Chapter 8
The Distinctive Role of NREM and REM Sleep in the Consolidation of Fear Memory

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Abstract Studies suggest that one-night sleep sufficiently evokes qualitative changes in the emotional memory systems and helps in remodeling the amygdalar and prefrontal neural circuitries after learning. REM sleep also potentiates the conditioned neural responses to fear mainly in the lateral amygdala, hippocampus, and medial geniculate nucleus. In addition, the coherence of oscillatory theta waves increases in the amygdala and hippocampus after conditioning, which presumably helps encode fear memory. Some of the symptoms of post-traumatic stress disorder (PTSD) such as hyperarousal reactions, flashing, and nightmare are developed as a result of over-consolidation of negative memories. Subjects falling into sleep soon after experiencing a life-threatening event may keep such negative emotional memories alive for years. Therefore, sleep deprivation following trauma can be one of the interventions to help prevent the development of PTSD. On the other hand, sleep architecture changes after the consolidation of fear memory. Within sleep, NREM sleep significantly increased, and REM sleep significantly decreased only after the consolidation of fear memory but did not change if the memory was impaired. Using the Bayesian law of conditional probability, we have observed that the consolidation of fear memory requires facilitated NREM sleep. In this chapter, we have reviewed the beneficial effects of sleep loss in the consolidation of fear-conditioned memory and sleep loss-mediated alteration in the possible underlying molecular mechanisms. We have also discussed that rats trained during the active phase exhibited better conditioning response compared to the rats trained during the inactive phase. In addition, we have discussed the correlation between augmented NREM sleep manifestations with the induced freezing response and Bayesian probabilistic theorem to predict, if within sleep, there is (a) a high probability of NREM sleep augmentation after the consolidation of fear memory and (b) a low probability of NREM sleep augmentation after impairment of fear memory.

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8.1 Introduction

Several studies suggest that post-learning sleep helps in better memory recall and task performance associated with different memory types such as declarative memory (Gais and Born 2004; Ellenbogen et al. 2006; Rasch et al. 2007; Lu and Goder 2012), procedural memory (Gais et al. 2000; Walker et al. 2003; Hill et al. 2008), trace-conditioned memory (Wamsley and Antrobus 2009), and emotional memory (Wagner et al. 2006; Holland and Lewis 2007; Walker and van der Helm 2009). Sleep deprivation alternatively impairs the consolidation of memory formation associated with these learning paradigms. For example, total sleep deprivation impairs consolidation of declarative memory (Backhaus et al. 2006), motor adaptation task memory (Hill et al. 2008), motor sequence task memory (Walker et al. 2003), visual discrimination task memory (Gais et al. 2000), and trace-conditioned memory (Chaudhury and Colwell 2002), while REM sleep deprivation induces memory deficit of spatial (Bjorness et al. 2005) and water maze learning (Smith and Rose 1996) tasks. In addition, it has been observed that reactivating the episodic memory's neural network during slow-wave sleep with cue helps reinforce memory retention (Rasch et al. 2007). These studies have thus demonstrated the significance of sleep in the consolidation of several discrete memory types.

Fear conditioning is a widely accepted model to study the neural basis of negative memory and has been used in numerous studies to explore the mechanisms that may underlie the psychophysiological changes associated with negative emotional memories (Madan et al. 2008). In fear conditioning, the neutral conditioned stimulus (CS) (e.g., tone, light, or context) is paired with an aversive unconditioned stimulus (US) (such as foot shock). Since both the stimuli are presented together during the conditioning, an emotionally neutral stimulus (context or tone/light) acquires aversive properties. The CS thus elicits fearful behavioral responses such as freezing, defecation, and piloerection. These responses are otherwise generated by the threatening stimuli (LeDoux 2000; Madan et al. 2008; Madan and Jha 2008). The information processing of emotion-event association is modulated by the hippocampus-dependent but amygdala-dominant memory system (Knight et al. 2009; Kuriyama et al. 2010). The basolateral amygdala and hippocampus seem to be the primary sites for cued and contextual fear conditioning, respectively, where the information of conditioned and unconditioned stimuli associations are formed and remain stored (Kim and Jung 2006).

Several factors including circadian phases influence learning and memory (Gerstner and Yin 2010). For example, rodents exhibit better learning of the active avoidance task if it is performed during their active phase and the passive avoidance task if it is performed during their rest period (Davies et al. 1973; Catala et al. 1985). Likewise, it has been reported that the circadian phase affects only the consolidation

of contextual fear memory but not the cued fear memory (Valentinuzzi et al. 2001). Furthermore, it has been reported that the hippocampal "per-2," a clock gene, is involved in the modulation of trace fear conditioning but not cued fear conditioning (CuFC) (Wang et al. 2009). Chaudhury and Colwell (2002) have reported that the circadian phases can also influence CuFC memory (Chaudhury and Colwell 2002). In subsequent studies, however, the similar effects of the time of day on fear conditioning were not observed at all (McDonald et al. 2002; Hagewoud et al. 2010).

Sleep is another factor, which potentially facilitates the consolidation of fearful memories and underlying neuronal plasticity (Hennevin et al. 1995, 1998; Graves et al. 2003; Vecsey et al. 2009). One study suggests that one-night sleep induces some discrete qualitative changes in the emotional memory system. It helps in remodeling the neural network in the amygdala and prefrontal cortex (Payne and Kensinger 2011). The conditioned neural responses to fear increase mainly in the lateral amygdala, hippocampus, and medial geniculate nucleus during REM sleep (Hennevin et al. 1995, 1998). The oscillatory theta rhythms, which possibly facilitate the consolidation of cued fear memories, exhibit increased coherence in the amygdalar and hippocampal circuitries after conditioning (Seidenbecher et al. 2003; Narayanan et al. 2007). Further, the consolidation of excessive negative memories includes many symptoms associated with post-traumatic stress disorder (PTSD) such as flashing, nightmare, hyperarousal reactions, etc. (Pitman 1989). Sleep soon after learning contributes in keeping the emotional memories alive for years (Wagner et al. 2006) suggesting that sleep immediately after experiencing a traumatic event may possibly contribute in developing PTSD in the traumatized patients. Therefore, sleep deprivation following trauma has been sought as an intervention to help prevent the development of PTSD (Wagner et al. 2006; Holland and Lewis 2007). On the other hand, sleep architecture changes after the consolidation of fear memory (Kumar and Jha 2012, 2017; Qureshi and Jha 2017). Within sleep, NREM sleep significantly increased, and REM sleep significantly decreased only after the consolidation of fear memory but did not change if the memory was impaired (Kumar and Jha 2017). Using the Bayesian law of conditional probability, we observed that the consolidation CuFC memory requires facilitated NREM sleep. In this chapter, we have reviewed the beneficial effects of sleep loss in the consolidation of fear-conditioned memory and sleep loss-mediated alteration in the possible underlying molecular mechanisms. We have also discussed that rats trained during the active phase exhibited better conditioning response compared to the animals trained during the inactive phase. In addition, we have discussed the correlation between augmented NREM sleep manifestations with the induced freezing response and Bayesian probabilistic theorem to predict, if within sleep, there is (a) a high probability of NREM sleep augmentation after the consolidation of fear memory and (b) a low probability of NREM sleep augmentation after impairment of fear memory.

8.2 Sleep Deprivation and Alteration of Fear Memory

Sleep deprivation is a standard method to study the role of sleep in learning and memory processes. Various studies have shown that sleep deprivation shortly after acquiring a new task has an adverse effect on memory consolidation.

8.2.1 Short-Term Sleep Deprivation Soon After Training Impairs the Consolidation of Fear Memory

Total sleep deprivation soon after fear conditioning induces memory deficit in rodents. Freezing is a hallmark of fear conditioning. We have reported that sleep-deprived animals showed a marked decrease in freezing response as compared to non-sleep-deprived and stress control animals (Kumar and Jha 2012; Qureshi and Jha 2017). Earlier it was reported that sleep deprivation selectively impairs contextual fear memory but not cued fear memory (Graves et al. 2003; Ruskin et al. 2004). Our report, however, suggests that sleep deprivation also alters cued fear-conditioned memory (Kumar and Jha 2012). We have observed that sleep-deprived, non-sleep-deprived, and stress control animals exhibited almost similar freezing response during the initial 5 min of CS presentation, but sleep-deprived animals showed a dramatic reduction in freezing response 5 min after the tone inception (Kumar and Jha 2012). In earlier studies, the freezing response to CS was assessed only during 2–3 min after tone presentation (Graves et al. 2003; Ruskin et al. 2004). However, we observed that a significant decrease in freezing response in cued-fear-conditioned and sleep-deprived animals occurred outside the 2–3 min time window.

Short-term total sleep deprivation also alters contextual fear memory (Fig. 8.1). Sleep deprivation for 5 h soon after contextual fear conditioning impairs contextual fear memory (Graves et al. 2003; Qureshi and Jha 2017). However, Cai et al. 2009 have reported that sleep deprivation does not induce a deficit in contextual fear memory (Cai et al. 2009). Cai et al. have used highly anxious mice strain *C57BL/6Jx129T2SvEms* in their study, whereas, Graves et al. and we have used low and moderately anxious mice. The highly anxious mice demonstrate fearful behavior even with gentle handling, and it is, therefore, challenging to assess the effects of sleep deprivation on the consolidation of fear memory in them.

8.2.2 Sleep Deprivation Performed 5 Hours After Training Does Not Impair Fear Memory

It will be intriguing to see if there is a specific time window soon after training, during which, the memory is susceptible to sleep deprivation. It has been found that animals sleep deprived 5–10 h after training did not alter fearful memory. The

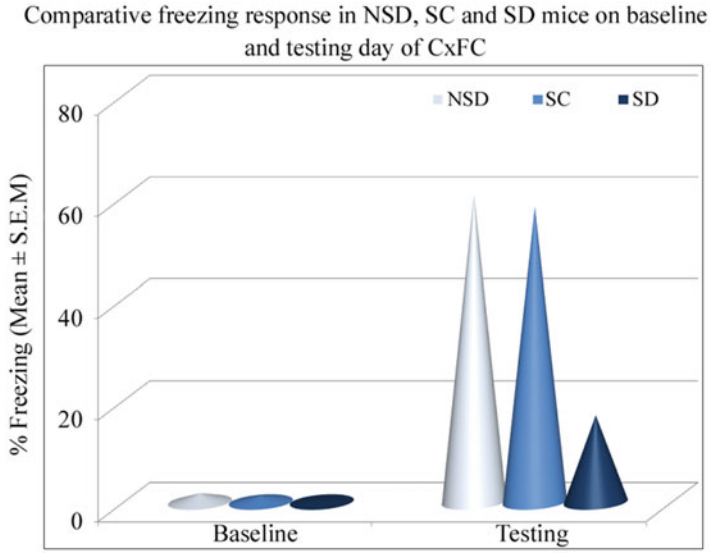

Fig. 8.1 The percent freezing response in the NSD, SC, and SD animals before and after contextual fear conditioning. The NSD, SC, and SD animals exhibited a comparable freezing response on the baseline day. The NSD and SC animals showed an increased freezing response on the testing day. Interestingly, the SD animals exhibited significantly less freezing response ($p < 0.001$, $F_{(2,18)} = 13.82$) as compared to the NSD (Tukey $p < 0.001$) and SC (Tukey $p < 0.01$) animals on the testing day. Abbreviations: *NSD* non-sleep-deprived animals, *SC* stress control animals, *SD* sleep-deprived animals. (Adapted from M. Quereshi's PhD Thesis)

induced freezing response in an altered context in sleep-deprived mice was also comparable to that of non-sleep-deprived animals. Furthermore, the freezing response to the cue was also comparable in the sleep-deprived and non-sleep-deprived mice. These findings suggest that sleep deprivation 5 h after training does not impair fearful memory (Graves et al. 2003).

8.2.3 Effect of Sleep Deprivation and Altered Circadian Cycle on Fear Memory

Sleep plays an essential role in the consolidation of fear memory but, during which circadian phase, is still debatable. Six-hour sleep deprivation immediately after training during the daytime impairs the consolidation of contextual fear memory. However, 6-h sleep deprivation soon after conditioning performed just before the dark onset did not induce memory deficit. Hagewoud et al. (2010) have observed that 6-h sleep deprivation during the night did not induce learning deficit, but 12 h of sleep deprivation indeed caused impairment (Hagewoud et al. 2010). Several other studies also support the view that 6-h sleep deprivation after training during the dark

phase did not induce memory deficit. Six-hour sleep deprivation soon after training in a novel object recognition task at the dark onset period does not affect recognition memory in rodents (Halassa et al. 2009; Palchykova et al. 2009). However, prolonged sleep deprivation after training during the night does impair memory consolidation. Rats sleep less (20–35%) during the dark phase compared to the light phase (65–80% (Borbély and Neuhaus 1979; Lancel and Kerkhof 1989). Therefore, it was argued that the amount of sleep loss during the 6-h sleep deprivation in the dark period is significantly less than the same period of sleep deprivation during the light period. The detrimental effect of sleep deprivation on memory consolidation hence depends on the total amounts of sleep loss during the deprivation period. Also, 6-h sleep deprivation during the late dark phase did not cause memory deficit, but 12-h sleep deprivation immediately after the training during the entire dark period impairs memory. Therefore, it has been proposed that the amount of sleep loss during deprivation is crucial for inducing the detrimental effect of sleep deprivation irrespective of the time of day.

It is not clearly known why sleep is required for memory consolidation. One of the possible explanations could be that sleep may be facilitating memory consolidation by preventing memory interference, which otherwise frequently occurs during wakefulness (Ellenbogen et al. 2006). The ongoing memory can be disrupted during wakefulness mainly due to the processing of various other sensory inputs associated with new experiences. It is also argued that sleep deprivation possibly blocks memory consolidation because of the stress allied with forced wakefulness. If it may be the reason, then sleep deprivation performed, either during the day or night, should have a similar detrimental effect on memory consolidation. But, studies have shown that 6-h sleep deprivation performed by gentle sensory stimulation during the day impairs memory consolidation but not during the dark period, even when the amount of stimulation used under both the conditions matches precisely. Six hours of sleep deprivation during the day and 12 h of sleep deprivation during the night may cause a comparable amount of sleep loss. Therefore, the disruption in memory formation can be attributed to the amount of sleep loss during the deprivation rather than the stress associated with forced wakefulness.

Alteration in the circadian cycle can also influence learning. We have reported that animals conditioned to fear during the night exhibit significantly better conditioning than the animals conditioned to fear during the daytime (Kumar and Jha 2012). Further, we have examined the combined effects of sleep loss and altered circadian phase on fearful learning. Animals were randomly divided into four groups: (a) non-sleep-deprived (NSD) group, (b) extended light period (ELP) group [animals were kept under constant 18 h light (12 h during the daytime +6 h during the dark period)], (c) stress control (SC) group (animals were kept in a movement restricted chamber for 6 h soon after contextual fear conditioning), and (d) sleep-deprived-extended light period (SD-ELP) group (animals were sleep deprived for 6 h soon after contextual fear conditioning under 6-h extended light condition). The percent mean (± SEM) conditioned freezing response in NSD, ELP,

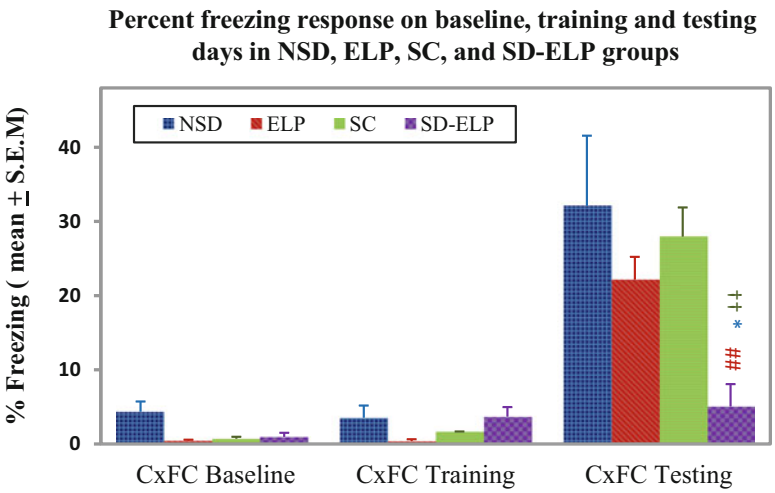

Fig. 8.2 Changes in % freezing response in NSD, ELP, SC, and SD-ELP animals on baseline, training, and testing day. SD-ELP animals exhibited significantly less freezing on CxFC testing day compared to NSD, ELP, and SC animals [$p < 0.05$, $F_{(1,15)} = 7.29$ compared to NSD animal; $p < 0.01$, $F_{(1,13)} = 22.12$ compared to SC animal; $p < 0.01$, $F_{(1,13)} = 15.12$ compared to ELP animal] (*one-way ANOVA followed by Tukey* post hoc *test*). Percent freezing response among NSD, ELP, and SC animals was statistically not significant on post-conditioning day. Percent freezing response on baseline and training days were comparable in all groups. * means $p < 0.05$ compared to NSD group. ## means $p < 0.01$ compared to ELP group. †† means $p < 0.01$ compared to SC group. Abbreviation: *CxFC* contextual fear conditioning, *NSD* non-sleep- deprived, *ELP* extended light period (7:00 PM–1:00 AM), *SC* stress control, *SD-ELP* sleep deprivation during extended light period (7:00 PM–1:00 AM). (Taken from G. Singh's PhD Thesis)

and SC groups were 32.00 ± 9.46, 22.15 ± 3.08, and 27.00 ± 3.93, respectively. However, SD-ELP animals demonstrated significantly less freezing ($5.05 \pm 3.02\%$) compared to NSD ($p < 0.05$; $F_{(1,15)} = 7.29$), ELP ($p < 0.01$; $F_{(1,13)} = 15.12$), and SC ($p < 0.01$; $F_{(1,13)} = 22.12$) (one-way ANOVA followed by Tukey post hoc test) groups during the post-conditioning day (Fig. 8.2). The SD-ELP animals exhibited 89.83%, 72.20%, and 81.30% less freezing compared to NSD, ELP, and SC animals. Freezing response within groups was not significant on the baseline and training days. Animals in the ELP group also exhibited significantly more freezing ($p < 0.001$; $F_{(1,11)} = 49.69$) (one-way ANOVA followed by Tukey post hoc test) on post-conditioning day compared to their baseline day. This suggests that extended light period (6 h) during active phase does not induce learning deficit. Similarly, SC group of animals also exhibited significantly more freezing response ($p < 0.001$; $F_{(1,11)} = 47.74$) (one-way ANOVA followed by Tukey post hoc test) on post-conditioning day suggesting that mild stress does not induce learning deficit. These results suggest that short-term sleep deprivation (6 h) with altered circadian phase induces learning deficit of fearful memory.

8.3 Molecular Mechanisms of Sleep-Dependent Memory Consolidation

The consolidation of fearful memory occurs over a period of hours to days and possibly depends on the short- and/or long-term reversible changes in the neuronal properties. Neuronal plasticity with increased synaptic strength is one of the underlying mechanisms of memory consolidation (Kushner et al. 2005). It has been observed that glutamatergic receptors are selectively upregulated in the synapses, which are involved in the processes of synapse strengthening after learning (Ljaschenko et al. 2013). Further, the sequence of neuronal activation seen during learning reoccurs during the post-learning sleep period as well. Several studies have provided evidence that the cellular and molecular events, which take place during learning, are replayed during post-learning sleep. We discuss below some of the molecular events, which are possibly associated with sleep-dependent memory consolidation.

8.3.1 Sleep Deprivation Alters N-Methyl-D-Aspartate (NMDA) Receptor and α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA) Receptor-Mediated Plasticity

All the three stages of memory, acquisition, consolidation, and retrieval require NMDA receptor activity. The consolidation phase, in which the memory transforms from labile to a stable state, requires significant NMDAR activity (Hernandez and Abel 2011). NMDARs increase Ca^{2+}influx into the cells and thus help induce the phenomenon of long-term potentiation (LTP) (Xia and Storm 2012). Sleep deprivation seems to affect the NMDAR activation by altering receptor subunit composition, surface expression, and hence, reduced Ca^{2+} influx (Chang et al. 2012). It was found that 72 h of sleep deprivation not only reduced the NMDA/AMPA receptor ratio in the hippocampal CA1 pyramidal cells in response to Schaffer collateral stimulation but also reduced the amplitude of NMDA receptor-mediated currents in the distal dendrites of CA1 neurons in the hippocampus. The reduction in the amplitude of NMDA receptor-mediated currents is primarily due to a reduction in the surface expression of NMDA receptors after sleep deprivation. It was observed that the majority of NR1 and NR2A subunits of NMDA receptors were present in the cytoplasm after sleep deprivation suggesting that the surface expression of NMDARs is altered in sleep-deprived animals (McDermott et al. 2006). Prolonged sleep deprivation (24 h) also disrupts NMDAR trafficking to the cell surface and a reduction in NMDA receptor-mediated current (Chen et al. 2006). Few other studies have also observed a sleep deprivation-induced reduction in NR1 subunit expression in the hippocampus. Apart from effects on NMDARs, sleep deprivation also causes

reduced phosphorylation of the GluR1 subunit of AMPA receptors (AMPARs) in the hippocampus. The phosphorylation at S845 is a prerequisite for the incorporation of AMPAR in the synaptic membrane. In addition, dephosphorylation of S845 may increase endocytosis of GluR1 containing AMPA receptors from the membrane. A reduction in phosphorylation of S845GluR1 after sleep deprivation suggests that sleep deprivation possibly alters the incorporation of GluR1 containing AMPA receptors in the membrane (Havekes et al. 2007).

8.3.2 Sleep Deprivation Alters and Attenuates mTOR Expression and Its Signaling

Mammalian target of rapamycin (mTOR) is a key regulator of the translational machinery. Translational regulation in the hippocampus is one of the key steps in memory consolidation (Bekinschtein et al. 2007). Sleep deprivation alters the levels of several proteins such as mTOR and 5′-adenosine monophosphate-activated protein kinase (AMPK). Short-term sleep deprivation decreases not only the total mTOR levels, but it also causes a huge decrease in phosphorylated mTOR level. The changed levels of total as well as phosphorylated mTOR return to their basal level after recovery of sleep from deprivation. These findings suggest that the decreases in total and phosphorylated mTOR could be exclusively associated with sleep loss (Vecsey et al. 2012). The two functionally distinct complexes of mTOR are mTORC1 and mTORC2, in which mTOR remains associated with Raptor and Rictor, respectively. Interestingly, it has been found that 5 h of sleep deprivation specifically decreased mTORC1 level, whereas mTORC2 remains unaltered. The kinase complex mTORC1 phosphorylates and inhibits the translation initiation factor 4E-binding protein-2 (4EBP2) and thereby initiates protein synthesis. The phosphorylated 4EBP2 does not bind to eukaryotic translation initiation factor 4E (eIF4E) and thus allows its binding to eukaryotic translation initiation factor 4G (eIF4G) and trigger translation. AMPK inhibits mTORC1 activity directly as well as indirectly. AMPK inhibits mTORC1 activity indirectly by activating the tuberous sclerosis complex (TSC) or directly by phosphorylating Raptor. It has been shown that 5 h of sleep deprivation significantly increased AMPK phosphorylation and significantly decreased the amount of phosphorylated 4EBP2 in the hippocampus. It has been found in sleep-deprived animals that mTORC1 activity, as well as the level of 4EBP2 phosphorylation, decreases in the hippocampus, suggesting that sleep deprivation could alter the binding efficacy of eIF4E with eIF4G. In a subsequent study, it was clearly shown that 5 h of sleep deprivation reduces eIF4E-eIF4G association in the hippocampus in mice. Therefore, it seems that sleep deprivation alters protein synthesis by influencing the AMPK-mTORC1-4EBP2 signaling pathway. Overall, these findings suggest that sleep deprivation targets 4EBP2-regulated protein synthesis resulting in sleep deprivation-induced memory deficit (Tudor et al. 2016).

8.3.3 Sleep Deprivation Alters Translational and Transcriptional Machinery

Sleep deprivation alters the expression of several genes. The most prominent downregulated gene clusters after sleep deprivation are (1) *Usp2*, *Usp24*, *Usp3*, and *Usp34* genes, which are involved in the regulation of ubiquitination/proteolysis; (2) *Eif2a*, *Eif3s6ip*, *Eif4el3*, and Eif5 genes associated with translation initiation factors; (3) mRNA processing and transport associated genes (*Rbm3* and *Denr*); (4) the nuclear mRNA shuttle *Hnrpdl gene*; and (5) *Cirbp* and *Rbm3* gene associated with cold-induced RNA-binding proteins. In addition, some other gene clusters such as AMP-activated kinase (AMPK) *Prkaa2* gene; *Vldlr* a very low-density lipoprotein receptor gene; genes associated with histone deacetylase activity such as *Sirt5*, *Sirt7*, *Hdac3*, and *Hdac9*; and ion-binding protein classes that require ion cofactors such as *Prkaa2*, *Kcnv1*, *Kcnk2*, *Camk4*, *Zswim1*, and *Nfx1* are also downregulated after sleep deprivation. Few of the gene clusters are upregulated after sleep deprivation, which are (1) nucleosomes/chromatin assembly having *Elk1* and *Fos* as transcription factors; (2) *H2afj*, *Hist1h2bc*, and *Hist3h2a* genes of histone family members; (3) *Rab8b*, *Rab15*, and *Rab21* genes of RAS oncogene family; and (4) *Hspa8*, *Hsp110*, and three *Hsp40* homologs from heat shock family of proteins. Some other genes such as (1) *Fos*, *Elk1*, *Nr4a1*, *Creb1*, and *Crem* associated with transcription factors and positive regulation of transcription; (2) *Nr4a1*, *Lats2*, and *Dusp19* genes involved in the negative regulation of kinase activity; and (3) genes associated with ion-binding (*Adamts2* and *Lats2*) and ATP/nucleotide binding (*Hspa8*, *Lats2*) are also upregulated after sleep deprivation (Vecsey et al. 2012). These studies have demonstrated that sleep deprivation simultaneously downregulates the gene clusters associated with the translation initiation genes and RNA-binding genes (many of which help mRNA to shuttle from the nucleus to ribosomes for translation) and upregulates the genes associated with the unfolded protein response. This suggests that inadequate sleep and sleep disturbances can directly alter the expression of many genes and proteins.

8.3.4 Sleep Deprivation Impairs cAMP Signaling

Sleep deprivation significantly reduces many signaling molecules including PKA activity and cAMP levels in the hippocampus. The levels of calcium and adenylyl cyclase enzyme, which are involved in the production of cyclic adenosine monophosphate (cAMP), transiently increase after learning (Xia and Storm 2012). The cAMP activates mainly three downstream targets, which play an essential role in protein synthesis as well as in memory consolidation: (a) protein kinase A (PKA), (b) exchange protein activated by cAMP (Epac), and (c) hyperpolarization-activated

cyclic nucleotide-gated channels (HCN) (Arnsten 2007). These downstream targets, along with other kinases, for example, calmodulin-dependent protein kinase (CAMKII), extracellular signal-regulated kinase (ERK1/ERK2), and mitogen-activated protein kinase (MAPK), are activated, which are primarily involved in the phosphorylation of transcription factors and in turn involved in protein translation (Enslen et al. 1994). It has been reported that the transcription factor "cAMP response element binding protein" (CREB) plays an essential role in memory conversion from a labile form to a stable state. Sleep deprivation significantly reduces both cAMP levels and PKA activity in the hippocampus, thereby, disrupting the downstream signaling pathway. Also, sleep deprivation alters phosphorylation of CREB by PKA at serine 133 in the hippocampus (Vecsey et al. 2009).

Phosphodiesterase-4 (PDE4) enzyme periodically degrades cAMP and thus plays an essential role in maintaining an optimum balance between PKA activity and cAMP. The level of cAMP decreases after sleep deprivation because of the increased activity of PDE4. Protein expression of PDE4A5, an isoform of PDE4, increases after short-term total sleep deprivation (Vecsey et al. 2009). It has also been found that blocking the PDE4 signaling during sleep deprivation rescues sleep deprivation-mediated deficit in LTP induction as well as the hippocampus-dependent memory consolidation suggesting that sleep deprivation alters cAMP-PKA pathway, which in turn affects memory.

8.3.5 Sleep Deprivation Increases Inhibitory G Protein-Coupled Receptor (GPCR) Signaling

Adenosine through its "A1" receptor activates Gi proteins and inhibits cAMP production in the cells. The extracellular levels of adenosine increase in the brain with increased metabolism (Radulovacki 1985). It has been found that the adenosine levels increase during wakefulness and decrease during sleep, and an increased level during wakefulness augments sleep drive (Porkka-Heiskanen et al. 1997). It has been reasoned that the prolonged wakefulness increases adenosine level, which acts through its A1 receptors and causes neuronal hyperpolarization and attenuation in neurotransmitter release around the surrounding excitatory presynaptic terminals (Newman 2003). The adenosine receptor A1 is coupled with Gi proteins resulting in the inhibition of cAMP production. Therefore, it is possible that the increased adenosine level with prolonged wakefulness will lead to increased G protein-coupled receptor (GPCR) signaling and decreased cAMP levels. The decreased cAMP levels would eventually impair memory consolidation. Caffeine, a nonselective A(1)/A(2) adenosine receptor antagonist, improves cognitive performance in humans. It facilitates memory retention when administered immediately after training, but not when administered 3 h later (Kopf et al. 1999).

8.3.6 *Restoration of Sleep Deprivation-Mediated Impairment in Memory Consolidation*

Sleep deprivation-mediated detrimental effects on memory consolidation can be rescued by activating specific molecular pathways associated with learning (Fig. 8.3). CREB-mediated gene transcription is one of the underlying mechanisms of long-term memory formation (Vecsey et al. 2007). An increased cAMP level activates PKA, which in turn enhances CREB-mediated gene transcription. Therefore, one possible mechanism for restoring the sleep loss-mediated impairment in memory consolidation could be the increased levels of cAMP in the hippocampus during sleep deprivation.

It has been reported that sleep deprivation associated deficits in cAMP signaling, synaptic plasticity, and hippocampus-dependent memory can be recovered if the animal is treated with PDE inhibitor (Vecsey et al. 2009). The memory

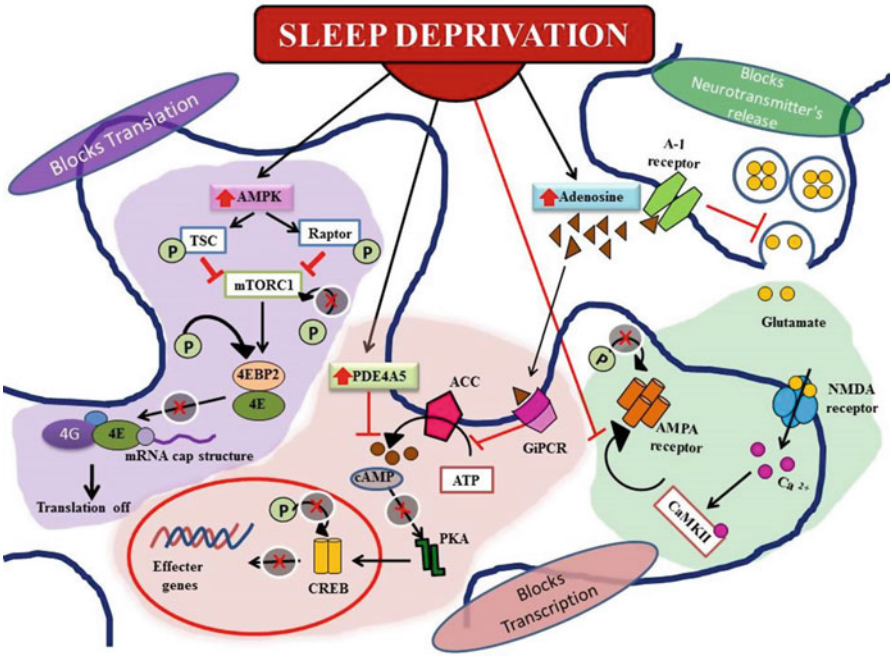

Fig. 8.3 A model showing that sleep deprivation affects transcriptional and translational machinery as well as neurotransmitter release in the neurons. (i) Sleep deprivation attenuates protein synthesis initiation by altering the AMPK-mTORC1-4EBP2 signaling pathway and eIF4E-eIF4G association in the hippocampus. (ii) In addition, sleep deprivation significantly reduces both, cAMP level and PKA activity in the hippocampus, thereby disrupting the downstream signaling pathway. Sleep deprivation also alters phosphorylation of CREB by PKA in the hippocampus. (iii) Sleep deprivation causes reduced phosphorylation of the GluR1 subunit of AMPA receptors in the hippocampus and thus impairs the recruitment of AMPA receptor in the membrane

consolidation processes become resistant to sleep loss after systemic injections of the nonspecific PDE4 inhibitor rolipram (Vecsey et al. 2009). It suggests that sleep deprivation alters the cAMP signaling, which may cause memory deficits. The increased adenosine level during prolonged forced wakefulness inhibits cAMP production through the activated adenosine A1 receptors. A1 receptors inhibit the production of adenylyl cyclase and thus decrease cAMP levels in the neurons. Interestingly, the chronic infusion of "8-cyclopentyl-1,3-dimethylxanthine" (CPT), a blocker of adenosine A1 receptor, prevents the sleep deprivation-mediated alteration in the memory consolidation and induction of hippocampal LTP (Havekes et al. 2012). It has also been reported that caffeine (an A1 receptor antagonist) helps prevent the sleep loss-mediated detrimental effects on the hippocampal synaptic plasticity and memory (Alhaider et al. 2011). Caffeine inhibits phosphodiesterase (PDEs) (such as PDE1, PDE4, and PDE5) and also promotes calcium release from intracellular stores and thereby helps in memory consolidation (Wu et al. 2009; Ribeiro and Sebastiao 2010).

The GluA1 subunit of the AMPA receptor is another candidate in the cAMP-PKA signaling pathway, which is involved in memory consolidation and synaptic plasticity (Havekes et al. 2007). Sleep loss impairs memory consolidation by reducing hippocampal AMPAR functioning including AMPAR GluA1 phosphorylation (Hagewoud et al. 2010). Therefore, if the levels of cAMP levels are increased with pharmacological intervention during sleep deprivation, it may restore the functioning of AMPAR and, in turn, facilitate memory consolidation. It, however, needs to be determined, if the increased cAMP levels during sleep deprivation reestablish the functions of AMPAR and levels of CREB and phosphorylated PKA and Epac substrates and rescue the sleep deprivation-induced memory deficit.

Histone deacetylase (HDAC) inhibitors can also be one of the potential candidates, which can restore sleep deprivation-mediated memory deficit. The administration of HDAC inhibitors reinstates learning-induced gene expression and memory function in aged animals (Peleg et al. 2010). Suberoylanilide hydroxamic acid (SAHA) is the first of the new HDAC inhibitors, which is being used to restore pathophysiology in cancer patients (Dokmanovic et al. 2007). It is also now well known that epigenetic machinery regulates contextual fear memory, and histone acetylation, specifically of H4K12, significantly increases after contextual fear conditioning (Peleg et al. 2010). Our lab has also further investigated (a) the effects of short-term sleep deprivation in an extended light period on acetylation of histone H4K12 soon after fear conditioning and (b) the role of HDAC inhibitor SAHA in rescuing the alteration in fear memory after sleep deprivation under altered circadian conditions (SD-ELP). We found that SAHA microinjection in the hippocampus soon after fear-conditioned training in the SD-ELP animals rescued sleep deprivation-dependent learning impairment. The SAHA-treated SD-ELP animals exhibited significantly more freezing response as compared to the vehicle-treated SD-ELP animals (Fig. 8.4).

Further, we observed that the acetylation of H4K12 significantly increased after contextual fear training compared to the control animals (Fig. 8.5). Interestingly, acetylation level decreased in SD-ELP animals after contextual fear training,

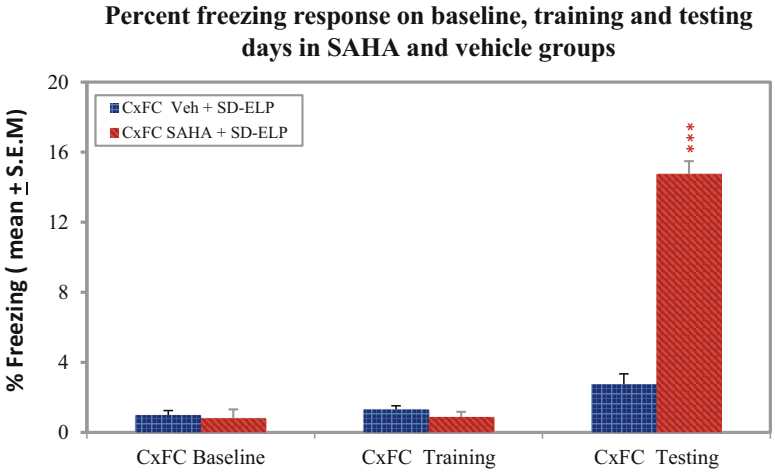

Fig. 8.4 Changes in % freezing response in SAHA + SD-ELP and vehicle + SD-ELP animals on baseline, training, and testing days. SAHA-treated animals exhibited significantly more freezing on testing day compared to baseline and training. The percent freezing response on baseline and training days was comparable in both group, but percent freezing response on testing day was significantly increased in SAHA + SD-ELP animals compared to vehicle + SD-ELP animals ($p < 0.001$, $F_{(1, 11)} = 156.612$) *** means $p < 0.001$ compared to CxFCVeh+SD-ELP group (*one-way ANOVA followed by Tukey* post hoc *test*). Abbreviations: *CxFC* contextual fear conditioning, *SD-ELP* sleep deprivation during extended light period (7:00 PM–1:00 AM). (Taken from G. Singh's PhD Thesis)

suggesting that sleep deprivation alters histone acetylation. SAHA microinjection in the dorsal hippocampus significantly increases H4K12 acetylation in normal and CxFC trained SD-ELP animals (Fig. 8.5). These results demonstrate that the sleep loss-mediated memory deficit can also be rescued by enhancing H4K12 histone acetylation.

8.4 Reactivation of Fear Memory During Sleep

Sleep possibly helps consolidate memories by facilitating the integration and strengthening of the neural circuitries (Diekelmann and Born 2010; Lewis and Durrant 2011; Rasch and Born 2013; Stickgold and Walker, 2013). Wilson and McNaughton (1994) have reported that the information acquired during acquisition is re-expressed in the hippocampal circuitry during sleep. They observed that some hippocampal place cells fired together when the animals explored a particular location in the environment. Interestingly, the cells which fired together during exploration exhibited an increased tendency to fire together during the subsequent sleep period (Wilson and McNaughton 1994). The *memory replay* during sleep might follow the same temporal order in which the cells fired during spatial exploration (Wilson and McNaughton 1994; Knierim 2009). Neuronal activity

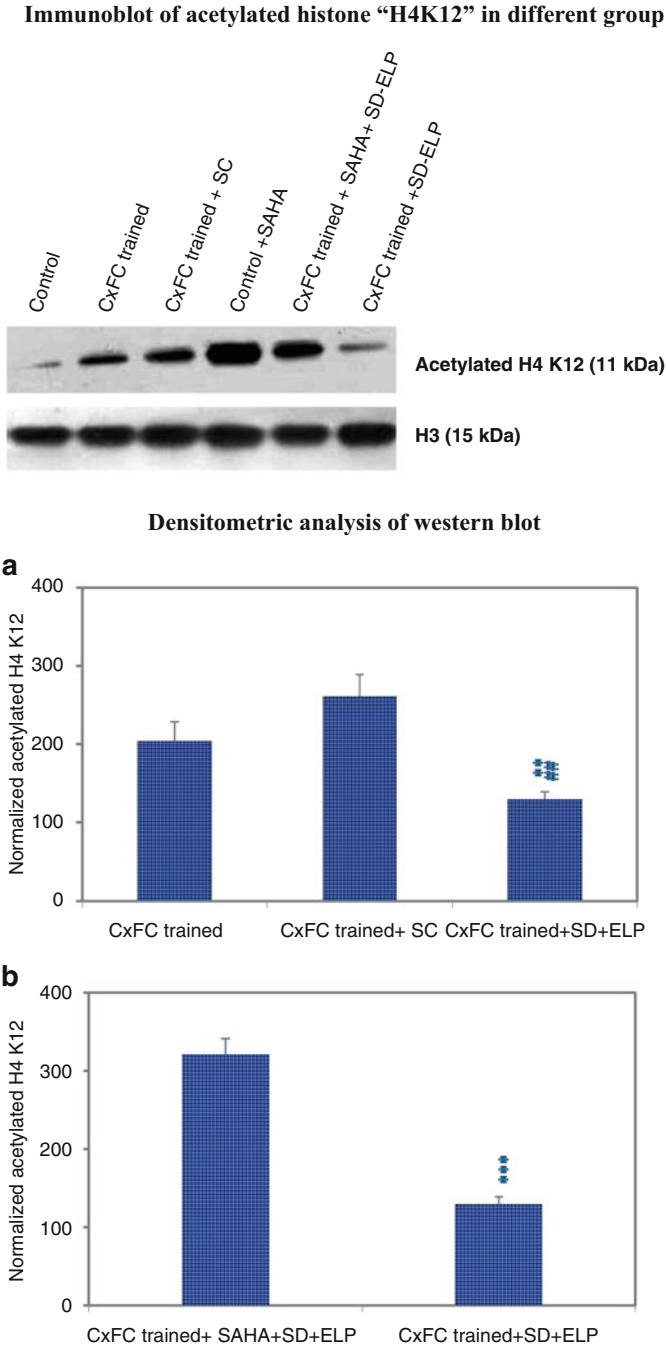

Fig. 8.5 Western blot and densitometric analysis of western blot showing the changes in acetylation of histone "H4K12" in different groups. The control animals (no CxFC) exhibited a lowest basal level of acetylation. The level of acetylated H4K12 increased in CxFC trained and CxFC trained stress control (CxFCtrained+SC) animals 6 h after training. Interestingly, it significantly decreased in CxFC trained sleep-deprived (CxFC+SD-ELP) animals after 6 h of sleep deprivation. In CxFC-training+SAHA-treated animals, level of acetylated H4K12 increased significantly, which also remained elevated in the CxFC trained sleep-deprived SAHA-treated animals (CxFCtrained+SAHA+SD-ELP). (Taken from G. Singh's PhD Thesis)

patterns observed during learning particularly in the hippocampus are replayed during sleep which seems to be an essential component of memory processing (Breton and Robertson 2013). The active system consolidation hypothesis posits that memory consolidation underlies the reactivation of the recently encoded memory representations (Rasch and Born 2013). In some human studies, sensory cues (auditory or olfactory) have been used to reactivate memories during the night, and it was observed that it robustly influenced memory strengthening during their subsequent recall (Rudoy et al. 2009; Antony et al. 2012).

8.5 Reactivation of Fear Memory During NREM Sleep

The spontaneous reactivation phenomena that have been shown to occur during NREM sleep mediates the transfer of temporarily stored information in the hippocampus to long-term storage sites (Lee and Wilson 2002). NREM sleep is characterized by events like sharp-wave ripples (SW-R) in the hippocampus along with slow neocortical and thalamic oscillations. The hippocampal SW-R plays an essential role in the consolidation of spatial memory. The disruption in the hippocampal SW-R activity during the post-learning period impairs memory, whereas, augmentation in neocortical slow-waves potentiates memory (Barnes and Wilson 2014). Sleep or sleep-like state is an ideal condition for memory consolidation because it helps in minimizing interference from external events as well as facilitates the flow of sensory information from the thalamus to the neocortex (Murakami et al. 2005). The re-exposure of the sensory stimuli associated with learning during NREM sleep can boost memory consolidation possibly by facilitating the effectiveness of repeated reactivations (Rasch et al. 2007). Nakashiba et al. have provided somewhat direct evidence regarding the facilitatory role of slow waves during NREM sleep in memory consolidation. They altered SW-R-associated reactivation of CA1 neurons by genetically blocking the hippocampal CA3 output. It was observed that SW-R reduction through genetic manipulation significantly impaired the consolidation of contextual fear memory (Nakashiba et al. 2009). A similar finding has been found in another study that re-exposure of the odor cues during NREM sleep in human subjects facilitated memory consolidation, whereas the re-exposure of the odor cue during REM sleep did not (Rasch et al. 2007). These reports clearly demonstrate that NREM sleep-associated events such as slow oscillatory activity, SW-R, sleep spindles, etc. facilitate neural reactivation during NREM sleep and thus contribute to memory stabilization (Cordi et al. 2014).

8.6 Reactivation of Fear Memory During REM Sleep

The experience-mediated neuronal activation during wakefulness also reoccurred in a similar way during REM sleep. Hennevin and his group have observed that neurons of the dorsal hippocampus and medial geniculate nucleus are highly

activated after cued fear conditioning. For fear conditioning, they have used tone as the conditioned stimulus paired with the unconditioned stimulus "foot shock." Following training, the non-awakening tone alone was represented during REM sleep. They observed that some neurons in the hippocampus and the auditory thalamus exhibited enhanced tone responsiveness during REM sleep (Hennevin et al. 1993). A similar study was carried out with multiunit recordings in the auditory thalamus and lateral amygdala. It was observed that the conditioned neural responses to fear largely increase in the lateral amygdala and medial geniculate nucleus during REM sleep. These results suggested that neurons involved in emotionally conditioned responses are capable of responding to the reinforcing stimuli during REM sleep (Hennevin et al. 1995).

Now, an intriguing question is, can the conditioned response be expressed during wake if the conditioning is performed during sleep? There is evidence suggesting that animals elicit conditioning response during the wake period when the animals are conditioned to lick behavior during sleep. This was ascertained by using the second-order conditioning protocol (Hennevin and Hars 1992). Animals were trained to lick-training along with low-level electro-tactile stimulation (ETS) to the ear during the alert condition. On the following day, the animals were exposed to second-order pairing with a tone signaling ETS delivery while they were either awake or asleep. Animals were subsequently tested for the acquisition of second-order association by monitoring lick-suppression response to the tone. It was reported that animals acquiring tone-ETS association during both, NREM and REM sleep, exhibited lick suppression to the tone while they were awake. In another similar study, Hennevin and colleagues (Hars et al. 1985) trained rats for active avoidance task, in which, an aversive foot shock (US) was associated either with a tone or a mild electrical shock to the ear (CS). During the post-learning period, the CS was redelivered to the rats during REM sleep episodes in the absence of US. Re-exposure of non-awakening ear shocks not only increased the duration of REM sleep but also significantly improved subsequent active avoidance performance (Hars et al. 1985). These results suggest that the paired external sensory stimuli presented either during NREM or REM sleep could induce conditioned behavioral response during wake.

Growing evidence undoubtedly suggest that memory reactivation induced by external cues during sleep evidently strengthened emotional memories. It is believed that neuronal activation occurs during both NREM and REM sleep, and sleep-stage-specific sleep components such as theta waves during REM sleep and delta waves during NREM sleep contribute to neuronal activation. Rats trained to shock avoidance learning showed increased theta power during REM sleep and subsequent increase in sleep spindles in NREM sleep (Fogel et al. 2009). On the other hand, a decrease in REM sleep theta power and increased delta power during NREM sleep were observed after fear conditioning (Hellman and Abel 2007). In addition, it has been found that synchronization of theta frequencies increases in the amygdalo-hippocampal network after fear conditioning (Seidenbecher et al. 2003). These studies suggest that sleep components are possibly enhancing the neuronal communication between different circuitries and inducing both, memory strengthening and memory stability.

8.7 The Role of Sleep in Fear Extinction

The conditioned fear responses can be weakened by a phenomenon called "fear extinction." Previously fear-conditioned animals/subjects, if repeatedly exposed to the conditioned context in the absence of any aversive stimulus, may weaken their negative conditioned memory (Myers et al. 2006). Fear extinction is not similar to forgetting; rather during the process, a new memory is formed where animals/subjects learn that the CS is not paired with the US, and thus the life-threatening feeling diminishes (Myers and Davis 2007). Extinction phenomena can be termed as "inhibitory learning," as a fear response is inhibited when the CS-US association is abolished (Quirk et al. 2010). It is well established that hippocampal-cortical circuits play a central role in the formation of CS representations, whereas CS-US associations are believed to be processed by the basolateral amygdala (Kim and Fanselow 1992; Phillips and LeDoux 1992; Debiec et al. 2010). It has also been shown that the PFC neurons are recruited during fear extinction, which inhibit the output regions of the amygdala and thereby reduce the fear response (Maren and Quirk 2004).

8.7.1 REM Sleep and Fear Extinction

REM sleep also plays an essential role in the consolidation of extinction memory. REM sleep-deprived animals exhibited normal retention of cued and contextual fear-conditioned memories but showed impairments in the extinction of cued fear memory (Silvestri 2005). Later, it was demonstrated more clearly that REM sleep deprivation impairs recall of the hippocampal-independent fear extinction memory, but not the hippocampal-dependent fear extinction memory (Fu et al. 2007). Interestingly, 6-h REM sleep deprivation immediately after training significantly altered extinction memory, but REM sleep deprivation 6 h after training had no effect (Fu et al. 2007). These results provide evidence that REM sleep may be playing a crucial role in the consolidation of extinction memory.

REM sleep deprivation possibly alters NMDA receptor sensitivity and/or its associated events, which may be involved in extinction memory. Administration of NMDA receptor agonist "d-cycloserine" to REM sleep-deprived animals partially reversed extinction memory deficit, suggesting that REM sleep deprivation-mediated effects on extinction memory could be partially mediated through the NMDA-dependent mechanisms (Silvestri and Root 2008). In addition, potentiation of phasic P-wave activity in the brainstem during post-training REM sleep plays a crucial role in the consolidation of fear extinction memory. It has been proposed that post-training increased sleep amount alone may not be enough to retain the fear extinction memory; the augmented sleep-associated events can also play a crucial role in memory consolidation (Datta and O'Malley 2013).

8.8 Fear Memory Interference During Sleep

The neural replay during sleep contributes to memory strengthening (Abel et al. 2013). It has been shown that *targeted memory reactivation* strengthens memories (Rasch et al. 2007; Rudoy et al. 2009; Antony et al. 2012), but it is not yet known if the same targeted memory reactivation tool can be used to weaken negative memories. Two recent studies in rats and humans have used this approach to abolish negative memories. Rolls and group (2013) have conditioned mice to fear, during which the foot shocks (US) were delivered in the presence of an odor. Twenty-four hours later, the CS odor was again presented during sleep. They observed an increased freezing response during wakefulness. Thereafter, they bilaterally injected protein synthesis inhibitor specifically into the basolateral amygdala before re-exposing the CS odor during sleep. Interestingly, blocking new protein synthesis during targeted memory reactivation led to a decline in the fear response on the following day. These findings suggest that interference in the reactivation processes of targeted fear memory during sleep can also cause memory deficit.

Furthermore, Hauner and colleagues conditioned 15 healthy humans differently (Hauner et al. 2013). All the volunteers underwent olfactory contextual conditioning. The images of different faces as CS were shown to them. Every image was paired with a neutral smell such as lemon or mint, but some of them were paired with mild electrical shock (US). The conditioning procedure was performed inside a fMRI scanner, and the skin conductance response (SCR) and the amygdala and hippocampal activities were monitored. Following conditioning, volunteers were allowed to sleep for about 70 min outside the scanner. During the nap time, the participants were re-exposed to one of the smells they had earlier experienced during the conditioning. It was observed that odor re-exposure gradually diminished the fear response, as measured by the changes in SCR and fMRI. When the subjects woke up, same face images were shown to them. Projection of the face images elicited fearful responses only with those images to which the corresponding odor was re-exposed during sleep. In addition, they observed that the amygdalar activity associated with fear extinction during sleep was entirely different from that observed at the time of conditioning. These findings suggest that the extinction of fear memory can be accomplished without having a conscious perception of the re-exposed context.

In another study, the role of sleep in the consolidation of fear extinction memory has been demonstrated (He et al. 2015). In their study, humans were subjected to cued fear conditioning, and an increase in skin conductance response to the conditioned stimulus was considered as an outcome measure of fear. All subjects were allowed to sleep for 4 h. During NREM sleep, volunteers were repeatedly re-exposed to the tone, which led to a decrease in the subsequent fear response, as compared to subjects who were either exposed to a different tone during NREM sleep or no tone at all. These studies support the view that presenting cues during sleep indeed gradually attenuates fear responses as well.

8.9 The Consolidation of Fear Memory Requires NREM Sleep

Within sleep, the proportion of NREM sleep significantly increases after the consolidation of fear memory, and sleep architecture does not change if memory is impaired (Kumar and Jha 2017). Further, REM sleep significantly decreases after the consolidation of fear memory, but REM sleep does not change if the consolidation of fearful memory processes is altered (Kumar and Jha 2017). These findings suggest that there could be a high probability of augmentation of NREM sleep after fear conditioning, and it could be associated with the consolidation of fearful memory. To demonstrate this, we have taken two independent approaches: (i) the expression of NREM and REM sleep were correlated with the amount of freezing response, and (ii) the Bayesian rule was applied to predict if there is a high probability of NREM sleep augmentation after successful consolidation of fear memory, and low probability of NREM sleep augmentation after fear memory was impaired.

8.9.1 The Expression Ratio of NREM/REM Sleep Correlates with the Freezing Response

The expression ratio of NREM/REM sleep significantly correlates with the freezing response in consolidated memory group, while it did not correlate in memory-impaired group (Fig. 8.6). We performed Pearson correlation test between NREM/REM ratio (out of sleep time) and percent freezing amount on the training and testing days. It was observed that NREM/REM ratio (out of total sleep time) did not correlate with percent freezing response on the training day ($r = 0.14$, $p = 0.28$). While, on the testing day, NREM/REM ratio significantly positively correlated with percent freezing response ($r = 0.36$, $p < 0.01$) (Fig. 8.6). On the other hand, the expression ratio of NREM/REM sleep did not significantly correlate in the memory-impaired group. A significant positive correlation between NREM/REM ratio and freezing response in the consolidated memory group suggests that the consolidation of fear memory is sleep-dependent.

8.9.2 The Bayesian Predictions for the Probability of NREM Sleep Augmentation in Memory-Consolidated and Impaired Groups

Bayesian probabilistic model is one of the popular approaches used increasingly to understand learning and cognition (Jacobs and Kruschke 2011; Shankle et al. 2013). We have used the Bayesian rule to predict if there is (a) a high probability of NREM

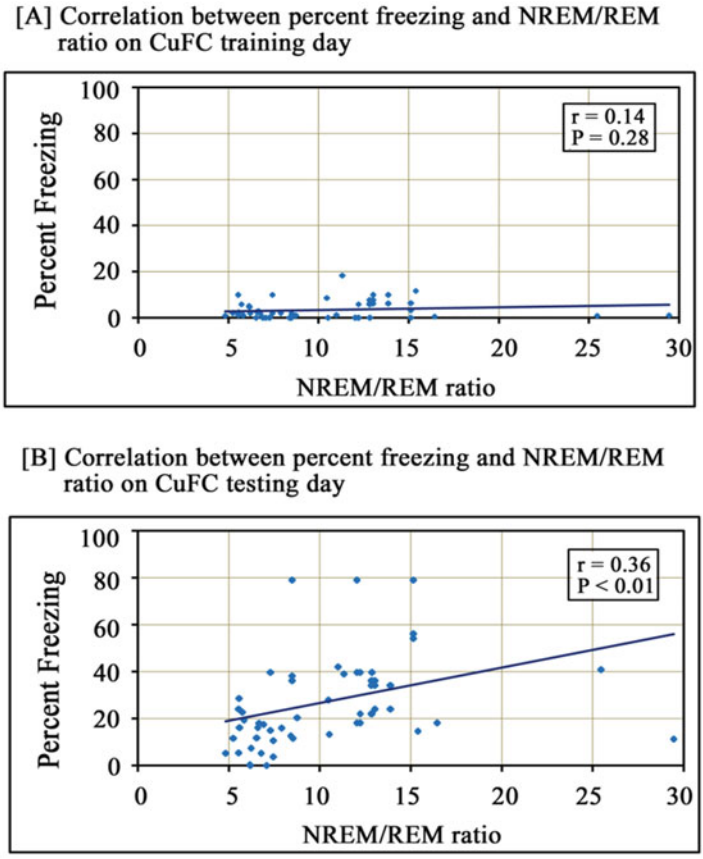

Fig. 8.6 Correlation between percent freezing response and NREM/REM ratio (out of total sleep time) ($n = 64$) on CuFC training and testing days. (**a**) NREM/REM ratio did not correlate with percent freezing response on the CuFC training day ($r = 0.14$; $p = 0.28$). (**b**) On the CuFC testing day, NREM/REM ratio significantly positively correlated with percent freezing response ($r = 0.36$, $p < 0.01$) in the memory-consolidated group. Abbreviations: *CuFC* cued fear conditioning. (Taken from T Kumar's PhD Thesis)

sleep augmentation after CuFC memory was consolidated and (b) a low probability of NREM sleep augmentation after CuFC memory was impaired (pharmacologically). Animals which demonstrated successful cued fear conditioning ($n = 41$) were placed in the memory-consolidated (MC) groups. On the other hand, we pharmacologically altered cued fear conditioning in 23 animals and placed them in memory-impaired (MI) group. We recorded sleep in all animals before and after conditioning and changes in NREM and REM sleep amount on the conditioning day were compared with before conditioning day. We found that REM sleep significantly decreased, but NREM sleep significantly increased out of total sleep time in a memory-consolidated group on the conditioning day. Statistically significant or nonsignificant increase in NREM sleep after CuFC training was determined in

Table 8.1 Number of animals divided into four groups in the Bayesian table

	MC	MI	Total
$NREM^{(inc)}$	31	2	33
$NREM^{(no\text{-}inc)}$	10	21	31
Total	41	23	64

$NREM^{(inc)}$= NREM sleep significantly increased
$NREM^{(no\text{-}inc)}$= NREM sleep did not increase
MC = Memory consolidated
MI = Memory impaired
Taken from Tankesh's PhD Thesis

each animal by computing mean of hourly NREM sleep and was compared with the baseline of the same animal using one-way RM-ANOVA followed by Tukey post hoc test. The animals were then accordingly assigned to any of the four groups: Group I, significant increase in % NREM sleep ($NREM^{inc}$) in MC group; Group II, nonsignificant changes in % NREM sleep ($NREM^{no\text{-}inc}$) in MC group; Group III, nonsignificant changes in % NREM sleep ($NREM^{no\text{-}inc}$) in MI group; and Group IV, significant changes in % NREM sleep ($NREM^{inc}$) in MI group (Table 8.1).

We found that out of total 41 animals in the memory-consolidated group, NREM sleep significantly increased in 31 animals, while it did not change in 10 animals. Accordingly, we placed them in the memory-consolidated group but under increased or no change in NREM sleep groups. Similarly, in 23 animals in the memory-impaired group, 2 animals showed significantly increased NREM sleep, but 21 animals showed no change in NREM sleep. Hence, we placed them in memory-impaired but under increased or no change in NREM sleep group, respectively (Table 8.1).

We applied Bayes' rule to predict if there is (a) high probability for the expression of augmented NREM sleep after the consolidation of cued fear memory and (b) low probability for the expression of augmented NREM sleep after cued fear memory was impaired.

Bayes' formula:

$$P(A/B) = \frac{P(B/A) \times P(A)}{P(B/A) \times P(A) + P(B/A) \times P(B)}$$

where $P(A)$ is the probability of event A; $P(B)$ is the probability of event B; $P(A/B)$ is the probability of event A when B is present; and $P(B/A)$ is the probability of event B when A is present.

(i) **The probability for NREM Sleep augmentation after CuFC memory consolidation:**

$$P\left(\mathrm{MC}/\mathrm{NREM}^{(\mathrm{inc})}\right) = \frac{P\left(\mathrm{NREM}^{(\mathrm{inc})}/\mathrm{MC}\right) \times P(\mathrm{MC})}{P\left(\mathrm{NREM}^{(\mathrm{inc})}/\mathrm{MC}\right) \times P(\mathrm{MC}) + P\left(\mathrm{NREM}^{(\mathrm{inc})}/\mathrm{MI}\right) \times P(\mathrm{MI})}$$

$$P\left(\mathrm{MC}/\mathrm{NREM}^{(\mathrm{inc})}\right) = \frac{\frac{31}{41} \times \frac{41}{64}}{\frac{31}{41} \times \frac{41}{64} + \frac{2}{23} \times \frac{23}{64}}$$

$$P\left(\mathrm{MC}/\mathrm{NREM}^{(\mathrm{inc})}\right) = \frac{0.48}{0.48 + 0.03} = 0.94$$

The high predictive value 0.94 suggests that there is a high probability of NREM sleep augmentation after CuFC memory consolidation.

(ii) **The probability for NREM Sleep augmentation after CuFC memory impairment:**

$$P\left(\mathrm{MI}/\mathrm{NREM}^{(\mathrm{inc})}\right) = \frac{P\left(\mathrm{NREM}^{(\mathrm{inc})}/\mathrm{MI}\right) \times P(\mathrm{MI})}{P\left(\mathrm{NREM}^{(\mathrm{inc})}/\mathrm{MI}\right) \times P(\mathrm{MI}) + P\left(\mathrm{NREM}^{(\mathrm{inc})}/\mathrm{MC}\right) \times P(\mathrm{MC})}$$

$$P\left(\mathrm{MI}/\mathrm{NREM}^{(\mathrm{inc})}\right) = \frac{\frac{2}{23} \times \frac{23}{64}}{\frac{2}{23} \times \frac{23}{64} + \frac{31}{41} \times \frac{41}{64}}$$

$$P\left(\mathrm{MC}/\mathrm{NREM}^{(\mathrm{inc})}\right) = \frac{0.03}{0.03 + 0.48} = 0.06$$

A minimal predictive value 0.06 suggests that there is no probability of NREM sleep augmentation after CuFC memory impairment.

The Bayes' rule clearly demonstrated that there is a high probability of NREM sleep augmentation after the consolidation of CuFC memory and low probability for NREM sleep augmentation after memory impairment. It further suggests that the consolidation of CuFC memory requires NREM sleep for memory consolidation.

8.10 Conclusions

It has been widely discussed globally since years to find out how can bad/negative memories could be erased in humans. Our findings, that post-learning NREM sleep facilitates fear memory, suggest that short-term sleep deprivation soon after experiencing traumatic events could potentially be used to prevent the consolidation of negative memories. It, however, needs further study mainly on human subjects before the approach is adapted for therapeutic use.

Further, we have found that REM sleep is decreased after fear conditioning, which may play the role of a negative modulator for the consolidation of fear memory. Our results show that decreased REM sleep after fear conditioning could be an obligatory phenomenon for the consolidation of fear memory. It thus suggests that the development and use of REM sleep inducing drugs can be used as potential

treatments to erase negative or traumatic memories soon after experiencing traumatic events.

Finally, our results also show that sleep modulates epigenetic regulation of gene expression underlying consolidation of contextual fear memory. Similar to cancer therapy, drugs which can alter histone acetylation at epigenetic level can possibly be used as a therapeutic measure to counteract the development of anxiety disorders such as PTSD in humans.

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