposure therapy in spider-phobic subjects would lead to greater retention of fear extinction for the spider to which they were repeatedly exposed. In addition, we predicted that sleep would enhance generalization of this extinction memory to a novel spider.

2. Methods and materials

2.1. Participants

Participants were 66 females (18–28 yrs, mean = 19.9) with significant fear of spiders operationally defined using thresholds of 80 on the Fear of Spiders Questionnaire [FSQ (Szymanski and O'Donohue, 1995)] and 15 on the Spider Phobia Questionnaire [SPQ (Klorman et al., 1974)]. Previous research has determined that these thresholds reflect significant fear of spiders (Muris and Merckelbach, 1996; Rodriguez et al., 1999; Guastella et al., 2007; Vansteenwegen et al., 2007). The FSQ was included in a large pre-screening for research participation by students at the University of Massachusetts, Amherst. Starting with the highest FSQ scores and working downward, such individuals were offered the opportunity to earn academic credit for participation if they confirmed eligibility via the SPQ.

Qualified respondents were pseudo-randomly assigned to Sleep ($N = 20$) and Wake ($N = 23$) experimental groups as well as Morning ($N = 11$) and Evening ($N = 12$) control groups. Invitations specified that participants must be non-smoking, without psychiatric, sleep, medical or neurological disorders and not using psychiatric or sleep-affecting drugs. A 23-item screening questionnaire administered at the first session queried these criteria. Only 12 individuals had one or more deviations from the invitation criteria (Table 1). However, because deviations were distributed between the groups and because co-morbidities are common in specific phobias (American Psychiatric Association, 2000), these individuals were included in analyses (see Supplementary methods for additional details). Physiological data could not be analyzed in 1 Wake, 2 Sleep and 1 Evening participants leaving $N = 18$ (Sleep), 22 (Wake), 11 (Morning) and 11 (Evening) for the physiological measures. This study was approved by the University of Massachusetts, Amherst, IRB and all participants provided written informed consent.

2.2. Procedure

Participants completed two sessions (Fig. 1) from approximately 8:00–9:00PM and 8:00–9:00AM the following morning (Sleep), 8:00–9:00AM and 8:00–9:00PM on a single day (Wake), 7:00–8:00 and 10:00–11:00AM (Morning) or 7:00–8:00 and 10:00–11:00PM (Evening). Procedures for the experimental groups (Sleep and Wake) and those for the control groups (Morning and Evening) were identical, except for the duration of the inter-session interval (12 h experimental vs. 2 h control). All participants were instructed to abstain from alcohol, recreational drugs and daytime napping from the day before Session 1 (S1) until completing Session 2 (S2). At S1 (Sleep and Wake) or immediately following S1 (Morning and Evening), participants completed a sleep diary that retrospectively queried sleep duration and quality on the 2 preceding nights. The Sleep group also completed this diary for the night between sessions. On the night before S1 (all groups) and between S1 and S2 (Sleep group), participants were instructed to allow themselves the opportunity for at least 7 h sleep and to have no caffeine after arising until the end of S2. Wake, Morning and Evening groups were specifically instructed to remain continuously awake between sessions. Between S1 and S2, all participants

Table 1
Demographic, self-reported habitual sleep, sleepiness and substance use, subjective sleep duration and psychological traits in the Sleep, Wake Morning and Evening groups.

Characteristic	Sleep (SD)	Wake (SD)	Morning (SD)	Evening (SD)	F(3,62)†
N	20	23	11	12	
Age	20.1 (1.5)	20.2 (2.1)	19.3 (1.4)	19.5 (1.1)	1.12
FSQ	101.4 (12.4)	107.1 (14.9)	110.8 (10.5)	110.3 (13.6)	1.72
SPQ	23.7 (3.3)	24.5 (4.3)	23.3 (2.4)	24.0 (3.2)	0.38
Habitual TST (hr)	7.6 (0.9)	7.4 (1.1)	7.7 (0.8)	7.7 (0.9)	0.38
Habitual SOL (min)	17.3 (10.9)	24.2 (23.7)	24.6 (15.3)	19.2 (13.5)	0.75
ESS	7.50 (3.29)	8.30 (4.42)	9.82 (4.36)	9.08 (3.80)	0.92
PSQI	4.80 (1.58)	5.09 (2.97)	5.67 (2.40)	4.92 (1.83)	0.30[a]
MEQ	39.50 (7.56)	44.83 (10.50)	43.00 (10.34)	39.13 (10.08)	1.52[b]
STAI-Trait	41.05 (8.41)	40.96 (10.11)	36.86 (11.49)	44.25 (10.11)	1.08
Disgust propensity	24.80 (5.14)	25.70 (5.87)	26.82 (4.75)	24.5 (3.09)	0.53
Disgust sensitivity	18.70 (4.51)	19.46 (7.88)	21.91 (5.84)	19.96 (3.48)	0.70
NEO-PI-R Neuroticism	103.63 (17.87)	101.52 (21.13)	95.64 (17.91)	101.67 (20.96)	0.75
NEO-PI-R Extraversion	118.40 (21.68)	128.28 (19.92)	138.45 (15.15)	123.92 (15.10)	2.79*
NEO-PI-R Openness	120.40 (14.54)	117.87 (18.71)	116.46 (21.09)	114.67 (15.61)	0.30
NEO-PI-R Agreeableness	120.75 (17.40)	116.30 (19.41)	110.09 (18.53)	116.25 (12.70)	0.87
NEO-PI-R Conscientiousness	111.20 (16.68)	116.74 (21.54)	120.55 (20.13)	108.83 (25.98)	0.86
Habitual daily caffeine (serv.)	1.5 (1.2)	1.5 (1.2)	1.1 (1.0)	1.0 (0.7)	0.44[c]
Habitual weekly EtOH (serv.)	3.3 (4.8)	2.4 (2.6)	3.6 (2.9)	3.8 (2.6)	0.50[d]
Diary sleep Day −1 (min)	489 (91)	458 (35)	455 (20)	555 (78)	6.87***[e]
Diary sleep Day −2 (min)	490 (68)	496 (98)	447 (109)	517 (116)	1.03[f]
Diary inter-session sleep	431 (25)				
(N) Criteria deviations	(3) Head injury, (1) Head injury & panic disorder & night terror, (1) "Sleep pill"	(3) Head injury, (2) Ritalin, (1) Head injury & Insomnia		(1) Head injury & Ritalin & Depression, (1) Fluoxetine	

*$p < .05$, Morning > Sleep ($p < .01$) and Evening ($p < .05$).
***$p < .001$, Evening > Morning ($p < .0001$), Wake ($p < .0001$) and Sleep ($p = .008$).
†Lower-case letters reflect smaller samples due to participant omission of data points: [a]$F(3,60)$ Morning $N = 9$; [b]$F(3,61)$ Morning $N = 10$; [c]$F(3,61)$ Wake $N = 22$; [d]$F(3,57)$ Wake $N = 21$, Morning $N = 10$, Evening $N = 10$; [e]$F(3,60)$ Sleep $N = 19$, Wake $N = 22$; [f]$F(3,59)$ Sleep $N = 19$, Wake $N = 22$, Morning $N = 10$.

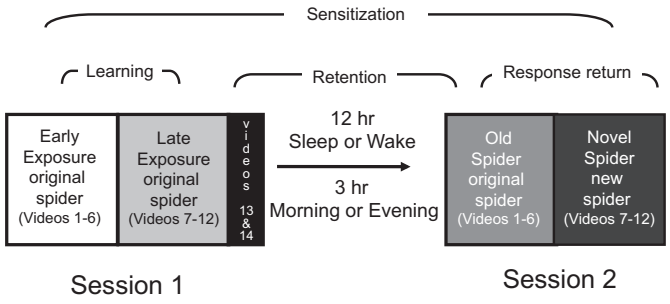

Fig. 1. Experimental protocol showing experimental phases and putative emotional memory processes accounting for changes across phases. In S1, spider videos 13-14 were added in order to maximize extinction learning following Vansteenwegen et al., (2007). However data for these videos were not analyzed so as to accommodate mixed ANOVA design with nested within-subject factors.

completed the Epworth Sleepiness Scale [ESS (Johns, 1994)], the Pittsburgh Sleep Quality Index [PSQI (Buysse et al., 1989)], the Morningness-Eveningness questionnaire [MEQ (Horne and Ostberg, 1976)], the Revised NEO Personality Inventory [NEO-PI-R (Costa and McCrae, 1992)], the Disgust Propensity and Sensitivity Scale [DPSS-R (van Overveld, de Jong, Peters, Cavanagh, & Davey, 2006)] and the STAI-Trait version [STAI-T (Spielberger et al., 1990)].

Participants completed the STAI State version (STAI-S) and the Stanford Sleepiness Scale [SSS (Hoddes et al., 1973)] at the beginning and end of both sessions. They were instructed to imagine themselves "being there with the spider" in the video and told that they "should not resist their fear" and that the "fear will go away by itself" (Vansteenwegen et al., 2007). Participants sat approximately 2 feet from a 17-inch computer screen. Following each video, a screen instructed participants to rate how disgusting, fearful and unpleasant they found the spider using three 11-point rating scales with maxima (+10), minima (−10) and midpoint (0) indicated and intervening increments of 2 indicated by bullets. The order of scales was counterbalanced across subjects. After 20 s, another screen briefly appeared instructing participants to await the next video followed by a 10 s inter-stimulus interval (ISI) after which the next video began. During S1, participants viewed 14 identical videos of a spider and, during S2, participants viewed six videos of this same, "old" spider followed by six videos of a novel spider.

2.3. Stimuli

Three *YouTube* videos of individual spiders (see Supplementary methods) were edited to remove sound, achieve a standard 60-sec length and optimize homogeneity of background. Each subject viewed two of these videos, one as the old spider and a second as the novel spider. The three videos were counterbalanced across participants. Three videos were used in order to reduce the effect of systematic differences in fearfulness between any specific pair of videos thereby increasing the generalizability of findings. In other words, to increase importance of the experimental assignment (old vs. novel) over any inherent differences between any specific pair.

To probe evoked sympathetic reactivity, during some videos and ISIs, a 10-msec, 83 dB white noise stimulus was delivered through headphones. During S1, this noise stimulus occurred during 9 of 14 videos and 5 of 14 ISIs. During S2, the noise stimulus occurred during 4 of 6 videos and 4 of 6 ISIs each for the old and novel spider. For habituation, each session began with 10 unpredictable repetitions of this noise stimulus. The noise stimulus occurred only during the last 30 s of a video with its latency pseudo-randomly varying between 5 and 26 s into these final 30 s. Similarly, the noise stimulus occurred with a latency of 2–8 s into the 10-sec ISI. Noise latencies were identical for all subjects.

2.4. Psychophysiological measurements

Skin conductance response (SCR), electrocardiography (ECG) and electromyography (EMG) were recorded using the MP150 data acquisition unit (BIOPAC Systems, Inc., Goleta, CA) and BIOPAC AcqKnowledge 3.9.2 software for the Macintosh (see Supplementary methods for hardware details). One event marker indicated the beginning of each video and another the onset of the noise stimulus allowing precise synchronization of each stimulus onset with ongoing physiological recording. Sampling rate was 2000 Hz.

Skin conductance level (SCL), a reliable index of sympathetic activation (Dawson et al., 2007), was recorded using disposable adhesive sensors, separated by 10 mm, attached to the hypothenar surface of the non-dominant hand. Two measures of SCR were computed. First, video-related skin conductance change (Video SCR) was computed by subtracting the mean SCL in microSiemens (μS) during the first 1 s of the video, the typical SCR latency (Dawson et al., 2007), from the maximum SCL during the subsequent 29 s (i.e., before any of the noise-stimuli occurred). Second, noise-related SCR (Noise SCR) was computed by subtracting the mean SCL during the 2 s preceding the noise-stimulus onset from the maximum SCL during sec 2-6 following stimulus onset during videos (Noise-SCR-in-Video) or during ISIs (Noise-SCR-in-ISI). SCRs were square-root transformed and, if the untransformed SCR was negative, the negative sign was retained after calculating the square root of the SCR's absolute value (Orr et al., 2000).

ECG electrodes were attached to the torso over the first intercostal space (right) and below the lowest rib (left). Heart-Rate acceleration (HRA) was defined as the maximum beats per minute (bpm) achieved during the first 30 s of each video (i.e., before any noise stimulus) minus the mean of the initial 2 s of the preceding 10-sec ISI (again before any noise stimulus). A correction for extreme outlying values was applied (see Supplementary methods).

Corrugator supercilli EMG is a reliable index of both overt and covert negative emotion (Bradley and Lang, 2007). Electrode attachment followed Fridlund and Cacioppo (1986). In the majority of participants, impedance was confirmed to be below 10 KOhm (see Supplementary methods). EMG was integrated over a 250-msec time constant and response to each video was calculated by subtracting the mean signal amplitude during the initial 2 s of the 10-sec ISI from the maximum during the first 30 s of the succeeding video.

2.5. Statistical analyses

A single self-report outcome measure, Composite Negative Ratings, was calculated by summing each participant's −10 to +10 ratings of disgust, fearfulness and unpleasantness for each video.

Data were analyzed in four phases (Fig. 1). "Early Exposure" contained responses during videos ("Trials") 1–6 of S1; "Late Exposure," Trials 7–12 of S1; "Old Spider," Trials 1–6 of S2; and "Novel Spider," Trials 7–12 of S2. Although 14 trials were presented in S1 in order to maximally promote extinction learning (Vansteenwegen et al., 2007), videos 13 and 14 were excluded from analyses to balance the nested analysis of variance (ANOVA) design described below.

Composite Negative Ratings, corrugator EMG, HRA and Video SCR were analyzed using 3-factor mixed ANOVA with one between-subjects variable, Group, and 2 within-subject variables, 6 Trials nested in 4 Phases (fewer trials for Noise SCR described in Supplementary methods). Because the inter-session duration differed between experimental and control groups, the Sleep vs. Wake groups and Morning vs. Evening groups were separately compared, however, their phase definitions and analyses were identical.

Fig. 1 illustrates operational definitions of emotional memory processes that were measured for each outcome variable. First, "Extinction Learning" was analyzed in S1 data by 2 separate methods using mixed ANOVA and the between-subjects variable, Group (see Supplementary methods). Next, each outcome variable was analyzed across all 4 phases using 3-factor mixed ANOVA (see above). Each group was then analyzed separately by repeated measures ANOVA and, when there was a significant main effect of Phase, the remaining 3 emotional memory processes were evaluated using post-hoc means comparisons between the 4 Phases. "Extinction Retention" was defined as the maintenance of lowered responding at Old Spider following the inter-session delay. "Response Return" was defined as the increase in responding at Novel Spider relative to Old Spider. "Sensitization" was defined as the degree to which responses at Novel Spider exceeded those to the old spider at Early Exposure during S1 when it was equally "novel." For both Response Return and Sensitization, change in the opposite direction represented "Generalization" of extinction learning.

Of specific interest were instances in which a significant Group × Phase interaction indicated group differences between one or more of the above processes. When such an interaction occurred, the above, within-group post-hoc comparisons of Phase were used to determine which specific processes differed between groups (e.g., occurred in one group but not the other). Such Group × Phase interactions, however, also reflected group differences in response magnitude at specific phases, differences that, in turn, were determined, in part, by individual differences in baseline reactivity. Additionally, the absolute magnitude of responses at baseline (S1) could potentially influence the ability to retain extinction across the delay. Therefore, the above 3-factor mixed ANOVA for each outcome measure was repeated with the inclusion of each subject's maximum S1 response as a covariate. Similarly, S1 differences in Extinction Learning might influence subsequent extinction memory processes. Therefore, when Group main effects or Group × Phase interactions occurred at S1, ANOVA was repeated across all 4 phases using only those individuals showing Absolute Extinction Learning (i.e., Early Extinction−Late Extinction > 0).

The Greenhouse-Geisser correction was applied to all within-subject main effects and their interactions. To examine outcome variables without individuals deviating from advertised inclusion criteria (see Table 1), analyses were repeated excluding these individuals in a step-wise manner (see Supplementary methods).

One-way ANOVA, followed, when significant, by Bonferroni-Dunn post-hoc tests, compared groups' questionnaire (e.g., FSC) results. Mixed ANOVA compared repeated measure questionnaires (e.g., SSS) between groups.

3. Results

3.1. Comparison of group characteristics

As shown in Table 1 and Supplementary results, the four groups showed highly similar spider fear, habitual sleep and psychological trait measures. All groups averaged above 100 on the FSQ and 20 on the SPQ (Table 1) indicative of a highly spider-fearful sample (Muris and Merckelbach, 1996). Notably, when Group main effects were present (NEO-PI-R Extraversion, sleep duration on night before S1), this reflected one control group differing from the remaining groups rather than differences between Sleep and Wake groups (Table 1, Supplementary results). Group similarity also extended to sleepiness (SSS) and state anxiety (STAI-S) measured at the beginning and end of each session (Supplementary results). Collapsing across groups, significantly greater STAI-S at the end vs. beginning of sessions [$F(1,61) = 74.59$, $p < .0001$] confirmed the impact of videos on participants' state anxiety.

3.2. Extinction learning, retention and generalization

Comparisons across both S1 and S2 (e.g., Extinction Retention) are detailed below for each outcome measure. However, Extinction Learning within S1 itself is summarized below with details provided in Supplementary results.

3.2.1. Rating scales

3.2.1.1. Session 1. Across S1, significant Extinction Learning for Composite Negative Ratings was evident in both the experimental (Sleep and Wake) and control (Morning and Evening) groups. However, there was no S1 Group main effect or Group interactions with Phase or Trial (Fig. 2A, Supplementary results).

3.2.1.2. Experimental groups. Across both Sessions, comparing Sleep and Wake groups for Composite Negative Ratings, there was

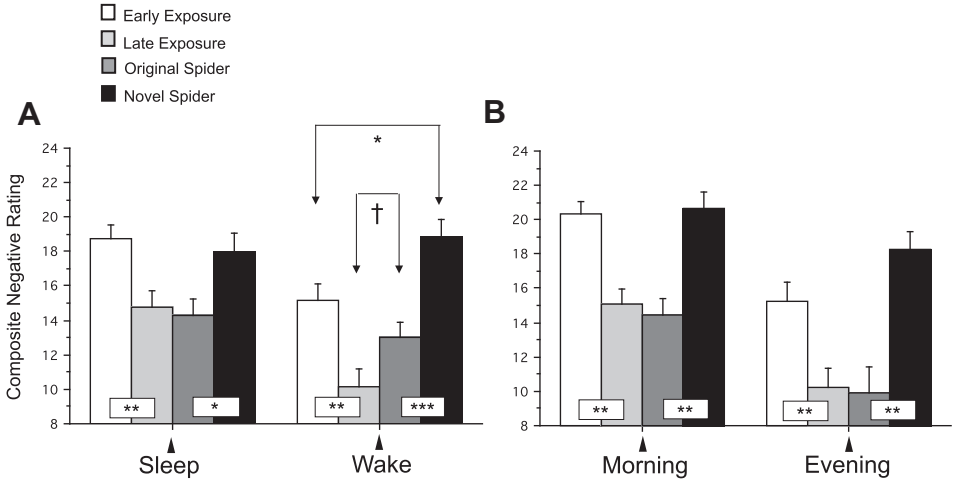

Fig. 2. Composite negative ratings (summed disgust, fear and unpleasantness ratings). A. Experimental (Sleep and Wake) groups. B. Control (Morning and Evening) Groups. Arrows and boxes superimposed on histograms indicate significance levels for post-hoc means comparisons of phases in separate repeated measures ANOVAs for each group. Arrows indicate means comparisons that produced differing results in the two groups when analyzed separately. Boxes indicate Extinction Learning in S1 (see Supplementary results) and Response Return in S2. Bars are standard error of the mean. †$p < .10$, *$p < .05$, **$p < .01$, ***$p < .01$.

a significant Group × Phase interaction [$F(3,123) = 3.43$, $p = .026$] that remained significant when maximum S1 Composite Negative Rating was included as a covariate [$F(3,120) = 2.99$, $p = .044$]. Main effects of Phase occurred in both Sleep [$F(3,57) = 6.23$, $p = .004$] and Wake [$F(3,66) = 14.45$, $p \leq .0001$] groups. Both groups showed significant Response Return during S2 with ratings during Novel Spider significantly greater than during Old Spider (Sleep: $F = 8.47$, $p = .012$; Wake: $F = 18.38$, $p = .0004$, Fig. 2A). However, only in the Wake group was Old Spider at S2 rated more negatively than at Late Exposure during S1 ($F = 4.35$, $p = .055$, Fig. 2A). Similarly, only in the Wake group was Novel Spider in S2 rated more negatively than was the old spider at Early Exposure during S1 when it was equally novel ($F = 7.23$, $p = .017$, Fig. 2A). Therefore, for Composite Negative Ratings, only in the Wake group was there incomplete Extinction Retention at Old Spider and significant Sensitization at Novel Spider.

3.2.1.3. Control groups. Comparing Composite Negative Ratings in the Morning and Evening (control) groups across both sessions, the Group × Phase interaction that was observed when comparing Sleep and Wake groups was absent [$F(3,63) = 0.38$, $p = p = .74$]. Both control groups showed significant Response Return as well as full Extinction Retention and no Sensitization (Fig. 2B).

3.2.2. Physiological measures

3.2.2.1. Video SCR

3.2.2.1.1. Session 1. Across S1, significant Extinction Learning in Video SCR was evident in both Sleep and Wake groups (Fig. 3A, Supplementary results). However, Extinction Learning was more pronounced in the Sleep relative to the Wake group as evidenced by a Group × Phase interaction trend [$F(1,38) = 3.48$, $p = .07$].

3.2.2.1.2. Experimental groups. Across both Sessions, comparing Sleep and Wake groups for Video SCR (Fig. 3A), there was a significant Group × Phase interaction [$F(3,114) = 3.16$, $p = .035$] that remained significant when maximum S1 Video SCR was included as a covariate [$F(3,111) = 3.20$, $p = .034$]. (Further analyses of the effects of the S1 Group × Phase interaction on S2 performance for Video SCR are provided in Supplementary results.) Main effects of Phase occurred in both Sleep [$F(3,51) = 12.57$, $p < .0001$] and Wake [$F(3,63) = 6.68$, $p = .002$] groups. Response Return during S2 was significant in the Sleep group ($F = 12.53$, $p = .001$) and a trend in the Wake group ($F = 3.03$, $p = .095$). However, only in the Wake group was Video SCR to Old Spider during S2 greater than at Late Exposure during S1 ($F = 6.97$, $p = .016$). This loss of extinction memory for the old spider across the inter-session interval in the Wake group may account for this group's lesser Response Return (i.e., the S2 old vs. novel comparison) because responding was already elevated to the Old Spider when Novel Spider was introduced. Notably, despite a lesser increase relative to Old Spider, only in the Wake group was Video SCR to Novel Spider in S2 greater than it was to the old spider during S1 at Early Exposure when it was equally novel ($F = 7.08$, $p = .015$). Therefore, for Video SCR, only in the Wake group was there incomplete Extinction Retention and significant Sensitization.

3.2.2.1.3. Control groups. In Morning and Evening groups (Fig. 3B), there was no Group × Phase interaction for Video SCR across both sessions ($p = .52$).

3.2.2.2. Noise SCR

3.2.2.2.1. Session 1. Across S1, Extinction Learning, as measured by Noise-SCR-in-Video, occurred in all 4 groups (Fig. 4 A, C). In the experimental groups, S1 Group main effect and Group × Phase interaction trends also occurred (Supplementary results).

3.2.2.2.2. Experimental groups. Across both sessions, comparing Noise-SCR-in-Video for Sleep and Wake groups, there was a significant Group × Phase interaction ($F[3,114] = 6.00$, $p = .002$). Although this interaction was due, in part, to the above baseline Group differences, when each subject's maximum S1 Noise-SCR-in-Video was added as a covariate to the mixed ANOVA model, the Group × Phase interaction remained significant ($F[3111] = 3.87$, $p = .017$). (Two further analyses of the effects of these S1 group differences on S2 Noise-SCR-in-Video are provided in Supplementary results.) Decomposing this interaction, the Sleep group showed a main effect of Phase [$F(3,51) = 18.73$, $p < .0001$] but this was absent in the Wake group ($p = .38$). In the Sleep group, there was a trend for Noise-SCR-in-Video at Old Spider to further decrease relative to Late Exposure ($F = 4.11$, $p = .056$, Fig. 4A). Additionally, at Novel Spider, Noise-SCR-in-Video was significantly lower than at Early Exposure when the old spider was equally novel ($F = 46.65$, $p < .0001$, Fig. 4A).

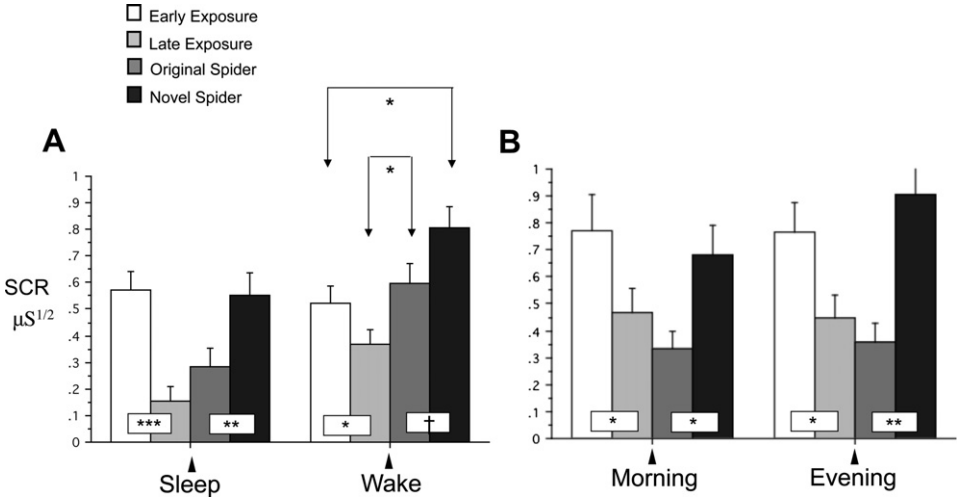

Fig. 3. Video SCR computed by subtracting the mean SCL during the first 1 s following video onset (the latency for SCR) from the highest SCL achieved during the following 29 s of video (during which the noise stimulus never occurred). A. Experimental (Sleep and Wake) groups. B. Control (Morning and Evening) Groups. Arrows indicate means comparisons that produced differing results in the two groups analyzed separately. Boxes indicate Extinction Learning in S1 (see Supplementary results) and Response Return in S2. Bars are standard error of the mean. †$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$, $\mu S^{1/2}$ square-root transformed SCR in micro-Siemens.

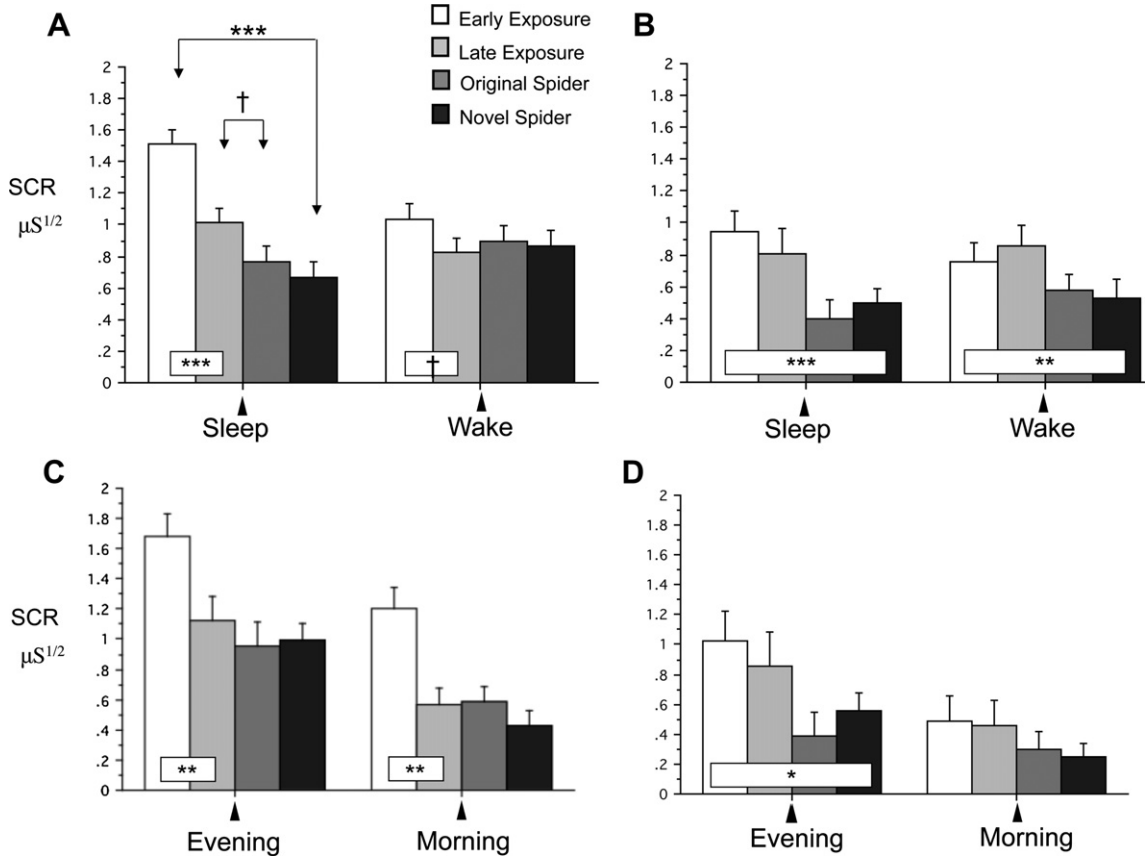

Fig. 4. Noise SCR to 10 msec, 83 dB white noise played through headphones during 60% of videos and ISIs. A. Noise-SCR-in-Video in experimental (Sleep and Wake) groups. B. Noise SCR-in-ISI in experimental groups. C. Noise-SCR-in-Video in control (Morning and Evening) groups. D. SCR-in-ISI in control groups. Arrows indicate means comparisons that produced differing results in the two experimental groups analyzed separately. Boxes indicate Extinction Learning in S1 (see Supplementary results) and Response Return in S2. Bars are standard error of the mean. †$p < .10$, *$p < .05$, **$p < .01$, ***$p < .01$, $\mu S^{1/2}$ square-root transformed SCR in micro-Siemens. (Because noise-containing portions of physiological recordings were intact for all evening subjects, $N = 12$ vs. 11 For other physiological measures in this group.)

Therefore, among experimental participants, only the Sleep group showed further augmentation of Extinction Retention for Noise-SCR-in-Video across the inter-session delay as well as "negative" Sensitization (i.e., Generalization) (Fig. 4A). That observed differences between Sleep and Wake groups were specific to noises presented during spider videos was indicated by the lack of such group effects for Noise-SCR-in-ISI (Fig. 4B, see Supplementary results).

3.2.2.2.3. Control groups. Analysis of Morning and Evening groups across both sessions showed no Group × Phase interaction for Noise-SCR-in-Video ($p = .72$, Fig. 4C) or Noise-SCR-in-ISI ($p = .49$, Fig. 4D).

3.2.2.3. Heart rate acceleration

3.2.2.3.1. Session 1. Across S1, significant Extinction Learning for HRA was evident in experimental and control groups (Fig. 5A, and see Supplementary materials). However, there was no S1 Group main effect or Group interactions with Phase or Trial.

3.2.2.3.2. Experimental groups. Across both sessions, comparing HRA in Sleep and Wake groups, there was a significant Group-× Phase interaction [$F(3,111) = 3.42$, $p = .025$] that remained significant when maximum S1 HRA was included as a covariate [$F(3,108) = 2.96$, $p = .042$]. There was a main effect of Phase in the Sleep [$F(3,51) = 4.89$, $p = .007$] but not the Wake ($p = .39$) group. In the Sleep group, HRA at Novel Spider was significantly lower than at Early Exposure when the old spider was equally novel ($F = 7.74$, $p = .01$, Fig. 5A). Therefore, for HRA in the Sleep group, there was "negative" Sensitization (i.e., Generalization) whereas, in the Wake group, phases did not significantly differ.

3.2.2.3.3. Control groups. In Morning and Evening groups, there was no Group × Phase interaction for HRA across both sessions ($p = .78$; Fig. 5B).

3.2.2.4. Corrugator EMG

Across S1, no significant Extinction Learning for corrugator EMG was evident in experimental or control groups (Fig. 6A, B, Supplementary results). Across both sessions, neither the experimental nor control groups showed a Group × Phase interaction ($p = .75$ and 0.57 respectively) or a Group main effect ($p = .39$ and 0.22). However, both the experimental and control groups showed Response Return and Sensitization (Fig. 6A, B, Supplementary results).

4. Discussion

In spider-fearing young adult women, simulated exposure therapy acutely diminished both subjective and physiological measures of negative affect. Following a 12-hr delay, those exposed in the evening, who then slept, showed better extinction retention and generalization when tested in the morning than those exposed in the morning who remained awake until tested in the evening. The majority of findings remained unchanged after exclusion of participants with potentially confounding factors (see

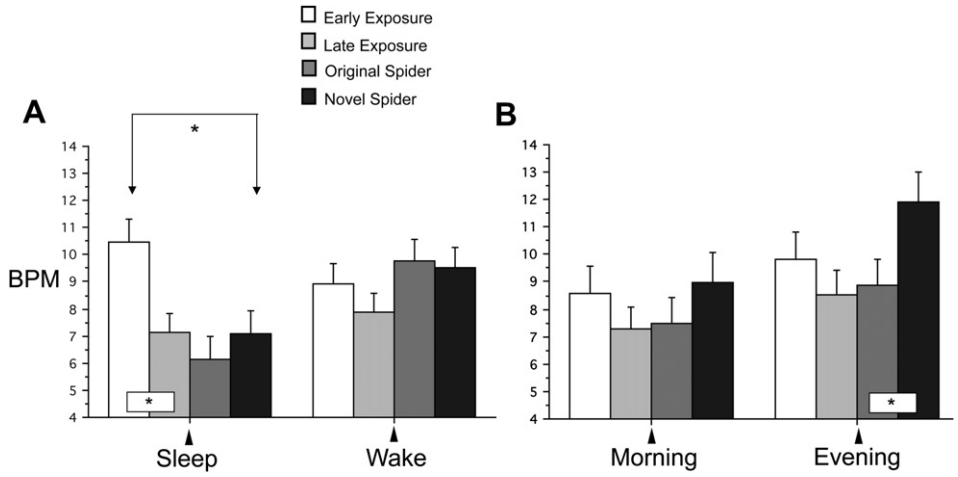

Fig. 5. Heart-Rate acceleration (HRA) in response to spider video relative to preceding ISI. Heart rate sampled from both ISI and video during initial period in which noise stimulus did not occur (first 2 and 30 s respectively). A. Experimental (Sleep and Wake) groups. B. Control (Morning and Evening) groups. Arrows indicate means comparisons that produced differing results in the two groups analyzed separately. Boxes indicate Extinction Learning in S1 (see Supplementary results) and Response Return in S2. Bars are standard error of the mean. $^{*}p < .05$, BPM beats per minute.

Supplementary results) and results from control groups are inconsistent with a circadian explanation of experimental group differences (see below).

4.1. Circadian vs. sleep-dependent processes

Because, among control groups, those both exposed and tested in the morning did not differ from those exposed and tested in the evening, intervening sleep rather than circadian effects better explains the differences between Sleep and Wake groups seen in 4 of 5 outcome variables. Nonetheless, certain circadian effects were evident. Most notably, at S1, the evening-exposed groups displayed greater Noise-SCR-in-Video than the morning-exposed groups (see Supplementary results) – a diurnal pattern of electrodermal reactivity that has previously been described for SCR to emotional images (Hot et al., 2005).

We chose Morning and Evening groups as circadian controls for two main reasons. First, when measuring physiological parameters as expression of emotional memory, major concerns are possible circadian effects upon these same parameters independent of memory processes (as noted for SCR). Because we define emotional memory processes by intra-group changes in each outcome variable (see Methods), the control groups tested whether time-of-day might influence changes in outcome variables from one phase to another despite having maintained the same behavioral state (wake) across the delay. Second, we ruled out use of an alternative 24-h circadian control (i.e., AM to AM and/or PM to PM) because work in animals has shown that extinction memory consolidation, pharmacologically blocked for 24 h, can consolidate across the subsequent 24 h (Santini et al., 2001). Therefore, in the current study, 24-h controls would confound the duration of wake prior to sleep with the effects of sleep itself.

Rather than circadian effects, we hypothesize that sleep-dependent processes allow new emotional learning, such as extinction, to influence pre-existing memories. A network of limbic and paralimbic, fear-related anatomic regions have repeatedly been shown to be activated by *de-novo* fear conditioning (Alvarez et al., 2008; LaBar et al., 1998), extinction learning for such conditioning (Knight et al., 2004; Phelps et al., 2004) and recall of memory for that extinction (Kalisch et al., 2006; Milad et al., 2007). A recent meta-analysis has shown greater amygdala and insula activity

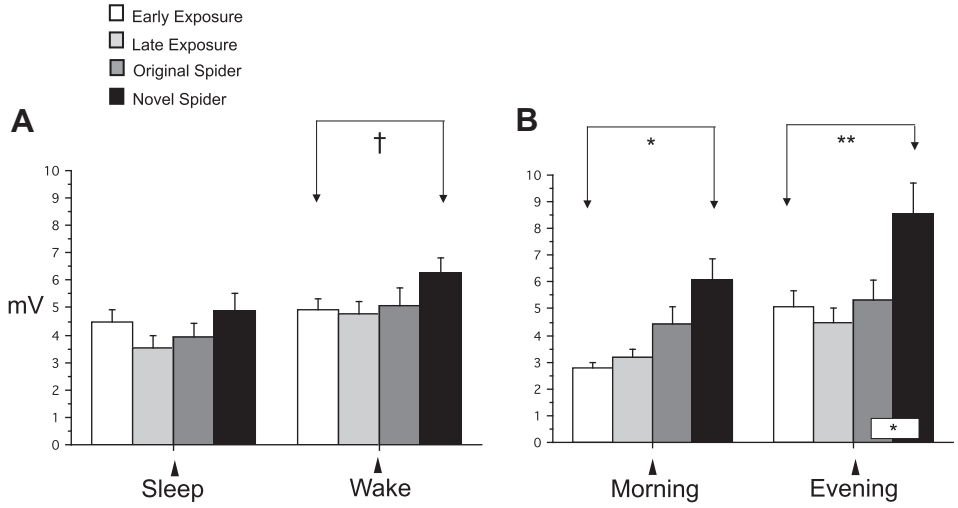

Fig. 6. Corrugator supercilii EMG in response to spider video relative to preceding ISI. EMG sampled from both ISI and video during initial period in which noise stimulus did not occur (first 2 and 30 s respectively). A. Experimental (Sleep and Wake) groups. B. Control (Morning and Evening) Groups. Arrows indicate Sensitization. Box indicates Response Return. Bars are standard error of the mean. $\dagger p < .10$, $^{*}p < .05$, $^{**}p < .01$, mV millivolts.

during emotional processing tasks in individuals with PTSD, Social Phobia and Specific Phobia compared with controls, suggesting underlying similarity in neural circuits affected by these anxiety disorders (Etkin and Wager, 2007). The amygdala and insula are components of an "anterior paralimbic REM activation area" that selectively reactivates during REM (Nofzinger et al., 2004) providing opportunity during sleep for new emotional learning, such as extinction, to be integrated with preexisting fear-related memory.

4.2. Extinction and habituation

Habituation and extinction, are overlapping forms of learning (McSweeney and Swindell, 2002) and its underlying neuroplasticity (Storvse et al., 2010). However, whereas habituation is a non-associative learning process whereby behavioral and physiological responses during initial exposure to a stimulus diminish with its repeated presentation (Grissom and Bhatnagar, 2009; Leussis and Bolivar, 2006), extinction constitutes new associative learning whereby a stimulus previously associated with danger also becomes associated with the absence of danger (Hermans et al., 2006). During exposure therapy, habituation learning occurs concurrently with extinction. For example, in the current study, *intra*-session habituation undoubtedly contributed to diminishing responses across S1 as well as, possibly, *inter*-session habituation to such diminution in SCR across the delay (see Pace-Schott et al., 2011). However, extinction learning is a key contributor to reduction of fear in exposure therapy (Hermans et al., 2006; McNally, 2007; Milad et al., 2006) and may be more important than habituation for the therapeutic efficacy of this treatment (Craske et al., 2008). Similarly, although declarative and vicarious learning may have contributed to participants' original spider fear, we assume that fear conditioning also played a role in its origin (Armfield, 2006; Fyer, 1998; Mineka and Oehlberg, 2008; Rachman, 2002). Other emotional processes that may have contributed to inter-individual variability in subjective and objective responses are considered in Supplementary discussion.

4.3. Clinical significance

Sleep-dependent generalization of extinction memories (Pace-Schott et al., 2009) suggests that conducting exposure therapy sessions in temporal proximity to sleep may enhance its efficacy. The current findings suggest an additional mechanism by which sleep might enhance exposure treatment, protection from sensitization. Sensitization-like processes play a role in the perpetuation of anxiety disorders. One such process is fear generalization, in which fear of a conditioned stimulus specifically associated with an inherently aversive stimulus comes to be elicited by stimuli that resemble but are not identical to the original conditioned stimulus (Lissek et al., 2008). A second such process is secondary conditioning whereby fear of a previously neutral stimulus is established by its association with a stimulus that was itself associated with the original, inherently aversive stimulus or event. Increased secondary conditioning has been demonstrated both in PTSD (Wessa and Flor, 2007) and in spider phobia (Schweckendiek et al., 2011).

5. Conclusions

A period of sleep following simulated exposure therapy resulted in better retention and generalization of subjective and physiological responses to phobic stimuli. Sleep may confer protection from sensitization to such stimuli. Findings encourage further clinical investigation into the timing of exposure therapy in relation to sleep.

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Contributors

Edward F. Pace-Schott designed experiments, designed stimuli, collected and analyzed data, and wrote manuscript. Patrick W. Verga collected and analyzed data. Tobias S. Bennett designed stimuli and collected and analyzed data. Rebecca M.C. Spencer analyzed data and wrote manuscript.

Conflict of interest

None of the authors report any potential conflicts of interest.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.jpsychires.2012.04.015.

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