Published in final edited form as:

Exp Brain Res. 2014 May ; 232(5): 1535–1545. doi:10.1007/s00221-014-3858-4.

Dorsal subcoeruleus nucleus (SubCD) involvement in context-associated fear memory consolidation

Donald F. Siwek^{1,3}, Clifford M. Knapp¹, Gurcharan Kaur^{1,4}, and Subimal Datta^{1,2,*}

¹Laboratory of Sleep and Cognitive Neuroscience, Department of Psychiatry, Boston University School of Medicine, 85 East Newton Street, Suite M-902, Boston, MA, 02118 USA

²Department of Neurology, Boston University School of Medicine, 85 East Newton Street, Suite M-902, Boston, MA, 02118 USA

³Department of Anatomy and Neurobiology, Boston University School of Medicine, 85 East Newton Street, Suite M-902, Boston, MA, 02118 USA

⁴Department of Biotechnology, Guru Nanak Dev University, Amritsar, India

Abstract

The neurobiological mechanisms of emotional memory processing can be investigated using classical fear conditioning as a model system, and evidence from multiple lines of research suggests that sleep influences consolidation of emotional memory. In rodents, some of this evidence comes from a common finding that sleep deprivation from 0 to 6 h after fear conditioning training impairs processing of conditioned fear memory. Here, we show that during a 6-h session of sleep–wake (S–W) recording, immediately after a session of context-associated fear conditioning training, rats spent more time in wakefulness (W) and less time in slow-wave sleep (SWS) and rapid eye movement (REM) sleep. This context-associated fear conditioning training-induced reduction in SWS lasts for 2 h, and the REM sleep reduction lasts throughout the entire 6-h post-training S–W recording period. Interestingly, these reductions in SWS and REM sleep during this 6-h period did not impair memory consolidation for context-associated fear conditioning. The results of this study show, for the first time, that lesions within the dorsal part of the subcoeruleus nucleus (SubCD), which were unintentionally caused by the implantation of bipolar recording electrodes, impair consolidation of context-associated fear conditioning memory. Together, the results of these experiments suggest that emotional memory processing associated with fear conditioning can be completed successfully within less than a normal amount of sleep, but it requires a structurally and functionally intact SubCD, an area in the brain stem where phasic pontine wave (P-wave) generating cells are located.

Keywords

Fear conditioning; Memory consolidation; Wakefulness; Slow-wave sleep; REM sleep; Subcoeruleus nucleus

Introduction

An impressive number of studies in both animals and humans have shown that sleep plays an important role in memory consolidation within multiple memory systems including that of emotional memory (Smith 1995; Bjorness et al. 2005; Hu et al. 2006; Wagner et al. 2006; Walker and Stickgold 2006; Datta 2010; Diekelmann and Born 2010; Poe et al. 2010). Emotional memories of fearful experiences can lead to enduring alterations in behavior, and many investigators have hypothesized that sleep plays a regulatory role in these fearful memories and emotional reactivity (Lavie 2001a, b; Koren et al. 2002; Rosales-Lagarde et al. 2012). Impaired regulation of emotional memory, especially fear memory, which involves remembering contexts that signal safety or threat, is a core symptom of a number of neuropsychiatric disorders, including anxiety disorders, depression, and schizophrenia (Lavie 2001a, b; Anderson and Insel 2006; Beck 2008; Walker and van der Helm 2009; Kring and Caponigro 2010). It is evident that in healthy individuals, adequate sleep is beneficial for processing emotional memories (Wagner et al. 2001; Hu et al. 2006; Holland and Lewis 2007; Baran et al. 2012; Diekelmann and Born 2010), and in individuals with the major neuropsychiatric disorders listed above, such processing is severely impaired (Mandal et al. 1998; Charney 2004; Lissek et al. 2005; Rauch et al. 2006; Soliman et al. 2010). To understand the underlying pathophysiological mechanisms that impair sleep-associated beneficial effects on emotional memory regulation in these neuropsychiatric disorders, it is important to examine the changes in S–W activity at the time of emotional memory consolidation.

A large number of studies in both humans and animals have used a classical fear conditioning (both cued and contextual) paradigm to study the mechanisms of emotional memory processing (LeDoux 1992, 2002; Mahan and Ressler 2011; Pattwell et al. 2012). Fear conditioning occurs when an emotionally neutral stimulus comes to be associated with an inherently aversive experience, thereby acquiring the capability of evoking a fearful response on its own. The aversive experience itself is termed as an unconditioned stimulus (US; such as foot-shock). The initially neutral stimulus, which becomes associated with the aversive experience, is termed as a conditioned stimulus (CS; such as context and/or cue, light or tone), and the subsequent response to it is referred to as a conditioned response (CR; such as freezing behavior in rodents). The examination of the development of conditioned fear memory has proved an ideal starting point for studies of emotional memory for several reasons. First, fear learning occurs in nearly every animal group in which it has been examined including rodents and human (Lazaro-Munoz et al. 2010; LeDoux 2002; Ressler 2010; Rosenkranz et al. 2010). Although we would not claim that the mechanisms are identical in all of these different species, it seems clear from studies to date that the pathways of fear are very similar in all mammals (Fendt and Fanselow 1999; LeDoux 2002; Phelps and LeDoux 2005). Since fear appears to be a well-conserved basic necessity for survival, fear conditioning studies done with rats bear relevance to the processes in humans as well. Another advantage of this type of conditioning is that the stimuli most commonly used are not signals that rats encounter in their daily lives, helping to ensure that the animals have not already developed strong emotional reactions to them. By engendering new emotional reactions, we can be confident that we are looking at valid models of true learning

and memory with minimal confounds from prior experience. Another benefit of these fear learning paradigms in rats is that they involve the amygdala, prefrontal cortex, and hippocampus (Morgan and LeDoux 1995; Fendt and Fanselow 1999; Maren and Quirk 2004; Phelps and LeDoux 2005; Herry et al. 2006; Heldt et al. 2007), but do not require complicated cognitive processing. Consequently, these paradigms provide access to the neurobiological mechanisms of emotional memory processing in a relatively pure form with a low degree of cognitive complexity. A final key feature of fear learning paradigms is that they involve the ability to recognize and remember contexts that signal safety or threat—skills that are of particular interest because they are notably impaired in anxiety disorders (such as post-traumatic stress disorder, panic disorder, and phobias), depression, and schizophrenia (Charney 2004; Lissek et al. 2005; Rauch et al. 2006; Soliman et al. 2010).

The studies investigating the role of sleep in memory can be grouped into three main categories: (1) behavioral studies that have examined how different types of learning affect subsequent sleep architecture and how pre- or post-training deprivation of total and/or specific sleep stages affects memory consolidation; (2) physiological studies that have investigated how neuronal transmission, activity, and plasticity are affected during different stages of sleep immediately after different types of learning; and (3) imaging, pharmacological, and biochemical–molecular studies that have examined the sleep state-specific changes occurring in the brain as a result of different types of learning training (for review and individual references, see Smith 1985, 1995; Datta 2006; Walker and Stickgold 2006). Although a vast number of studies have examined the role of sleep in memory consolidation, few have examined the role of sleep in conditioned fear memory. Human studies have used negative emotional stimuli as well as conditioned fear to demonstrate that the absence of post-training REM sleep impairs consolidation of emotional memories (Wagner et al. 2001; Hu et al. 2006; Payne et al. 2008; Groch et al. 2013). Studies on mice and rats have shown that, immediately after fear conditioning, deprivation of REM sleep or total sleep for 4–6 h impairs conditioned fear memory consolidation (Graves et al. 2003; Silva et al. 2004; Vecsey et al. 2009; Florian et al. 2011; Kumar and Jha 2012). However, if the deprivation period is delayed by more than 6 h, then the consolidation of the conditioned fear memory is not impaired. Unfortunately, none of these studies recorded polygraphic signs of S–W activity during the consolidation of contextual fear memory, and none have accounted for the non-specific stress induced by the sleep deprivation. Nonetheless, these results could be interpreted as evidence that there is a sleep window for conditioned fear memory consolidation during which sleep deprivation can impair subsequent recall. In light of the gaps in the existing research, the aim of the present study was to use polygraphic recordings to examine the temporal changes in S–W stages, during a sleep window for conditioned fear memory consolidation, following contextual fear conditioning training.

Methods

Animals

Twenty-eight naive adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA), weighing between 250 and 350 gm each, were used in the experiments. Rats were housed individually at 24 °C on a 12/12 h light/dark cycle (lights on

at 7:00 a.m.) with free access to food and water. Experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Boston University Animal Care Committee (AN-14085). Additional care was taken to ensure that any potential discomfort was eliminated and the number of animals used was minimized. To reduce additional stress that might be imposed by experimental handling, animals underwent a period of habituation during which they were handled gently daily for 15–20 min between 9:00 a.m. and 10:00 a.m., beginning 1 week prior to surgery and continuing up to the experimental recording sessions. Twenty-two of these 28 animals were initially processed for an another study (Datta and O'Malley 2013), but during their habituation polygraphic recording sessions, they did not exhibit pontine waves (P-waves) and thus were not used for that study. Interestingly, the absence of P-waves during REM sleep provided an important opportunity to use these animals for the present study. The other six rats were processed specifically for the completion of the present study.

Surgical procedures

All surgical procedures were performed stereotactically under aseptic conditions. Animals were anesthetized with pentobarbital (40 mg/kg, i.p; Ovation Pharmaceuticals, Deerfield, IL, USA), placed in the stereotaxic apparatus and secured using blunt rodent ear bars, as described elsewhere (Paxinos and Watson 1997). The appropriate depth of anesthesia was judged by the absence of palpebral reflexes and lack of response to tail pinch. Core body temperature was maintained at 37 ± 1 °C with a thermostatic heating pad and a rectal thermister. In the 22 rats (which were initially prepared for the earlier study), cortical electroencephalogram (EEG), neck muscle electromyogram (EMG), hippocampal EEG (to record theta wave activity), and pontine EEG (to record P-wave activity) recording electrodes were chronically implanted to record behavioral states of vigilance, as described previously (Datta 2000). The surgical procedures for the other 6 rats were identical to the initial 22 except that in these 6 rats, P-wave recording electrodes were not implanted. All electrodes were secured to the skull with dental acrylic, and the electrodes were attached to mini-connector pins and brought together in a plastic connector. Upon completion of the surgical procedure, animals received saline (5 cc, s.c.) to prevent dehydration, ampicillin (50 mg/rat, i.m.; Bristol-Myers Squibb Company, Princeton, NJ, USA) to control potential postsurgical infection and buprenorphine (0.05 mg/kg, s.c; Ben Venue Laboratories, Bedford, OH, USA) to control potential postoperative pain. Immediately after surgery, animals were placed in recovery cages and monitored for successful recovery from anesthesia and surgery as judged by purposeful movement and grooming behaviors.

Polygraphic recordings and determination of behavioral states

To record cortical EEG, EMG, hippocampal EEG, and pontine EEG under free-moving conditions, each head plug was mated to a male connector attached to a commutator. Signals from the commutator passed to a polygraph (Grass Model 15 Neurodata Amplifier System, Astro-Med, Inc., West Warwick, RI, USA), located in the next room, via its electrode board. To identify experimental effects on sleep and wakefulness, polygraphic data were captured online in a computer using “Gamma” software (Grass product group, Astro-Med, Inc.). One investigator, blinded to the treatment conditions, scored this captured data visually using “Rodent Sleep Stager” software (Grass product group). Three behavioral states were

distinguished: W, SWS, and REM sleep; these states were scored in successive 5-s epochs. The physiological criteria used to identify these S–W states are described in detail in an earlier publication (Datta et al. 2004).

Adaptation recording session

After 3–7 days of postsurgical recovery, rats were habituated to the experimenter, the sound-attenuating recording cage (electrically shielded: $76.2 \times 45.7 \times 45.7$ cm), and the free-moving polygraphic recording (Grass Model 15) conditions for 7–10 days. During these recovery and habituation periods, rats were housed in the same 12/12 h light/dark cycle with free access to food and water. Rats were polygraphically recorded during these habituation sessions, which took place between 10:00 a.m. and 4:00 p.m., a time when the rats are normally sleeping. Baseline recordings for electrode testing and monitoring of daily variations in S–W activity were recorded during the 6-h habituation sessions. The last day of these adaptation recording sessions was determined when, for 3 consecutive days, day-to-day variation in the percentage of time spent in REM sleep was less than 5 % of the total amount of time spent in REM sleep. During these final 3 recording sessions, the pontine EEG data collected from the P-wave recording electrode were studied carefully to identify rats with or without good quality P-wave activity during REM sleep. All of these 22 rats produced very few or no P-waves during REM sleep.

Contextual fear conditioning apparatus and training

A standard testing chamber ($30.5 \times 24.1 \times 21.0$ cm; Standard modular test chamber, ENV-008; Sound-attenuating cubicle, ENV-022MD; Med Associates, St. Albans, VT, USA) constructed from aluminum and Plexiglas was used. The floor of the chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart, which were wired to a programmable shock generator and solid-state grid scrambler for the delivery of foot-shocks. This chamber was situated in a sound-attenuating cabinet, which itself was located in a brightly lit and isolated room. A ventilation fan in the cabinet provided fresh air and background noise (65 db), and a closed circuit video camera mounted inside the cabinet videotaped the behavior of the rat inside the chamber. Freeze responses were video-recorded and analyzed online using customized computer software (Med Associates). Freezing behavior was defined as the absence of movement with the exception of breathing (sample rate: 30 frames/s; min freeze duration: 15 frames; % freezing in bin durations of 5 min; described earlier in Huang et al. 2010).

The contextual fear acquisition procedure began with the transportation of naïve rats from their housing room to the behavioral testing room. Five minutes after arrival, rats were placed inside the testing chamber for 3 min of adaptation. Following adaptation, the foot-shock-receiving rats ($n = 22$ rats; rats implanted with P-wave recording electrodes) underwent 3 trials of shock exposure (1.0-min interval between trials), consisting of unannounced (no light or tone) 1-s scrambled foot-shocks (1.0 mA). One minute after the end of the last trial, rats were removed from the testing chamber. The procedure for the control rats ($n = 6$ rats; rats without P-wave recording electrode) was identical, except that no shock was administered. Each rat was alone inside the testing chamber for a period of 6 min (between 9:49 a.m. and 9:55 a.m.). The testing chamber was cleaned and disinfected

with soapy water and chlorine dioxide disinfectant (MB-10, Quip Laboratories, Wilmington, DE, USA) before and after each use.

Post-training sleep–wake recording

Immediately after contextual fear and control training, rats were removed from the fear conditioning chamber and transported (in their home cages) to the sleep recording chamber, located in the next room. Animals were then transferred to the polygraphic recording chamber and then connected to the recording systems. These rats were then recorded for a 6-h period (between 10:00 a.m. and 4:00 p.m.) of undisturbed S–W activity, as described earlier (Datta and O'Malley 2013).

Contextual fear memory testing

Approximately 24 h after fear acquisition, rats were brought to the behavioral testing room for fear memory testing. Five minutes after arrival (at 9:55 a.m.), each of the 22 fear-acquired rats and 6 control rats was placed singly in the testing chamber (original shocked context) for 5 min, without any shock. During this contextual fear memory testing, rat behavior was recorded and analyzed as the total percentage of freezing time in 5 min. Freezing is the most commonly used behavioral measure to assess fear during shock training or context/cue re-exposure. A greater percentage of freezing during contextual fear conditioning test is interpreted as stronger fear memory (Blanchard and Blanchard 1969; Doyere et al. 2000; Choi et al. 2010; Peters et al. 2010).

Histological localization of lesion sites in the brain stem

At the end of fear memory testing, the rats with P-wave recording electrodes ($n = 22$ rats) were deeply re-anesthetized with pentobarbital (60 mg/kg, i.p.) and perfused transcardially with heparinized cold phosphate buffer (0.1 M, pH 7.4) followed by 4 % paraformaldehyde in 0.1 M phosphate buffer. The brains were then removed, and trimmed brain stem blocks (between anterior 2.5 mm and posterior 2.5 mm, in relation to the P-wave electrode track) were stored in sucrose saturated 10 % formalin solution for 24–48 h. The brain stem blocks were then cut in a transverse plane in 40- μ m-thick serial sections using a cryostat. Sections were then stained with cresyl violet, dehydrated, cleared, mounted, and coverslipped using Permount® and processed for staining and histological localization of the P-wave recording electrode penetration track and lesion areas. The sections were examined under a microscope (Eclipse E800; Nikon Corp., Tokyo, Japan), and images were captured with a color CCD camera (Optronics, Goleta, CA) using Image-Pro Plus v5.1 imaging software (Media Cybernetics, Inc. Silver Spring, MD, USA). In the Nissl stained sections, the pontine nuclei and relevant landmarks were readily apparent and could be followed microscopically through serial sections. Electrode tracks were clearly evident as dense linear accumulations of glial cells and obvious, localized disturbance of the tissue immediately surrounding the electrode track (See Fig. 1a). All nuclei and brain structures were identified using the rat brain atlas of Paxinos and Watson (1997).

Statistical analysis

Freezing data were tabulated as total percentage of time spent in freezing behavior in 5 min of fear memory testing. Sleep–wake data were tabulated as described in our earlier publication (Datta et al. 2004). Results are presented as means $\pm$ SD. Statistical significance was assessed using a one-factor or two-factor ANOVA followed by a post hoc Bonferroni multiple comparison test to determine the difference between groups. All statistical analyses were performed using GraphPad Prism statistical software (v5.0; GraphPad Software, Inc., La Jolla, CA, USA).

Results

In the baseline polygraphic recording session, before contextual fear conditioning memory, rats implanted bilaterally with pontine EEG recording electrodes ($n = 22$ rats), displayed good quality cortical and hippocampal EEG and neck muscle EMG activities during all stages of S–W cycle. If the tips of pontine EEG recording electrodes are within the P-wave generator zone, these electrodes record P-waves during REM sleep. But, in these animals, these pontine EEG recording electrodes failed to register any P-wave activity during REM sleep episodes. This absence of P-wave activity in the pontine EEG during REM sleep indicates that the tips of these P-wave recording electrodes were not within the P-wave generator zone. Another possibility is that implanted electrodes may have damaged neurons within the P-wave generator zone.

P-wave recording electrode implantation-induced histological changes

Histological examination revealed that eight of the 22 rats with P-wave recording electrodes sustained unintended bilateral damage to the SubCD, a region that generates P-waves (Fig. 1). In these eight animals, based on the electrode tracks and tissue damage, P-wave recording electrodes pierced through the P-wave generating zone and reached about 0.5- to 1.0-mm ventral to our intended target, the center of the P-wave generator (in relation to stereotaxic “0”—posterior 0.5–0.9; lateral: 1.1–1.6; horizontal/dorsoventral: 2.0–1.0). Since these electrodes pierced through the center of the P-wave generating zone, they damaged most of the neurons that generate P-wave activity during REM sleep. In contrast, in 6 other animals, the tip of the P-wave electrodes did not reach the P-wave generator zone—the tip of those electrodes remained 0.5–0.8 mm above the dorsal extent of P-wave generator. In two animals, electrodes were slightly anterior (0.5–1.1 mm) to the P-wave generator; in two animals, electrodes were slightly posterior (0.5–0.9 mm) to the P-wave generator; and in another four animals, electrodes were slightly medial and lateral to the P-wave generator. In summary, in 8 of the P-wave electrode implanted rats, implantation of these electrodes caused tissue damage within the P-wave generator, and in another 14 P-wave electrode implanted rats, these electrodes damaged brain tissue in areas very close to the P-wave generator zone. Based on these histological analyses, these 22 animals were then divided into two groups: (1) animals with SubCD-Lesion and 2) animals with lesions in areas close to but not within the SubCD (Other-Lesion). In addition to these two groups, another group of animals were chronically prepared for the S–W recording, but without P-wave electrodes. Therefore, these animals had no lesion in the brain stem (No-Lesion/Control). Hereafter,

these animal groups will be labeled as “No-Lesion/Control,” “SubCD-Lesion,” and “Other-Lesion.”

Effects of P-wave generator damage and contextual fear conditioning training on W, SWS, and REM sleep

Before training trials, in the final 6-h baseline-recording session (last day of adaptation recording), the total percentages of time spent in W, SWS, and REM sleep were not significantly different (one-factor ANOVA) among No-Lesion/Control, SubCD-Lesion, and Other-Lesion groups of animals. These results indicate that before contextual fear conditioning training, animals were initially equal in terms of time spent in W, SWS, and REM sleep for the final 6-h baseline-recording session (between 10:00 a.m. and 4:00 p.m.). Additionally, the time to stabilize sleep during the baseline-recording period, after 3–7 days of postsurgical recovery, was comparable between No-Lesion/Control, SubCD-Lesion, and Other-Lesion groups of animals. These results also suggest that the brain damage caused by the implantation of electrodes targeted for the P-wave generating site did not alter the time spent in W, SWS, and REM sleep for the final 6-h baseline-recording session.

To determine the effects of contextual fear conditioning training on W, SWS, and REM sleep, immediately after fear conditioning and control training, 6-h (between 10:00 a.m. and 4:00 p.m.) undisturbed S–W were analyzed from SubCD-Lesion, Other-Lesion, and No-Lesion/Control group of animals. During these recording periods, the total percentages of time spent in W indicated significant (two-factor ANOVA) main effects of group ($F_{(2,75)} = 10.23, p < 0.001$), time ($F_{(2,75)} = 323.3, p < 0.001$), and group $\times$ time interaction ($F_{(4,75)} = 9.89, p < 0.001$). During the first 2 h, compared with the No-Lesion/Control, SubCD-Lesion ($t_{(13)} = 7.14, p < 0.001$; Bonferroni post-test) and Other-Lesion ($t_{(19)} = 6.65, p < 0.001$; Bonferroni post-test) animals spent significantly more time in W (Fig. 2a), indicating that the contextual fear conditioning training increased W. However, a similar comparison revealed that the percentages of time spent in W during this first 2-h period after fear conditioning training were not significantly different between SubCD-Lesion and Other-Lesion animals ($t_{(21)} = 0.45, p > 0.05$; Bonferroni post-test), indicating that the contextual fear conditioning training increased W equally in SubCD-Lesion and Other-Lesion animals. Unlike during the first 2 h, during the 2- to 4- and 4- to 6-h periods, the total percentages of time spent in W were comparable between No-Lesion/Control, SubCD-Lesion, and Other-Lesion animals (Fig. 2a). These results suggested that the increase in W induced by contextual fear conditioning training lasted only for the first 2 h.

A two-factor ANOVA on the total percentages of time spent in SWS indicated a significant main effects of time ($F_{(2,75)} = 248.6, p < 0.001$) and group $\times$ time interaction ($F_{(4,75)} = 14.15, p < 0.001$). During the first 2 h, compared with the No-Lesion/Control, both SubCD-Lesion ($t_{(13)} = 5.94, p < 0.001$; Bonferroni post-test) and Other-Lesion ($t_{(19)} = 5.25, p < 0.001$; Bonferroni post-test) animals spent significantly less time in SWS (Fig. 2b), indicating that the contextual fear conditioning training decreased SWS. Similar comparison revealed no significant difference between SubCD-Lesion and Other-Lesion animals ($t_{(21)} = 0.28, p > 0.05$; Bonferroni post-test) on the percentages of time spent in SWS during this first 2-h period after fear conditioning training, indicating that the contextual fear

conditioning training decreased SWS equally in SubCD-Lesion and Other-Lesion animals. Unlike during the first 2 h, during the 2- to 4- and 4- to 6-h periods, the total percentages of time spent in SWS were comparable between No-Lesion/Control, SubCD-Lesion, and Other-Lesion animals (Fig. 2b), indicating that the contextual fear conditioning training-induced decrease in SWS lasted only for the first 2 h.

A two-factor ANOVA on the total percentage of time spent in REM sleep indicated significant main effects of group ($F_{(2,75)} = 193.4, p < 0.001$), time ($F_{(2,75)} = 112.3, p < 0.001$), and a group $\times$ time interaction ($F_{(4,75)} = 12.4, p < 0.001$). Post hoc comparisons (Bonferroni post-test) revealed that compared with the No-Lesion/Control animals, SubCD-Lesion animals spent significantly less time in REM sleep during the 0- to 2-h ($t_{(13)} = 5.57, p < 0.001$), 2- to 4-h ($t_{(13)} = 13.8, p < 0.001$), and 4- to 6-h ($t_{(13)} = 8.74, p < 0.001$) blocks of time after contextual fear conditioning training (Fig. 2c). Similarly, Other-Lesion animals also spent significantly less time during the 0- to 2-h ($t_{(19)} = 6.17, p < 0.001$), 2- to 4-h ($t_{(19)} = 15.6, p < 0.001$), and 4- to 6-h ($t_{(19)} = 11.0, p < 0.001$) blocks of time after contextual fear conditioning training (Fig. 2c). These results indicate that fear conditioning training decreased total percentage of time spent in REM sleep in both SubCD-Lesion and Other-Lesion animals equally and this effect lasted for the entire 6-h period of recordings.

Effects of P-wave generator lesion on contextual fear memory

To determine the effect of P-wave generator lesion on the development of contextual fear memory, 24 h after fear conditioning and control training, SubCD-Lesion, Other-Lesion, and No-Lesion/Control animals were re-exposed to the original training context without any foot-shock for a 5-min period (contextual fear memory testing session). A one-factor ANOVA (Kruskal–Wallis test) on the percentages of time spent in freezing during this 5-min test session revealed a significant difference between SubCD-Lesion, Other-Lesion, and No-Lesion/Control animals ($F_{(2,25)} = 20.4, p < 0.001$). Post hoc analysis (Bonferroni multiple comparison test) revealed that the total percentages of time spent in freezing behavior were not significantly different between No-Lesion/Control and SubCD-Lesion animals ($t = 0.29$; not significant), indicating that SubCD animals failed to develop contextual fear memory (Fig. 2D). On the contrary, compared with No-Lesion/Control animals, Other-Lesion animals spent significantly much higher percentages of time ($t = 17$; $p < 0.001$) in freezing behavior, indicating development of a strong contextual fear memory in Other-Lesion animals (Fig. 2d). Post hoc analysis also revealed that compared with SubCD-Lesion animals, Other-Lesion animals spent significantly more time in freezing behavior ($t = 18$; $p < 0.001$; Fig. 2d). These collective data indicate that animals with electrode implantation-induced damage within the P-wave generator failed to develop contextual fear memory. Additionally, these data also indicate that electrode implantation-induced brain damage near the P-wave generator area does not prevent development of contextual fear memory.

Discussion

The major finding of the present study is that bilateral damage within the SubCD, although unintentional, interrupted retention of context-associated fear memory. In addition, it was

observed that in both intact SubCD and SubCD-damaged animals, context-associated fear conditioning resulted in a decrease in the amount of time spent in SWS and REM sleep during the 6-h period after training. Recent studies in both rats and mice have shown that total sleep or REM sleep deprivation during the 6-h period following contextual and cued fear conditioning impairs fear memory consolidation (Graves et al. 2001; McDermott et al. 2003; Ruskin et al. 2004; Silvestri 2005; Kumar and Jha 2012). However, in contrast, the results of this study show that in animals with intact SubCD nuclei, context-associated fear conditioning-induced reduction in SWS and REM sleep did not affect the consolidation of context-associated fear memory.

Context-associated fear conditioning is an associative learning task in which animals learn to fear an environment (context) through development of an association between a particular context and an aversive foot-shock (for review see Maren 2001). When exposed to the same context sometime after training, animals exhibit a variety of fear responses, including freezing behavior (Blanchard and Blanchard 1969; Fanselow 1980). In recent decades, important progress has been made in identifying the essential neuronal circuits and cellular-molecular mechanisms for the consolidation of context-associated fear memory (reviewed in Kim and Jung 2006; Pape and Pare 2010; Maren et al. 2013). Many years of research on rodents and humans, using lesion, neurophysiological, functional neuroanatomical, molecular, and pharmacological methods, have produced a large body of evidence, suggesting that a neural circuit including the hippocampus, amygdala, and medial prefrontal cortex is involved in the learning and memory processes that enable context-associated behavior (Kim et al. 1993; Logue et al. 1997; Bourchouladze et al. 1998; Abel and Lattal 2001; Maren and Quirk 2004; Kim and Jung 2006; Wiltgen et al. 2006; Pape and Pare 2010; Maren et al. 2013). The present study, for the first time, demonstrates that damage to the SubCD prevents the retention of context-associated fear memory. This result suggests that in addition to other areas of the brain, the SubCD is an element in the neuronal circuitry supporting the consolidation of context-associated fear memory. However, it is certainly possible that damage to the SubCD caused by the implantation of P-wave recording electrodes might have also damaged passing fibers and vasculature and had an impact on the experimental outcome. Therefore, to support or to rule out this possibility, future studies are necessary to confirm our interpretation by producing small localized lesions with microinjection of ibotenic acid directly into the SubCD. Indeed, one study has shown that the selective elimination of cell bodies, by microinjection of ibotenic acid directly into the SubCD, impairs the consolidation of two-way active avoidance memory (Mavanji et al. 2004), thus suggesting a role in memory processing by SubCD. Another possibility is that the damage to the SubCD caused by the implantation of P-wave recording electrodes might have damaged the locus coeruleus (LC). This suggestion is based on the fact that the SubCD is very close to the LC, and it has been already suggested that the LC contributes to learning and cognitive flexibility (Watts et al. 2012). Therefore, in this study, the damage to the LC might have impaired consolidation of context-associated fear memory. However, our histological examinations revealed very little or no damage in the LC of animals that were implanted with P-wave electrodes. Future localized lesion study, described above, will also be able to support or rule out the involvement of LC in this SubCD-Lesion-induced impairment of context-associated fear memory consolidation. In evaluating the significant

outcome of this study, having no “Lesioned-No-Shocked” and “No-Lesion-Shocked” groups could be viewed as a weakness. However, the lack of “Lesioned-No-Shocked” animals is mitigated by using the baseline S–W for the no-shocked condition. Also, the lack of No-Lesion-Shocked animals may not be a serious concern because there are several studies that have already shown that non-lesioned control animals, like lesioned animals in this study, spent significantly less time in sleep after context-associated fear conditioning training (Huang et al. 2010; Maclean and Datta 2007; Wellman et al. 2008).

At this time, based on the existing literature, we can only speculate about the mechanisms of how SubCD is involved in the consolidation of context-associated fear memory. For example, various cellular and molecular studies have demonstrated that glutamate-induced neuronal activation-dependent gene expression and protein synthesis in the dorsal hippocampus and amygdala are necessary for memory processing and behavioral plasticity (Guzowski et al. 2001; Kandel 2001; Rebeiro et al. 2002; Lee et al. 2004; Datta et al. 2008). Also, brain-derived neurotrophic factor (BDNF) has been shown to contribute to neuronal activity-dependent processes such as long-term potentiation (Bramham and Messaoudi 2005; Heldt et al. 2007) and associative memory formation (Bliss and Collingridge 1993; Whitlock et al. 2006). There is increasing behavioral evidence indicating that BDNF gene expression is induced in the hippocampus and amygdala after contextual and spatial learning and that this mechanism is essential for normal learning and memory (Hall et al. 2000). More importantly, consolidation of fear memory requires plasticity within the prefrontal cortex, hippocampus, and amygdala, which in turn depends on *N*-methyl-D-aspartate (NMDA) receptors, protein kinases, and protein synthesis (Morris et al. 1986; Tsien et al. 1996; Bourchouladze et al. 1998; Lee and Kim 1998; Fendt and Fanselow 1999; Lee and Kesner 2002; Kim and Jung 2006; Choi et al. 2010). Additionally, it has been shown that the dorsal hippocampus and amygdala receive direct anatomical projections from P-wave generating cells and activation of the P-wave generator increases both glutamate release in the dorsal hippocampus and frequency of hippocampal theta wave activity (Datta et al. 1998; Karashima et al. 2004; Datta 2006). More recently, studies have shown that the activation of the P-wave generator increases expression of phosphorylated cyclic adenosine mono phosphate response element binding (pCREB), activity-regulated cytoskeletal-associated (Arc), and BDNF proteins and early growth response-1 (Egr-1), Arc, and BDNF mRNA expressions in the dorsal hippocampus and amygdala (Datta et al. 2008). The synthesis of this information suggests the hypothesis that the activation of P-wave generating cells in the SubCD is a neurochemical switch that increases glutamate release in the forebrain areas, especially in the dorsal hippocampus and amygdala, to activate NMDA receptors. Such activation could then ultimately initiate or intensify the cellular and molecular steps in the forebrain that are necessary for the consolidation of context-associated fear memory. This mechanism may explain why in this study, damage to the P-wave generating cells in the SubCD has impaired context-associated fear memory consolidation.

In recent years, a number of studies in rats and mice have shown that sleep deprivation from 0 to 5 h after fear conditioning training is sufficient to impair consolidation of fear conditioning memory (Florian et al. 2011; Graves et al. 2003; Ruskin et al. 2004; Silva et al.

2004; Vecsey et al. 2009; Kumar and Jha 2012). These studies suggest a requirement for discrete periods of undisturbed sleep following fear conditioning to successfully achieve consolidation of the fear memory. The results of the present study have demonstrated that fear conditioning training significantly reduced the amount of both SWS and REM sleep, yet these animals were able to successfully consolidate context-associated fear memory. Collectively, based on the results of earlier sleep deprivation studies and the present study, we now suggest that consolidation of conditioned fear memory requires post-training sleep, but it can be completed within a period of sleep shorter than the normal duration of sleep. Therefore, the consolidation processes for the contextual fear memory might be different than many other types of memory, because most other known memory consolidation processes require an additional amount of post-training sleep (Smith 1985, 1995; Datta 2010). However, these data do not preclude the possibility that contextual fear conditioning training may have increased and/or decreased SWS and/or REM sleep in another time period (between the 7th h and the beginning of testing at the 24th h) when we did not record polygraphic signs of S–W activity (Smith et al. 1980; Gisquet-Verrier and Smith 1989; Smith 1995, 2003; Fogel et al. 2009).

In summary, we have identified an anatomical site within the brain stem, the SubCD, that, when damaged, produces impaired consolidation of context-associated fear memory. Since the REM sleep P-wave generating cells are located in this part of the brain, the results of our experiments suggest that the P-wave generating cells might be involved in the consolidation of context-associated fear memory. Thus, the results of these experiments lay the behavioral and anatomical foundation for subsequent detailed examination of the cellular and molecular underpinnings of sleep-dependent emotional memory processing.

Acknowledgments

This study was supported by Boston University School of Medicine and National Institute of Health Research Grant (MH59839). We thank Dr. D. A. Ciraulo and Dr. E. H. Patterson for critical reading and constructive suggestions to improve the quality of this manuscript. We also thank Matthew W. O'Malley for his technical support.

References

- Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol.* 2001; 11:180–187. [PubMed: 11301237]
- Anderson KC, Insel TR. The promise of extinction research for the prevention and treatment of anxiety disorders. *Biol Psychiatry.* 2006; 60:319–321. [PubMed: 16919521]
- Baran B, Pace-Schott EF, Ericson C, Spencer RM. Processing of emotional reactivity and emotional memory over sleep. *J Neurosci.* 2012; 32:1035–1042. [PubMed: 22262901]
- Beck AT. The evolution of the cognitive model of depression and its neurobiological correlates. *Am J Psychiatry.* 2008; 165:969–977. [PubMed: 18628348]
- Bjorness TE, Riley BT, Tysor MK, Poe GR. REM restriction persistently alters strategy used to solve a spatial task. *Learn Mem.* 2005; 12:352–359. [PubMed: 15897251]
- Blanchard RJ, Blanchard DC. Crouching as an index of fear. *J Comp Physiol Psychol.* 1969; 67:370–375. [PubMed: 5787388]
- Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature.* 1993; 361:31–39. [PubMed: 8421494]
- Bourtchouladze R, Abel T, Berman N, Gordon R, Lapidus K, Kandel ER. Different training procedures for contextual memory in mice can recruit either one or two critical periods for memory

- consolidation that require protein synthesis and PKA. *Learn Mem.* 1998; 5:365–374. [PubMed: 10454361]
- Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol.* 2005; 76:99–125. [PubMed: 16099088]
- Charney DS. Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. *Am J Psychiatry.* 2004; 161:195–216. [PubMed: 14754765]
- Choi DC, Maguschak KA, Ye K, Jang SW, Myers KM, Ressler KJ. Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proc Natl Acad Sci USA.* 2010; 107:2675–2680. [PubMed: 20133801]
- Datta S. Avoidance task training potentiates phasic pontine-wave density in the rat: a mechanism for sleep-dependent plasticity. *J Neurosci.* 2000; 20:8607–8613. [PubMed: 11069969]
- Datta S. Activation of phasic pontine-wave generator: a mechanism for sleepdependent memory processing. *Sleep Biol Rhythms.* 2006; 4:16–26.
- Datta, S. Sleep: learning and memory. *Encyclopedia of behavioral neuroscience.* Vol. 3. New York: Elsevier Inc., Oxford Academic Press; 2010. p. 218-226.
- Datta S, O'Malley MW. Fear extinction memory consolidation requires potentiation of pontine-wave activity during REM sleep. *J Neurosci.* 2013; 33:4561–4569. [PubMed: 23467372]
- Datta S, Siwek DF, Patterson EH, Cipolloni PB. Localization of pontine PGO wave generation sites and their anatomical projections in the rat. *Synapse.* 1998; 30:409–423. [PubMed: 9826233]
- Datta S, Mavanji V, Ulloor J, Patterson EH. Activation of phasic pontinewave generator prevents rapid eye movement sleep deprivation-induced learning impairment in the rat: a mechanism for sleep-dependent plasticity. *J Neurosci.* 2004; 24:1416–1427. [PubMed: 14960614]
- Datta S, Li G, Auerbach S. Activation of phasic pontine-wave generator in the rat: a mechanism for expression of plasticity-related genes and proteins in the dorsal hippocampus and amygdala. *Eur J Neurosci.* 2008; 27:1876–1892. [PubMed: 18371081]
- Diekelmann S, Born J. The memory function of sleep. *Nat Rev Neurosci.* 2010; 11:114–126. [PubMed: 20046194]
- Doyere V, Gisquet-Verrier P, de Marsanich B, Ammassari-Teule M. Age-related modifications of contextual information processing in rats: role of emotional reactivity, arousal and testing procedure. *Behav Brain Res.* 2000; 114:153–165. [PubMed: 10996056]
- Fanselow MS. Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci.* 1980; 15:177–182. [PubMed: 7208128]
- Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev.* 1999; 23:743–760. [PubMed: 10392663]
- Florian C, Vecsey CG, Halassa MM, Haydon PG, Abel T. Astrocyte-derived adenosine and A1 receptor activity contribute to sleep loss-induced deficits in hippocampal synaptic plasticity and memory in mice. *J Neurosci.* 2011; 31:6956–6962. [PubMed: 21562257]
- Fogel SM, Smith CT, Beninger RJ. Evidence for 2-stage models of sleep and memory: learning-dependent changes in spindles and theta in rats. *Brain Res Bull.* 2009; 79:445–451. [PubMed: 19559345]
- Gisquet-Verrier P, Smith C. Avidance performance in rat enhanced by postlearning paradoxical sleep deprivation. *Behav Neural Biol.* 1989; 52:152–169. [PubMed: 2803171]
- Graves LA, Pack AI, Abel T. Sleep and memory: a molecular perspective. *Trends Neurosci.* 2001; 24:237–243. [PubMed: 11250009]
- Graves LA, Heller EA, Pack AI, Abel T. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learn Mem.* 2003; 10:168–176. [PubMed: 12773581]
- Groch S, Wilhelm I, Diekelmann S, Born J. The role of REM sleep in the processing of emotional memories: evidence from behavior and event-related potentials. *Neurobiol Learn Mem.* 2013; 99:1–9. [PubMed: 23123802]
- Guzowski JF, Setlow B, Wagner EK, McGaugh JL. Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. *J Neurosci.* 2001; 21:5089–5098. [PubMed: 11438584]

- Hall J, Thomas KL, Everitt BJ. Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nat Neurosci.* 2000; 3:533–535. [PubMed: 10816306]
- Heldt SA, Stanek L, Chhatwal JP, Ressler KJ. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry.* 2007; 12:656–670. [PubMed: 17264839]
- Herry C, Trifilieff P, Micheau J, Luthi A, Mons N. Extinction of auditory fear conditioning requires MAPK/ERK activation in the basolateral amygdala. *Eur J Neurosci.* 2006; 24:261–269. [PubMed: 16882022]
- Holland P, Lewis PA. Emotional memory: selective enhancement by sleep. *Curr Biol.* 2007; 17:R179–R181. [PubMed: 17339020]
- Hu P, Stylos-Allan M, Walker MP. Sleep facilitates consolidation of emotional declarative memory. *Psychol Sci.* 2006; 17:891–898. [PubMed: 17100790]
- Huang MP, Radadia K, Auerbach SH, Datta S. Effects of eszopiclone and zolpidem on sleep–wake behavior, anxiety and contextual memory in rats. *Behav Brain Res.* 2010; 210:54–66. [PubMed: 20153782]
- Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. *Science.* 2001; 294:1030–1038. [PubMed: 11691980]
- Karashima A, Nakao M, Honda K, Iwasaki N, Katayama N, Yamamoto M. Theta wave amplitude and frequency are differentially correlated with pontine waves and rapid eye movements during REM sleep in rats. *Neurosci Res.* 2004; 50:283–289. [PubMed: 15488291]
- Kim JJ, Jung MW. Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neurosci Biobehav Rev.* 2006; 30:188–202. [PubMed: 16120461]
- Kim JJ, Rison RA, Fanselow MS. Effects of amygdala, hippocampus, and periaqueductal gray lesions of short- and long-term contextual fear. *Behav Neurosci.* 1993; 107:1093–1098. [PubMed: 8136063]
- Koren D, Arnon I, Lavie P, Klein E. Sleep complaints as early predictors of posttraumatic stress disorder: a 1-year prospective study of injured survivors of motor vehicle accidents. *Am J Psychiatry.* 2002; 159:855–857. [PubMed: 11986142]
- Kring AM, Caponigro JM. Emotion in schizophrenia: where feeling meets thinking. *Curr Dir Psychol Sci.* 2010; 19:255–259. [PubMed: 22557707]
- Kumar T, Jha SK. Sleep deprivation impairs consolidation of cued fear memory in rats. *PLoS ONE.* 2012; 7(e47042):1–8.
- Lavie P. Sleep disturbances in the wake of traumatic events. *N Engl J Med.* 2001a; 345:1825–1832. [PubMed: 11752360]
- Lavie P. Eye movements during REM sleep. *Exp Brain Res.* 2001b; 192:657–667.
- Lazaro-Munoz G, LeDoux JE, Cain CK. Sidman instrumental avoidance initially depends on lateral and basal amygdala and is constrained by central amygdala-mediated Pavlovian processes. *Biol Psychiatry.* 2010; 67:1120–1127. [PubMed: 20110085]
- LeDoux JE. Brain mechanisms of emotion and emotional learning. *Curr Opin Neurobiol.* 1992; 2:191–197. [PubMed: 1638153]
- LeDoux JE. Emotion, memory and the brain. *Scientific American, Special Edition.* 2002:62–71.
- Lee I, Kesner RP. Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. *Nat Neurosci.* 2002; 5:162–168. [PubMed: 11780144]
- Lee H, Kim JJ. Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. *J Neurosci.* 1998; 18:8444–8454. [PubMed: 9763487]
- Lee JL, Everitt BJ, Thomas KL. Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science.* 2004; 304:839–843. [PubMed: 15073322]
- Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, Pine DS. Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behav Res Ther.* 2005; 43:1391–1424. [PubMed: 15885654]
- Logue SF, Paylor R, Wehner JM. Hippocampal lesion cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behav Neurosci.* 1997; 111:104–113. [PubMed: 9109628]

- MacLean RR, Datta S. The relationship between anxiety and sleep–wake behavior after stressor exposure in the rat. *Brain Res.* 2007; 1164:72–80. [PubMed: 17644077]
- Mahan AL, Ressler KJ. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends Neurosci.* 2011; 35:24–35. [PubMed: 21798604]
- Mandal MK, Pandey R, Prasad AB. Facial expressions of emotions and schizophrenia: a review. *Schizophr Bull.* 1998; 24:399–412. [PubMed: 9718632]
- Maren S. Neurobiology of Pavlovian fear conditioning. *Ann Rev Neurosci.* 2001; 24:897–931. [PubMed: 11520922]
- Maren S, Quirk GJ. Neuronal signalling of fear memory. *Nat Rev Neurosci.* 2004; 5:844–852. [PubMed: 15496862]
- Maren S, Phan KL, Liberzon I. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci.* 2013; 14:417–428. [PubMed: 23635870]
- Mavanji V, Ulloor J, Saha S, Datta S. Neurotoxic lesions of phasic pontine-wave generator cells impair retention of 2-way active avoidance memory. *Sleep.* 2004; 27:1282–1292. [PubMed: 15586781]
- McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *J Neurosci.* 2003; 23:9687–9695. [PubMed: 14573548]
- Morgan MA, LeDoux JE. Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behav Neurosci.* 1995; 109:681–688. [PubMed: 7576212]
- Morris RG, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature.* 1986; 319:774–776. [PubMed: 2869411]
- Pape HC, Pare D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol Rev.* 2010; 90:419–463. [PubMed: 20393190]
- Pattwell SS, Duhoux S, Hartley CA, Johnson DC, Jing D, Elliott MD, Ruberry EJ, Powers A, Mehta N, Yang RR, Soliman F, Glatt CE, Casey BJ, Ninan I, Lee FS. Altered fear learning across development in both mouse and human. *Proc Natl Acad Sci (USA).* 2012; 109:16318–16323. [PubMed: 22988092]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1997.
- Payne JD, Stickgold R, Swanberg K, Kensinger EA. Sleep preferentially enhances memory for emotional components of scenes. *Psychol Sci.* 2008; 19:781–788. [PubMed: 18816285]
- Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. Induction of fear extinction with hippocampal-infralimbic BDNF. *Science.* 2010; 328:1288–1290. [PubMed: 20522777]
- Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron.* 2005; 48:175–187. [PubMed: 16242399]
- Poe GR, Walsh CM, Bjorness TE. Cognitive neuroscience of sleep. *Prog Brain Res.* 2010; 185:1–19. [PubMed: 21075230]
- Rauch SL, Shin LM, Phelps EA. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research—past, present, and future. *Biol Psychiatry.* 2006; 60:376–382. [PubMed: 16919525]
- Rebeiro S, Mello CV, Velho T, Gardner TJ, Jarvis ED, Pavlides C. Induction of hippocampal long-term potentiation during waking leads to increased extrahippocampal zif-268 expression during ensuing rapid-eye-movement sleep. *J Neurosci.* 2002; 22:10914–10923. [PubMed: 12486186]
- Ressler KJ. Amygdala activity, fear, and anxiety: modulation by stress. *Biol Psychiatry.* 2010; 67:1117–1119. [PubMed: 20525501]
- Rosales-Lagarde A, Armony JL, Del Rio-Portilla Y, Trejo-Martinez D, Conde R, Corsi-Cabrera M. Enhanced emotional reactivity after selective REM sleep deprivation in humans: an fMRI study. *Front Behav Neurosci.* 2012; 6(25):p1–p13.
- Rosenkranz JA, Venheim ER, Padival M. Chronic stress causes amygdala hyperexcitability in rodents. *Biol Psychiatry.* 2010; 67:1128–1136. [PubMed: 20378100]

- Ruskin DN, Liu C, Dunn KE, Bazan NG, LaHoste GJ. Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated cued learning in rats. *Europ J Neurosci*. 2004; 19:3121–3124.
- Silva RH, Chehin AB, Kameda SR, Takatsu-Coleman AL, Abilio VC, Tufik S, Frussa-Filho R. Effects of pre- or post-training paradoxical sleep deprivation on two animal models of learning and memory in mice. *Neurobiol Learn Mem*. 2004; 82:90–98. [PubMed: 15341794]
- Silvestri AJ. REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiol Behav*. 2005; 84:343–349. [PubMed: 15763570]
- Smith C. Sleep states and learning: a review of the animal literature. *Neurosci Biobehav Rev*. 1985; 9:157–168. [PubMed: 3892377]
- Smith C. Sleep states and memory processes. *Behav Brain Res*. 1995; 69:137–145. [PubMed: 7546305]
- Smith, CT. The REM sleep window and memory processing. In: Maquet, P.; Smith, CT.; Stickgold, R., editors. *Sleep and brain plasticity*. Oxford: Oxford University Press; 2003. p. 117–133.
- Smith CT, Young J, Young W. Prolonged increases in paradoxical sleep during and after avoidance task acquisition. *Sleep*. 1980; 3:67–81. [PubMed: 7466127]
- Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, Jing D, Tottenham N, Amso D, Somerville LH, Voss HU, Glover G, Ballon DJ, Liston C, Teslovich T, Van Kempen T, Lee FS, Casey BJ. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science*. 2010; 327:863–866. [PubMed: 20075215]
- Tsien JZ, Huerta PT, Tonegawa S. The essential role of hippocampal CA1. NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell*. 1996; 87:1327–1338. [PubMed: 8980238]
- Vecsey CG, Baillie GS, Jaganath D, Havekes R, Daniels A, Wimmer M, Huang T, Brown KM, Li XY, Descalzi G, Kim SS, Chen T, Shang YZ, Zhuo M, Houslay MD, Abel T. Sleep deprivation impairs cAMP signalling in the hippocampus. *Nature*. 2009; 461:1122–1125. [PubMed: 19847264]
- Wagner U, Gais S, Born J. Emotional memory formation is enhanced across sleep intervals with high amounts of rapid eye movement sleep. *Learn Mem*. 2001; 8:112–119. [PubMed: 11274257]
- Wagner U, Hallschmid M, Rasch B, Born J. Brief sleep after learning keeps emotional memories alive for years. *Biol Psychiatry*. 2006; 60:788–790. [PubMed: 16806090]
- Walker MP, Stickgold R. Sleep, memory, and plasticity. *Annu Rev Psychol*. 2006; 57:139–166. [PubMed: 16318592]
- Walker MP, van der Helm E. Overnight therapy? The role of sleep in emotional brain processing. *Psychol Bull*. 2009; 135:731–748. [PubMed: 19702380]
- Watts A, Gritton HJ, Sweigart J, Poe GR. Antidepressant suppression of non-REM sleep spindles and REM sleep impairs hippocampus-dependent learning while augmenting striatum-dependent learning. *J Neurosci*. 2012; 32:13411–13420. [PubMed: 23015432]
- Wellman LL, Yang L, Tang X, Sanford LD. Contextual fear extinction ameliorates sleep disturbances found following fear conditioning in rats. *Sleep*. 2008; 31:1035–1042. [PubMed: 18652099]
- Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning induces long-term potentiation in the hippocampus. *Science*. 2006; 313:1093–1097. [PubMed: 16931756]
- Wiltgen BJ, Sanders MJ, Anagnostaras SG, Sage JR, Fanselow MS. Context fear learning in the absence of the hippocampus. *J Neurosci*. 2006; 26:5484–5491. [PubMed: 16707800]

Fig. 1.

Example of histological localization of P-wave recording electrode implantation-induced brain damage. **a** A representative coronal section from a rat brain stem showing tissue damage caused by the implantation of P-wave recording electrode. **b** A magnified photomicrograph of electrode track in “a” demonstrating the neuronal cell loss in the middle of SubCD. **c** Schematic coronal section through the brain stem at interaural level -0.80 mm (Paxinos and Watson 1997), illustrating P-wave recording electrode implantation-induced areas damage in the SubCD (red areas). LC locus coeruleus, Me5 mesencephalic 5 nucleus,

Mo5 motor trigeminal nucleus, *PnC* pontine reticular nucleus, caudal, *scp* superior cerebellar peduncle

Fig. 2.

Effects of P-wave recording electrode implantation-induced brain damage and context-associated fear conditioning training on percentage of time spent in wakefulness, slow-wave sleep, and REM sleep, and also retention of context-associated fear memory. The wakefulness (**a**), slow-wave sleep (**b**), and REM sleep (**c**) effects were observed during the a 6-h sleep–wake recording period following context-associated fear conditioning. Note the significant increase in wakefulness (**a**) and decrease in slow-wave sleep (**b**) during the first 2-h block of sleep–wake recordings in rats that received context-associated fear conditioning training (SubCD-Lesion and Other-Lesion) than rats that received control training (No-Lesion/Control). Rats that received context-associated fear conditioning training (SubCD-Lesion and Other-Lesion) spent significantly less time in REM sleep (**c**) during all three 2-h blocks of sleep–wake recordings than rats received control training (No-Lesion/Control). **d**

Percentages of time spent in freezing behavior (% freezing) during context-associated fear memory testing. Note that compared with control rats (without P-wave recording electrodes, No-Lesion/Control), significantly higher levels of freezing were seen in rats in the Other-Lesion group, but not in rats of the SubCD-Lesion group. This finding suggests strongly that the SubCD-Lesion alone impaired context-associated fear conditioning memory. Post hoc test (Bonferroni): $*p < 0.001$, compared with No-Lesion/Control; $p < 0.001$, compared with SubCD-Lesion