Neurobiology of Learning and Memory 132 (2016) 9–17

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Sleep supports cued fear extinction memory consolidation independent of circadian phase

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ARTICLE INFO

Article history:
Received 4 March 2016
Revised 6 April 2016
Accepted 19 April 2016
Available online 19 April 2016

Keywords:
Fear extinction
Memory consolidation
Circadian phases
Sleep
Sleep deprivation

ABSTRACT

Sleep promotes memory, particularly for declarative learning. However, its role in non-declarative, emotional memories is less well understood. Some studies suggest that sleep may influence fear-related memories, and thus may be an important factor determining the outcome of treatments for emotional disorders such as post-traumatic stress disorder. Here, we investigated the effect of sleep deprivation and time of day on fear extinction memory consolidation. Mice were subjected to a cued Pavlovian fear and extinction paradigm at the beginning of their resting or active phase. Immediate post-extinction learning sleep deprivation for 5 h compromised extinction memory when tested 24 h after learning. Context-dependent extinction memory recall was completely prevented by sleep-manipulation during the resting phase, while impairment was milder during the active phase and extinction memory retained its context-specificity. Importantly, control experiments excluded confounding factors such as differences in baseline locomotion, fear generalization and stress hormone levels. Together, our findings indicate that post-learning sleep supports cued fear extinction memory consolidation in both circadian phases. The lack of correlation between memory efficacy and sleep time suggests that extinction memory may be influenced by specific sleep events in the early consolidation period.

1. Introduction

Learned fear responses enable the organism to adapt to dangerous situations and increase changes for survival. However, it is equally important to extinguish fear to stimuli and situations that do not predict danger anymore. Failure to do so underlies pathologies such as phobias and post-traumatic stress disorder (PTSD) (Mahan & Ressler, 2012; Pitman et al., 2012). Pavlovian fear conditioning and extinction are widely used laboratory models to investigate fear memories in humans and rodents (Maren, 2001; Pape & Pare, 2010; Phelps & LeDoux, 2005). In cued fear conditioning, an initially neutral conditioned stimulus (CS) is associated with an aversive unconditioned stimulus (US), and can later alone evoke a conditioned fear response. Fear extinction learning occurs when the CS is repeatedly presented without the US, leading to a disconfirmation of the danger expectation and a decrease in acquired fear (Myers & Davis, 2007; Quirk & Mueller, 2008). Fear can resurface after a shift of context (renewal), with the passage of time (spontaneous recovery), or a re-exposure to the US (reinstatement), indicating that extinction creates a new competing memory that suppresses the original fear memory (Bouton, Westbrook, Corcoran, & Maren, 2006; Maren, 2011). Clinically, extinction learning forms the basis for behavioral exposure therapies to treat phobias and PTSD in humans (VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). Therefore, it is important to understand circumstances under which extinction memories can lead to a more efficient inhibition of fear.

A reciprocally connected network, including the amygdala, the ventromedial prefrontal cortex (vmPFC), and the hippocampus plays a major role in fear extinction in both humans and rodents (Herry et al., 2010; Maren & Quirk, 2004). While the amygdala has an important role in both fear and extinction memories, the vmPFC plays is major role in extinction recall, due to its inhibitory action upon the amygdala activation (Marek, Strobel, Bredy, & Sah, 2013; Paré, Quirk, & Ledoux, 2004). Additionally, the hippocampus is believed to contribute contextual information and is implicated in context-dependent fear renewal (Ji & Maren, 2007; Orsini, Kim, Knapska, & Maren, 2011).

After the initial acquisition, memory traces are stabilized, a process referred to as consolidation. The consolidation period may extend from several hours until days or weeks, where a number of molecular, synaptic, cellular, and network alterations occur, that support durable and resistant long-term memories (Abel & Lattal,

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http://dx.doi.org/10.1016/j.nlm.2016.04.007
1074-7427/

2001). A large body of work in humans and animals indicates that sleep supports memory in multiple learning tasks (Rasch & Born, 2013; Smith, 1996; Walker & Stickgold, 2004). Sleep may affect memory formation through modulation of consolidation-related plasticity mechanisms and reactivation of neural ensembles formed during recent learning experiences (Abel, Havekes, Saletin, & Walker, 2013; Walker & Stickgold, 2004). Different sleep stages have been postulated to preferentially support consolidation of specific memories. A prominent role for slow wave sleep (SWS) and SWS-related events has been demonstrated for declarative memories, while rapid eye movement (REM) sleep and REM-related events are thought to preferentially support emotional memories (Diekelmann & Born, 2010; Genzel, Spoormaker, Konrad, & Dresler, 2015; Walker & van der Helm, 2009). However, some data suggest that memory consolidation for different tasks may benefit from both SWS and REM sleep (Ackermann & Rasch, 2014; Hauner, Howard, Zelano, & Gottfried, 2013).

There is growing evidence that sleep plays a role in fear-related memories. Fear learning is accompanied by significant changes in sleep architecture (Hellman & Abel, 2007; Kumar & Jha, 2012; Sanford, Tang, Ross, & Morrison, 2003), and a comorbidity is observed among people who suffer from sleep disorders and anxiety disorders, such as PTSD (Spoormaker & Montgomery, 2008). Several studies now converge on suggesting that post-learning sleep supports consolidation of contextual (Graves, Heller, Pack, & Abel, 2003; Hagewoud et al., 2010) and cued fear memories (Kumar & Jha, 2012; Menz et al., 2013). However, how sleep affects fear extinction consolidation is still unclear. In rodents, REM sleep deprivation before or after training impaired either extinction learning or recall (Fu et al., 2007; Silvestri, 2005). In humans, post-learning sleep supported cue generalization in extinction (Pace-Schott et al., 2009), and in rats, post-learning REM sleep related events positively correlated with contextual extinction memory (Datta & O'Malley, 2013).

As the majority of studies were performed in the resting phase, a key open question is the role of sleep in fear and extinction consolidation during different circadian phases. This is highly relevant for understanding the outcome of exposure therapies that are typically performed during the daytime active phase in humans. For example, cued fear memory was impaired with 6 h of sleep deprivation during the active phase, while the same manipulation was insufficient to impair contextual fear memory (Hagewoud et al., 2010; Kumar & Jha, 2012). Currently, nothing is known about the role of sleep in consolidation of fear extinction memory during the active phase. Therefore, we asked how post-learning sleep affects cued fear extinction recall during different circadian phases in mice, by applying sleep deprivation during the resting or active phase. Our data suggest that sleep supports consolidation of cued extinction memory independent of circadian phase.

2. Material and methods

2.1. Animals and housing conditions

Wild type C57BL/6J male mice (7–9 weeks old) were bred in house (breeders purchased from Harlan, Netherlands). Animals were group-housed with food and water ad libitum, under a 12:12 h light/dark cycle (lights on at 7:00 am), with a room temperature of 21–23 °C and 55–65% humidity. One week before the experiment, animals were individually housed and submitted to 4 or 5 handling sessions (one session per day), in the beginning of their resting or active phase. All procedures were in accordance with the EU directive on use of animals in research and approved by the Regierungspraesidium Tuebingen, state of Baden-Wuerttemberg, Germany.

2.2. Experimental design

Animals were trained in a Pavlovian fear conditioning and extinction paradigm with a total duration of 3 days (Fig. 1, top). The experimental design consisted of four experimental groups: Two groups were trained at the beginning of their resting phase at 8:00 am (sleep resting phase (SL-R) and sleep deprived resting phase (SD-R)), and the other two groups were trained at the beginning of their active phase at 8:00 pm (sleep active phase (SL-A) and sleep deprived active phase (SD-A)). Immediately following extinction learning, one group from the resting (SD-R) and one from the active phase (SD-A) were sleep deprived for 5 h by gentle handling, while the corresponding control groups were allowed to sleep undisturbed (SL-R and SL-A; Fig. 1, bottom).

2.3. Behavioral protocols

2.3.1. Fear conditioning

Discriminative fear conditioning was performed on the day 1 in context A, consisting of a square arena (625 cm^2) with a grid floor and transparent plexiglass walls, cleaned with 70% ethanol and illuminated with dim white light (17 Lux). After an exploration period of 120 s, 5 conditioned stimuli (CS+) were paired with unconditioned stimuli (US), interleaved by presentation of non-paired control stimuli (CS−). The CS+ was a 30 s auditory stimulus (7.5 kHz tone, 80 dB sound pressure level). The CS− was a different 30 s auditory stimulus (white noise, 80 dB sound pressure level). Both auditory stimuli were composed of 50 ms pips repeated at 0.9 Hz (Asede, Bosch, Luthi, Ferraguti, & Ehrlich, 2015; Herry et al., 2008). Stimuli were delivered at random inter-stimulus intervals (ISI, 30–120 s). The US was a 1 s scrambled foot shock (0.4 mA) coinciding with the offset of the CS+, delivered by a shocker through a shock floor for mice (Coulbourn Instruments, Whitehall, PA, USA). The entire apparatus was placed in a black soundproof box.

2.3.2. Fear recall and fear extinction learning

Twenty-four h after fear conditioning, animals were tested for fear memory and trained for fear extinction. Fear memory was tested by presentation of 4 CS− followed by 4 CS+, and an additional 8 CS+ were presented in this session to induce extinction. Animals were submitted to a second extinction session with 4 CS− and 12 CS+ after a 30 min interval. All stimuli were delivered at random ISI (30–120 s). Fear memory recall and extinction learning took place in context B, consisting of a round arena (452.16 cm^2) with a smooth transparent floor covered with clean bedding and transparent plexiglass walls, cleaned with 1% acetic acid, and in the presence of dim green light (15 Lux). The full context was placed in a light-gray soundproof box.

2.3.3. Extinction recall and fear renewal

Twenty-four h after fear extinction training, the animals were tested for fear extinction memory in context B by presentation of 4 CS− followed by 4 CS+. One hour after the extinction memory test, animals were tested for fear renewal by presenting 4 CS− followed by 4 CS+. Fear renewal took place in context C, consisting of a square arena (625 cm^2) with a smooth white floor and plexiglass walls with black and white stripes, cleaned with lemon incense, and illuminated by dim white light (17 Lux). The full context was placed in a black soundproof box. In both sessions, CSs were presented at random ISI (30–120 s).

2.3.4. Fear measurement and assessment of learning

Fear behavior was assessed as freezing, which was measured as time in % in which the animals were immobile during the 30 s of CS presentation. Baseline freezing was measured in the 120 s exploration period before any stimulus onset. Pretone freezing was mea-