A time for learning and a time for sleep: the effect of sleep deprivation on contextual fear conditioning at different times of the day

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ABSTRACT

Sleep deprivation (SD) negatively affects memory consolidation, especially in the case of hippocampus-dependent memories. Studies in rodents have shown that 5 hours of SD immediately following foot shock exposure selectively impairs the formation of a contextual fear memory. In these studies, both acquisition and subsequent SD were performed in the animals' main resting phase. However, in everyday life, subjects most often learn during their active phase. Here we examined the effects of SD on memory consolidation for contextual fear in rats when the task was performed at different times of the day, particularly, at the beginning of the resting phase or right before the onset of the active phase. Results show that SD immediately following training affects consolidation of contextual fear, independent of time of training. However, in the resting phase memory consolidation was impaired by 6 hours of post-training SD, whereas, in the active phase, the impairment was only seen after 12 hours of SD. Since rats sleep at least twice as much during the resting phase compared with the active phase, these data suggest that the effect of SD depends on the amount of sleep that was lost. Also, control experiments show that effects of SD were not related to the amount of stimulation the animals received and were therefore not likely an indirect effect of the SD method. These results support the notion that sleep immediately following acquisition, independent of time of day, promotes memory consolidation and that SD may disrupt this process depending on the amount of sleep that is lost.
INTRODUCTION

There is growing evidence that sleep plays a role in learning and memory processes (Graves et al., 2001; Maquet, 2001; Stickgold, 2005; Ellenbogen et al., 2006; Walker and Stickgold, 2006). A common approach to study the role of sleep in learning and memory processes is the use of sleep deprivation (SD). This approach is important not only from a fundamental perspective, but also from a social and clinical perspective, since disrupted and restricted sleep are major problems in our society (Bonnet and Arand, 1995; Rajaratnam and Arendt, 2001). Numerous studies have shown that SD after acquisition of a learning task has a negative effect on memory consolidation and subsequent performance in both humans (Karni et al., 1994; Stickgold et al., 2000; Ferrara et al., 2006; Mograss et al., 2009) and animals (Smith and Rose, 1996, 1997; Graves et al., 2003; Bjorness et al., 2005; Hairston et al., 2005; Palchykova et al., 2006; Alvarenga et al., 2008; Vecsey et al., 2009).

A well-known learning task to study the role of sleep in memory and the effects of SD in rodents is the fear conditioning paradigm. In this task, animals learn to associate a specific context (the test environment) or a conditioned stimulus (for example, a tone cue) with an aversive unconditioned stimulus (foot shock). When the animals are later exposed to the same context or cue, aversive learning is expressed, and animals will exhibit a fear-related freezing response (Blanchard and Blanchard, 1969; Fanselow, 1980). Both contextual and cued fear learning involve the amygdala. However, contextual fear learning also depends on the hippocampus (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Chen et al., 1996). Studies in mice have examined the effect of 5 hours of SD immediately following training in a fear conditioning paradigm and have shown that SD selectively impairs memory consolidation for contextual fear but not cued fear (Graves et al., 2003; Vecsey et al., 2009). This demonstrates that SD has a negative effect on memory consolidation, particularly when it involves the hippocampus. This finding is in line with several other studies, using spatial and nonspatial versions of the Morris water maze, showing that SD selectively affects consolidation of hippocampus-dependent spatial memory and does not affect performance in the hippocampus-independent nonspatial versions of the task (Smith and Rose, 1996, 1997; Hairston et al., 2005). Altogether, these studies suggest that sleep plays a critical role in hippocampus functioning.

Importantly, the fear-conditioning studies in mice showed that the formation of contextual fear memory was disrupted by 5 hours of SD immediately after the acquisition but not by delayed SD (5-10 hours after acquisition) (Graves et al., 2003). The finding that SD immediately following training affects memory consolidation, whereas delayed SD does not, has also been reported in several other studies with a variety of learning paradigms (Smith and Rose, 1996, 1997; Bjorness et al., 2005; Palchykova et al., 2006). It suggests there is a critical time window immediately following training during which memory consolidation is sensitive to sleep loss and indicates that the timing of sleep after learning might be important for memory consolidation.

Most experimental studies that have examined the role of sleep in memory consolidation performed the task near or in the main resting phase and sleep deprive the subjects immediately following training. However, in real life, subjects do not only learn right before they go to sleep, but often learn during their active phase. In the present study, we examined the effects of SD on memory...
consolidation for contextual fear in rats, not only when the task was performed at the beginning of the resting (light) phase, but also right before the onset of the active (dark) phase. The nocturnal rat spends about 65% to 80% of the light phase asleep and about 20% to 35% of the dark phase (Borbély and Neuhaus, 1979; Lancel and Kerkhof, 1989; Franken et al., 1991; Tang et al., 2007). If, indeed, sleep plays a role in memory consolidation, how does it fulfill this role in case learning takes place at the start of the dark phase when it normally is followed by very little and often fragmented sleep?

METHODS

Animals and housing conditions
All experiments were performed with adult male Wistar rats (Harlan, Horst, The Netherlands), weighing 300 to 340 g at the start of the experiment. Animals were individually housed in standard macrolon cages (42.5 × 26 × 15.5 cm), and a layer of sawdust served as bedding. Food and water were provided ad libitum. Animals were maintained on a 12-hour light/12-hour dark cycle. Light intensity in the light phase was 45 lux. The dark phase consisted of dim red light conditions (1-2 lux). All procedures described in the present study were approved by the Animal Experiment Committee of the University of Groningen in compliance with Dutch law and regulations.

Experiment set-up
Several experiments were performed to examine the effects of SD on memory consolidation for contextual fear when training was performed at 2 different time points of the day. In the first experiment, we examined whether SD affects memory consolidation when training is performed at the beginning of the light phase (i.e., resting phase), when rats sleep about 65% to 80% of the time (Borbély and Neuhaus, 1979; Lancel and Kerkhof, 1989; Franken et al., 1991; Tang et al., 2007). This first experiment was aimed to replicate earlier studies in mice showing that SD adversely affects the formation of contextual fear memory (Graves et al., 2003; Vecsey et al., 2009). In the following experiments, we examined if and how SD affects memory consolidation when training is performed immediately before the onset of the dark phase (i.e., active phase), when rats sleep only 20% to 35% of the time (Borbély and Neuhaus, 1979; Lancel and Kerkhof, 1989; Franken et al., 1991; Tang et al., 2007). In both conditions, the training and test sessions of the contextual fear-conditioning paradigm were carried out in the light (45 lux), either at the beginning of the light phase or at the end, right before the onset of the dark phase. In the second case, we chose to perform the test at the end of the light phase rather than the beginning of the dark phase to avoid differences in light conditions as an additional factor that might affect the strength of the conditioning. Testing was always performed 24 hours after training. In all experiments, 2 groups of animals were used, a group subjected to 6 or 12 hours of SD after training and a non-sleep-deprived control group.
Contextual fear conditioning
One week prior to the start of the fear conditioning experiments, all animals were handled daily. Contextual fear conditioning was performed in a black quadrilateral Plexiglas chamber (40 × 40 × 40 cm), which was located in a separate experiment room. Background noise (60 dB) was present in the room. During training, an animal was placed in the chamber and exposed to the conditioning context for 3 minutes followed by a mild electric foot shock (0.7 mA, 2 sec) delivered through a stainless-steel grid floor. The animal was removed from the chamber and returned to its home cage 30 seconds after the shock. The chamber was thoroughly cleaned with 70% ethanol between subjects. Twenty-four hours later, the animal was placed in the same chamber for 5 minutes without shock presentation. Contextual memory was tested by assessing freezing behavior, defined as complete lack of movement except for respiration. Behavior was recorded on videotapes, which were analyzed afterward by an experimenter who was unaware of the treatment of the animals. The amount of time the animals displayed freezing behavior was expressed as a percentage of the total test time.

Sleep deprivation
Animals were sleep deprived after training for either 6 hours during the light or for 6 or 12 hours during the dark phase. SD was accomplished by mild stimulation, which involved tapping on the cage, gently shaking the cage, or, when this was not sufficient to keep the animals awake, disturbing the sleeping nest (Van der Borght et al., 2006; Hagewoud et al., 2010). Previous studies have shown that this procedure is effective in keeping rodents awake for several hours, as established by electroencephalographic recordings (Meerlo et al., 2001), without being a major stressor (Meerlo and Turek, 2001; Van der Borght et al., 2006; Hagewoud et al., 2010).

Plasma corticosterone Levels
To assess effects of SD by mild stimulation on plasma levels of the stress hormone corticosterone (CORT) under the current experimental conditions, separate groups of animals were sleep deprived for either 6 hours in the light phase or 6 or 12 hours in the dark phase; the results were compared with home-cage control animals. Blood samples were taken from the tail at the end of the SD period and collected in precooled plastic centrifuge tubes containing 0.01% ethylenediaminetetraacetic acid (EDTA) as anticoagulant and antioxidant. Blood was centrifuged at 4°C for 15 minutes at 2600 g, and plasma was stored at -80°C until further processing. CORT levels were determined by radioimmunoassay (MP Biomedicals, Orangeburg, NY).

Statistical analysis
Behavioral freezing responses and plasma levels of CORT in all experiments were analyzed using an independent-samples t-test. The total number of stimulations per hour needed to keep animals awake was analyzed using a repeated-measures analysis of variance (ANOVA) with a between-subject factor ‘treatment’ (6 hours of SD light/6 hours of SD dark) and a within-subject factor ‘time’ (1 hour blocks). All data in text and figures are expressed as mean ± S.E.M. p < 0.05 was considered as significant.
Chapter 6

RESULTS

Experiment 1: Training and 6 hours of sleep deprivation in the light phase
The first experiment examined whether 6 hours of SD immediately following contextual fear conditioning at the beginning of the light phase would affect consolidation of contextual fear memory in rats (Fig. 1A).

The number of stimulations that was needed to keep the animals awake during the 6-hour SD period is shown in Fig. 1B. Initially, the animals required little stimulation and spent most of their time exploring their cage. The number of stimulations needed to keep the animals awake gradually increased during ongoing SD (F<sub>5,45</sub> = 31,273, p < 0.001), suggesting an increased drive for sleep. Rats that were sleep deprived for 6 hours immediately following training showed reduced freezing upon re-exposure to the shock context 24 hours after training, compared with control animals (20.8% ± 4.8% vs. 36.9% ± 5.7%, respectively; n = 10 in both groups; t<sub>18</sub> = 2.165, p = 0.044; Fig. 1C).
results are thus in agreement with previous studies in mice showing impairments of fear memory when SD is performed immediately after training in the light phase (Graves et al., 2003; Vecsey et al., 2009).

Experiment 2: Training and 6 hours of sleep deprivation during the dark phase

Experiment 2 addressed the question whether the memory-impairing effect of SD is independent of the actual time of training. Animals were exposed to a foot shock right before the onset of the dark phase, and half of the animals were sleep deprived for 6 hours immediately afterwards (Fig. 2A). Since rats spontaneously sleep far less during the first 6 hours of the dark phase, in comparison with the first 6 hours of the light phase, significantly fewer stimulations were needed to keep the animals awake in this experiment, compared with the first experiment (compare Fig. 2B with Fig. 1B; effect of treatment and interaction effect: $F_{1,18} = 185.060$, $F_{5,90} = 17.143$, $p < 0.001$ in both cases). Upon re-exposure to the shock context 24 hours after training, the animals subjected to 6 hours of SD immediately following training showed a freezing response similar to that of control animals (32.5% ± 5.9% vs. 32.0% ± 5.6%, respectively; $n = 10$ in both groups; $t_{18}= -0.051$, $p > 0.9$; Fig. 2C). The data suggest that 6 hours of SD immediately following training does not negatively affect memory consolidation for contextual fear conditioning performed right before the onset of the dark phase.

Experiment 3: Training and 6 hours of sleep deprivation with high-intensity stimulation during the dark phase

One possible explanation for the finding that 6 hours of SD during the light phase in Experiment 1 impaired memory formation, whereas 6 hours of SD during the dark phase in Experiment 2 did not, is the fact that animals that are sleep deprived during the light phase received far more stimulations to keep them awake. In other words, the memory impairment may have been, in part, a consequence of the interfering stimulation rather than sleep loss per se. To test this possibility, we repeated the preceding experiment with 6 hours of SD in the dark phase (Fig. 2D); however, we now subjected the animals to the same amount of stimulation that was needed to keep animals awake for a 6-hour period at the beginning of the light phase (compare Fig. 2E with Fig. 1B). The results show that memory for contextual fear was not affected by a higher number of stimulations. Upon re-exposure to the shock context the next day, animals subjected to 6 hours of SD during the dark phase with high-intensity stimulation displayed amounts of freezing similar to that of control animals (36.3% ± 6.2% vs. 34.5% ± 6.1%, respectively; $n = 10$ in both groups; $t_{18}= -0.206$, $p > 0.8$; Fig. 2F). These results suggest that the SD-induced effects during the light phase in the first experiment were not likely to be due to the number of stimulations animals received during the SD procedure. If the effect was a consequence of the amount of stimulation, we would have expected to have seen an effect of it during the dark phase as well.
Figure 2. (A and D) Scheme of the contextual fear conditioning paradigm in Experiment 2 and 3 with training right before the onset of the active (dark) phase. (A) In Experiment 2, half of the animals were subjected to sleep deprivation (SD) for 6 hours immediately following training; 24 hours after training, animals were tested for contextual fear during a 5-minute test phase. (B) The mean number and type of stimulations needed to keep the animals awake during the first half of the dark phase. (C) SD animals (n = 10) did not differ in the amount of freezing in response to the shock context, compared with control animals (n=10). (D) In Experiment 3, half of the animals were subjected to SD for 6 hours immediately following training but, in this case, with the same amount and type of stimulation as needed to keep animals awake for 6 hours during the light phase; 24 hours after training, animals were tested for contextual fear during a 5-minute test phase. (E) Number and type of stimulations used during the 6-hour SD period (matched with the number and type of stimulations given during the 6 hours of SD in the light phase, as shown in Figure 1B). (F) SD animals (n = 10) did not differ in the amount of freezing in response to the shock context, compared with control animals (n = 10). Data are expressed as mean ± S.E.M. ** indicates SD period with increased number of stimulations.
Experiment 4: Training and 12 hours of sleep deprivation during the dark phase

An alternative explanation for the finding that 6 hours of SD during the dark phase failed to affect the formation of contextual fear memory might be the fact that the actual sleep loss was far less, as compared with the amount lost with 6 hours of SD during the light phase. To induce a similar amount of sleep loss as during the 6-hour period in the light phase, animals should be sleep deprived over a longer period. For this reason, we performed an experiment in which a group of animals was sleep deprived immediately following fear conditioning during the entire dark phase (12 hours of SD; Fig. 3A). Results show that animals sleep deprived from 0 to 12 hours after training, on average, showed less freezing behavior in the shocked context, compared with control animals. However, due to large variation in the SD group, this trend did not reach statistical significance (sleep-deprived animals, 25.0% ± 6.9%; control, 43.5% ± 6.1%; n = 10 in both groups; t_{18}= 1.766, p = 0.09). We therefore repeated the experiment to increase the sample size, and the combined data indeed show that 12 hours of SD immediately following training, during the complete dark phase, results in significantly less freezing behavior, compared with control animals (24.6% ± 5.5% vs. 39.5% ± 4.4% with n = 20 and n = 19, respectively; t_{37}= 2.137, p = 0.039, Fig. 3C). The number of stimulations needed to keep the animals awake over the 12-hour period in the dark phase is shown in Fig. 3B. Despite the longer period of SD, the total number of stimulations that was required to keep the animals awake for the 12-hour dark phase was still lower (33.7 ± 2.0) than during the 6 hours of SD at high-stimulus intensity (68.9 ± 0.8) in the previous experiment. Yet, only the 12 hour SD period in the dark phase affected contextual fear memory, which suggests that this effect is not dependent on the amount of stimulation but, rather, on the total amount of sleep that was lost.

Since 12 hours of SD covering the entire dark phase impaired consolidation of contextual fear memory, whereas 6 hours of SD during the first half of the dark phase did not, it might, in theory, be that the effect of the 12 hour of SD was caused by SD during the second half of the dark phase. Although this seems unlikely, we nevertheless performed an additional experiment to exclude this possibility. We subjected a new batch of animals to the fear conditioning paradigm right before the onset of the dark phase and subjected half of them to a delayed 6 hour SD (6-12 hour) period. No significant differences were found in freezing response between animals in the SD group and control animals (35.2% ± 5.8% vs. 30.0% ± 4.9%, respectively; n = 10 in both groups; t_{18}= 0.690, p > 0.4). Thus, this confirms that, with training near the start of the dark phase, consolidation of fear memory is only disrupted with 12 hours of SD.

Experiment 5: Sleep deprivation and plasma corticosterone levels

It is often suggested that the effects of SD on learning and memory processes in animals may be related to stress induced by the SD procedure rather than to sleep loss per se. To examine whether SD by our mild sensory-stimulation method increases plasma CORT levels, we performed an experiment in which separate groups of animals were sleep deprived for 6 hours in the light phase or for 6 or 12 hours in the dark phase and compared these results with those of home cage control animals. At the end of the SD period, blood was collected. Results show that 6 hours of SD in the light phase had no effect on plasma CORT levels (animals subjected to 6 hours of SD light: 3.7 ± 0.4 μg/dL.
vs. control animals: 4.2 ± 0.6 μg/dL; n = 10 and n = 9 respectively; t_{17} = 0.627, p > 0.5), nor did 6 hours or 12 hours of SD in the dark phase (6-h SD-dark: 8.8 ± 0.9 μg/dL vs. control animals: 7.9 ± 0.9 μg/dL with n = 10 in each group; 12-h SD-dark: 4.3 ± 1.1 μg/dL vs. control animals: 2.5 ± 1.0 μg/dL with n = 10 in each group; p > 0.4 in both cases). These data show that our SD method by mild stimulation, independent of time of day and SD length, does not lead to persistent increases in plasma levels of CORT.

Figure 3. (A) Scheme of the contextual fear conditioning paradigm in Experiment 4 with training right before the onset of the active (dark) phase. Half of the animals were subjected to sleep deprivation (SD) for 12 hours immediately following training; 24 hours after training, animals were tested for contextual fear during a 5-minute test phase. (B) Mean number and type of stimulations needed to keep the animals awake for 12 hours during the entire dark phase. (C) Animals sleep deprived for 12 hours (n = 20) during the dark phase displayed significantly less freezing behavior in response to the shock context than did control animals (n=19). Data are expressed as mean ± S.E.M. * p < 0.05.
DISCUSSION

Previous studies in mice have shown that sleep selectively promotes the formation of contextual fear memory and that SD immediately after acquisition selectively impairs the formation of this memory (Graves et al., 2003; Cai et al., 2009; Vecsey et al., 2009). We continued on these findings and examined the effect of SD in rats after training in a contextual fear conditioning paradigm at different times of the day, particularly the beginning of the resting phase or right before the onset of the active phase. We show that 6 hours of SD immediately following training in the light phase, the main resting phase, impairs consolidation of contextual fear memory in rats. Memory for contextual fear conditioning performed right before the onset of the dark phase was affected when training was immediately followed by 12 hours of SD in the dark phase, but not by 6 hours of SD.

Our finding that 6 hours of SD after training during the dark phase had no effect on memory, in contrast with 6 hours of SD after training during the light phase, is in line with the results of other recent studies showing that 6 hours of SD immediately following training in a novel object recognition task near or in the beginning of the dark phase does not affect recognition memory in mice and rats (Halassa et al., 2009; Palchykova et al., 2009). These studies suggested that sleep immediately following novel object recognition training is not required for the memory per se. However, in the present study, we showed that a longer period of SD following training does negatively affect memory consolidation. Since rats only sleep about 20% to 35% of the time during the dark phase, compared with 65% to 80% of the time during the light phase (Borbély and Neubaus, 1979; Lancel and Kerkhof, 1989; Franken et al., 1991; Tang et al., 2007), the amount of sleep we deprived the animals of during the 6 hours of SD period in the dark phase is far less than the 6 hours of SD during the light phase. Thus, the different effects on memory consolidation may have been due to the different amount of sleep that was lost. Importantly, a delayed 6 hours of SD during the second half of the active phase did not affect memory, showing that a full 12-hour SD period immediately following training during the dark phase is necessary to impair memory. Together, these findings suggest that not only the timing of SD after training, but also the duration of SD, is important for an effect on memory.

It is still a matter of debate whether sleep plays an active role in memory consolidation or merely a passive role by preventing waking interference (Ellenbogen et al., 2006). Waking interference and disruption of ongoing memory consolidation might result from mental activity related to sensory input and processing of new information. Particularly in animal studies on sleep and memory, which often rely on forced SD, it is sometimes argued that interference might occur as a consequence of the stimulations required to keep the animal awake. In our study, we considered this possibility; however, our findings are not in line with this view. Memory consolidation during the light phase (i.e., main resting phase) was disrupted by a 6-hour period of mild sensory stimulation, whereas memory formation during the dark phase (i.e., active phase) was not disrupted, even when the amount of stimulation was exactly matched. Also, memory consolidation in the dark phase was only significantly affected by a much longer period of 12 hours of mild stimulation even though, in that case, the total amount of stimulation was still lower than the amount of stimulation used during 6 hours of SD in the light phase. Importantly, the 6 hours of SD in the light phase and 12 hours of SD in
the dark phase are associated with comparable amounts of sleep loss. Thus, the disruption of memory formation is perhaps better explained by the amount of sleep that was lost than by the amount of stimulation the animals received.

Along the lines of the waking-interference concept, stress is another commonly proposed factor to mediate the effects of SD. However, it is unlikely that stress induced by our SD method caused impairments in memory. First of all, one might expect that the strongest stimulation would lead to the greatest impairment, which, as we discussed in the previous paragraph, was not the case. Furthermore, we show that plasma levels of the stress hormone CORT after SD in the resting and active phase were low and not different from those of control animals. This is in line with the results of previous studies using the same SD method of mild stimulation, showing no significant elevations in stress hormone levels (Van der Borght et al., 2006; Hagewoud et al., 2010). In fact, these levels are in the same range or even lower than after spontaneous waking activities such as feeding and grooming (Shiraishi et al., 1984). In addition, contrary to the view that stress has adverse effects on learning and memory processes, it is well known that glucocorticoids contribute to contextual fear conditioning in a positive way. Indeed, administration of glucocorticoid-receptor antagonists immediately before or after training, as well as adrenalectomy, impairs the formation of contextual fear memory (Pugh et al. 1997a, 1997b), whereas administration of glucocorticoids immediately following fear conditioning facilitates the formation of contextual fear (Abrari et al., 2009). Therefore, if the SD-induced effect had been due to a small acute increase of glucocorticoids instead of sleep loss per se, we would likely have found results opposite of the present findings.

The mechanisms through which SD affects hippocampus function and memory consolidation are largely unknown. Several studies have indicated discrete periods of time after training for fear conditioning that are sensitive to inhibitors of protein kinase A (PKA) and protein synthesis (Bernabeu et al., 1997; Bourtchouladze et al., 1998; Wallenstein et al., 2002). For example, administration of a PKA inhibitor immediately after or 4 hours after training impairs the formation of contextual fear memory (Bourtchouladze et al., 1998). Since it has been shown that SD during a similar time period after training disrupts memory for contextual fear, it is suggested that SD might act on memory consolidation via these mechanisms (Graves et al., 2003). Indeed, a recent study in mice showed that SD selectively impairs hippocampal 3', 5'- cyclic AMP (cAMP) and PKA-signaling (Vecsey et al., 2009). By affecting these pathways, SD may alter the activity of transcription factors and expression of genes involved in synaptic plasticity (Guzman-Marin et al., 2006; Ribeiro et al., 1999; Ribeiro et al., 2002) and, ultimately, influence memory storage (Vecsey et al., 2009). Thus, the molecular mechanism through which SD following training in the light phase may affect memory is now partly unraveled. Here we show that SD immediately following training near the onset of the dark or active phase impairs contextual fear memory as well. To our knowledge, the time course of different signal transduction pathways underlying memory consolidation when training occurs near or in the active phase and the time periods of sensitivity to inhibitors of protein synthesis and PKA are unknown. Therefore, in future research, it would be of great interest to identify the molecular processes underlying the role of sleep in memory consolidation for training performed in the active phase.

Our data suggest that, in rats, sleep immediately following training, independent of time of day,
is involved in memory consolidation. This leaves us with the intriguing question of how this works in humans, who generally have a fairly monophasic sleep pattern with little or no sleep during the active phase. How do sleep and SD affect memory consolidation when training is performed early in the active phase and is not immediately followed by a substantial amount of sleep? Could it be that some forms of learning in humans are less effective when acquisition takes place early in the active phase because it often is not followed by sleep? Indeed, in line with this thought, it has been shown that napping during the day, after learning during the first half of the day, improves memory compared with no napping (Tucker et al., 2006; Nishida and Walker, 2007; Lahl et al., 2008; Mednick et al., 2008). Further studies will be needed to unravel mechanisms of sleep-dependent memory formation and to address the question of optimal timing for learning and sleep.

In summary, our experiments in rats show that SD immediately following acquisition impairs the consolidation of contextual fear memory independent of time of training. The data further suggest that the deficit in memory depends on the amount of sleep that is lost after training. A 6-hour SD period immediately after training impairs memory consolidation for contextual fear conditioning in the resting phase. However, with training right before the onset of the active phase, a 12-hour SD period -but not a 6-hour SD period- results in impairment in contextual fear memory. We conclude that both timing and quantity of sleep after learning may be important in the process of memory consolidation in rats.

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