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Exposure to extinction-associated contextual tone during slow-wave sleep and wakefulness differentially modulates fear expression

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ABSTRACT

Recent research has used context cues (odor or auditory cues) to target memories during sleep and has demonstrated that they can enhance declarative and procedural memories. However, the effects of external cues re-presented during sleep on emotional memory are still not fully understood. In the present study, we conducted a Pavlovian fear conditioning/extinction paradigm and examined the effects of re-exposure to extinction memory associated contextual tones during slow-wave sleep (SWS) and wakefulness on fear expression. The participants underwent fear conditioning on the first day, during which colored squares served as the conditioned stimulus (CS) and a mild shock served as the unconditioned stimulus (US). The next day, they underwent extinction, during which the CSs were presented without the US but accompanied by a contextual tone (pink noise). Immediately after extinction, the participants were required to take a nap or remain awake and randomly assigned to six groups. Four of the groups were separately exposed to the associated tone (i.e. SWS-Tone group and Wake-Tone group) or an irrelevant tone (control tone, CtrT) (i.e. SWS-CtrT group and Wake-CtrT group), while the other two groups were not (i.e. SWS-No Tone group and Wake-No Tone group). Subsequently, the conditioned responses to the CSs were tested to evaluate the fear expression. All of the participants included in the final analysis showed successful levels of fear conditioning and extinction. During the recall test, the fear responses were significantly higher in the SWS-Tone group than that in the SWS-No Tone group or the SWS-CtrT group, while the Wake-Tone group exhibited more attenuated fear responses than either the Wake-No Tone group or Wake-CtrT group. Otherwise, re-exposure to auditory tones during SWS did not affect sleep profiles. These results suggest that distinct conditions during which re-exposure to an extinction memory associated contextual cue contributes to differential effects on fear expression.

1. Introduction

Fear conditioning occurs when an originally neutral conditioned stimulus (CS) is associated with an inherently aversive unconditioned stimulus (US), and the conditioned fear response is elicited by the presence of CS after conditioning. Extinction occurs when the CS is presented repeatedly in the absence of the US (Bouton, 1993; Herry, Ferraguti, Singewald, Letzkus, Ehrlich, & Luthi, 2010). Previous studies have shown that extinction creates a new safety association rather than erasing conditioned fear (Myers & Davis, 2002; Quirk & Mueller, 2008), as evidenced by the return of fear following the passage of time (spontaneous recovery), following presentation of the US (reinstatement), or when cues were

Abbreviations: AASM, American Academy of Sleep Medicine; ANOVA, analysis of variance; BDI, Beck Depression Inventory; BMI, Body Mass Index; CS, conditioned stimulus; CtrT, control tone; EEG, electroencephalography; EMG, electromyography; EOG, electrooculography; ESS, Epworth Sleepiness Scale; FFTs, fast-Fourier transforms; HAMA, Hamilton Anxiety Scale; PSG, polysomnography; PSQI, Pittsburgh Sleep Quality Index; REM, rapid-eye-movement; SCR, skin conductance response; SSS, Stanford Sleepiness Scale; SWS, slow-wave sleep; TMR, targeted memory reactivation; US, unconditioned stimulus.

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encountered outside the extinction context (renewal) (Bouton, Westbrook, Corcoran, & Maren, 2006; Ji & Maren, 2007). Therefore, fear expression depends on the competition between reactivated memory from conditioning and reactivated memory from extinction.

Recent research suggests that sleep benefits memory consolidation (Maquet, 2001; Rasch & Born, 2007; Stickgold, 2005). A large body of evidence indicates that sleep promotes both declarative and procedural memory consolidation in various tasks (Marshall & Born, 2007; Smith, 2001; Walker, Brakefield, Hobson, & Stickgold, 2003). Compared with an equal duration of wakefulness, post-learning sleep enhances the retention of declarative memory and improves performance in procedural skills (Plihal & Born, 1997; Walker et al., 2003). Additionally, sleep and emotional memory have broad associations. Sleep contributes to emotional memory learning, including facilitating the acquisition of negative memory, promoting habituation to emotional stimuli, and improving the retention of previously encoded emotional information (Pace-Schott et al., 2009, 2011; Payne, Stickgold, Swanberg, & Kensinger, 2008). Moreover, sleep is considered to be a specific state during which memory-related arousal decreases, and it has been proposed that rapid eye movement (REM) sleep after an emotional experience can reduce the emotional response, but strengthen the content of the respective representations in memory (Walker, 2009). However, there are several reports that do not support this hypothesis. For example, one study showed that emotional reactivity to previously viewed aversive pictures was amplified after REM sleep-rich sleep (Wagner, Fischer, & Born, 2002), while selective REM sleep deprivation reduced arousal ratings to negative pictures on the next morning (Lara-Carrasco, Nielsen, Solomonova, Levrier, & Popova, 2009). Overall, these studies indicate that sleep plays a critical role in the modulation and integration of emotional memories (Walker, 2008; Walker, 2009; Wamsley & Stickgold, 2010), suggesting that sleep may be a good target for manipulating fear memory expression.

Recent studies have reported a role for targeted memory reactivation (TMR) which is applied during sleep, especially slow-wave sleep (SWS), in memory regulation (Antony, Gobel, O'Hare, Reber, & Paller, 2012; Rasch, Buchel, Gais, & Born, 2007; Rudoy, Voss, Westerberg, & Paller, 2009). Participants exhibited enhanced declarative memory retention after re-exposure to an odor or auditory cue that had been presented during prior learning during SWS but not REM sleep or wakefulness (Rasch et al., 2007; Rudoy et al., 2009). Concerning the effect of cued fear memory reactivation during sleep on fear expression, two animal studies showed that re-presenting an odor cue during SWS distinctly increased fear response (Barnes & Wilson, 2014; Rolls et al., 2013). However, it has been demonstrated that re-exposure to an odor cue during SWS reduced the fear response in human subjects who underwent an olfactory contextual fear conditioning paradigm (Hauner, Howard, Zelano, & Gottfried, 2013). Our recent study also showed that CS re-exposure during SWS promoted fear memory extinction (He et al., 2015). Overall, these results further demonstrate that new memories, including emotional memories, are highly labile and susceptible to modulation during SWS. Formation of extinction memories is presumed to be the neurocognitive basis for the efficacy of exposure therapy, a first-line behavioral treatment for anxiety disorders (McNally, 2007). However, to our knowledge, there have been no examinations of sleep-based extinction memory reactivation. In the present study, we conducted fear conditioning and extinction training to determine the effects of re-exposure to an extinction memory-associated contextual cue during SWS and wakefulness on fear expression.

2. Methods

2.1. Participants

One hundred and forty-nine undergraduate students were recruited through advertisements. During the week before the experiment, all of the participants were required to fill out a sleep diary. They all slept habitually for 1/2–2 h in the afternoon. Subjects were excluded if they had current sleep-related issues, including shift work within 1 week prior to the experiment, a history of any major mental disorders or physical diseases, < an average of 6 h of sleep per night, or excessive caffeine or alcohol consumption (i.e. at most five alcoholic beverages per week and one cup of coffee or one caffeine-containing beverage per day). During the study, ten subjects were excluded because of an inadequate level of fear conditioning or fear extinction. The exclusion criteria were based on differential responses to the CS^+ and CS^- in the second half of acquisition and extinction. That is, subjects were excluded if during acquisition the difference was in the opposite direction ($CS^- > CS^+$) or smaller than 0.1 μs. Subjects also were excluded if during extinction the difference was in the opposite direction ($CS^+ > CS^-$) or larger than 0.1 μs (Raio, Brignoni-Perez, Goldman, & Phelps, 2014; Schiller et al., 2010). Four subjects who reported noticing the auditory tones during sleep were excluded from the final analysis. Two subjects also were excluded because tone re-exposure occurred during sleep stage 2, based on the offline manual sleep stage analysis. A final total of 133 subjects were included in our analysis (76 females; age, 23.3 ± 2.10 years). To avoid the influences of menstrual cycle on memory (Milad et al., 2006), we only included females who were in the early follicular phase (i.e. at days 0–4 after onset of menstruation) when they participated in the study (Bayer, Schultz, Gamer, & Sommer, 2014). The ratios of females to males in each group are presented in Table 1. Each subject provided written informed consent and was paid 150 RMB (equivalent to USD $24) upon completion of the study. The study protocol was approved by the Institutional Review Board of Peking University.

Table 1
Demographic data, psychological traits, and sleepiness evaluation in the six groups.

	SWS-No Tone (n = 24)	SWS-Tone (n = 24)	SWS-CtrT (n = 22)	Wake-No Tone (n = 21)	Wake-Tone (n = 21)	Wake-CtrT (n = 21)
Age (years)	23.3 ± 1.9	23.1 ± 2.4	23.7 ± 1.7	23.7 ± 1.4	23.1 ± 1.7	23.4 ± 2.3
Male (n (%))	10 (41.7)	12 (50.0)	7 (31.8)	9 (42.9)	11 (52.4)	8 (38.1)
BMI (kg/m^2)	21.6 ± 2.7	21.5 ± 3.3	20.9 ± 2.9	21.3 ± 2.1	21.1 ± 2.8	21.2 ± 2.2
Education (years)	16.7 ± 1.7	16.5 ± 1.7	17.0 ± 2.0	16.5 ± 1.9	16.2 ± 1.6	16.6 ± 1.5
HAMA score	3.21 ± 3.79	4.04 ± 3.86	4.95 ± 4.65	3.57 ± 3.33	4.14 ± 3.78	3.00 ± 3.63
BDI score	1.79 ± 1.91	2.67 ± 3.12	2.82 ± 4.22	1.67 ± 2.44	1.67 ± 1.62	1.90 ± 2.43
ESS score	6.88 ± 3.14	6.17 ± 3.33	8.45 ± 3.78	6.95 ± 3.81	6.67 ± 2.85	6.29 ± 2.49
PSQI score	4.21 ± 2.19	4.50 ± 2.43	5.68 ± 2.38	3.81 ± 1.99	4.43 ± 2.38	4.76 ± 2.62

The data are expressed as mean ± standard deviation.
BMI = Body Mass Index; HAMA = Hamilton Anxiety Scale; BDI = Beck Depression Inventory; ESS = Epworth Sleepiness Scale; PSQI = Pittsburgh Sleep Quality Index.