Sleep to Find Your Way: The Role of Sleep in the Consolidation of Memory for Navigation in Humans

Michele Ferrara,1,2* Giuseppe Iaria,3 Daniela Tempesta,2 Giuseppe Curcio,4 Fabio Moroni,4 Cristina Marzano,4 Luigi De Gennaro,4 and Claudio Pacitti5

ABSTRACT: Although a large body of evidence indicates that sleep plays an important role in learning and memory processes, the actual existence of a sleep-dependent spatial memory consolidation has been not firmly established. Here, by using a computerized 3D virtual navigation tool, we were able to show that topographical orientation in humans largely benefits from sleep after learning, while 10 h of wakefulness during the daytime do not exert similar beneficial effects. In particular, navigation performance enhancement needs sleep in the first post-training night, and no further improvements were seen after a second night of sleep. On the other hand, sleep deprivation hinders any performance enhancement and exerts a proactive disruption of spatial memory consolidation, since recovery sleep do not revert its effects. Spatial memory performance does not benefit from the simple passage of time, and a period of wakefulness between learning and sleep does not seem to have the role of stabilizing memory traces. In conclusion, our results indicate that spatial performance improvement is observed only when learning is followed by a period of sleep, regardless of the retention interval length. © 2008 Wiley-Liss, Inc.

KEY WORDS: cognitive map; environment; hippocampus; virtual reality; spatial performance

INTRODUCTION

More than 80 years ago, a pioneer study by Jenkins and Dallenbach (1924) provided evidence that the strength of a memory trace may be enhanced by periods of sleep compared to equivalent periods of wakefulness. These sleep dependent benefits were interpreted as the prevention of additional acquisition of sensory information that would have impaired retention of previously acquired material. To date, this hypothesis has been replaced by the idea that sleep plays instead a very active role in memory consolidation. Indeed, according to Moruzzi’s hypothesis (1966), sleep does not concern the fast recovery processes in routine synapses underlying stereotyped activities, but the slow recovery of “learned synapses” (i.e., new contacts between neurons). This hypothesis is supported by a large body of evidence confirming the active role of sleep in learning and memory for both the declarative (e.g., Rasch et al., 2007) and nondeclarative domain (e.g., Walker et al., 2003). Despite such a widely growing of experimental findings, however, the extent to which sleep plays a critical role in memory remains hotly debated (e.g., Siegel, 2001). In fact, it is still poorly understood which aspects of memory function are affected by sleep, and which processes underly the consolidation, i.e., the series of changes by which a memory trace becomes more stable and less subject to disruption, giving way to a better performance.

In rodents, the relations between sleep and memory consolidation have been mostly investigated by taking into account spatial learning, which is strictly dependent on the hippocampal formation (Squire et al., 2004). More specifically, extensive investigation has been done in the context of post-training spontaneous reactivations of the so-called “place cells,” hippocampal neurons firing selectively when the animal occupies a specific location in space (O’Keefe and Nadel, 1978). At the cellular level, it has been observed that neuronal ensembles activated during the waking behavior are then reactivated during post-training slow-wave sleep (SWS) (e.g., Pavlides and WINSON, 1989; Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996) and REM sleep (Poe et al., 2000; Louie and Wilson, 2001). Consequently, it has been hypothesized that the offline replay of hippocampal activity during sleep in rodents may be involved in the consolidation of newly encoded spatial information, gradually transferred from short term hippocampal stores to long term neocortical memory stores (Sutherland and McNaughton, 2000). In humans, it has been suggested that spatial memory acquisition involves the same neuroanatomical substrate as in rodents (Burgess et al., 2002). Indeed, Ekstrom et al. (2003) directly recorded from neurons in mediotemporal lobes during navigation in a virtual environment, demonstrating the existence of cells that respond at specific spatial locations and cells that respond to views of landmarks. In recent years, the

1 Dipartimento di Medicina Interna e Sanita’ Pubblica, Universita’ degli Studi dell’Aquila, L’Aquila, Italy; 2 Laboratorio di Psicofisiologia del Sonno, Facolta’ di Psicologia, Universita’ degli Studi dell’Aquila, L’Aquila, Italy; 3 Human Vision and Eye Movement Laboratory, Faculty of Medicine, University of British Columbia, Vancouver, Canada; 4 Dipartimento di Psicologia, “Sapienza” Universita’ di Roma, Rome, Italy; 5 Dipartimento di Scienze e Tecnologie Biomediche, Universita’ degli Studi dell’Aquila, L’Aquila, Italy

Grant sponsors: The Department of Internal Medicine and Public Health (Ateneo ex 60%), Alzheimer Society of Canada (ASC), The Michael Smith Foundation for Health Research (MSFHR).

*Correspondence to: Michele Ferrara, Laboratorio di Psicofisiologia del Sonno, Facolta’ di Psicologia, Universita’ degli Studi dell’Aquila, Piazzale S. Tommasi 1, Blocco 11E, 67010 Coppito (AQ), Italy.
E-mail: Michele.Ferrara@cc.univaq.it
Accepted for publication 18 March 2008
DOI 10.1002/hipo.20444
Published online 20 May 2008 in Wiley InterScience (www.interscience.wiley.com).

© 2008 WILEY-LISS, INC.
neural mechanisms underlying spatial navigation have been investigated also by means of neuroimaging techniques such as functional Magnetic Resonance Imaging (fMRI). Neural activity within the right hippocampus has shown to be strongly related with knowing accurately where places are located in a complex virtual reality town, and with the individual’s ability of navigating between spatial locations (Maguire et al., 1998). The encoding of spatial information from the ground level perspective (route) recruits several bilateral medial temporal lobes (MTL) regions (Shelton and Gabrieli, 2002). In addition, it has been demonstrated that the pattern of brain activity involved in navigation changes as a function of the cognitive strategy used by subjects while solving a place-learning task in a virtual environment (Hartley et al., 2003; Iaria et al., 2003). These studies showed that activity within the hippocampus is detected only when the participants used spatial landmarks to navigate in the early phase of training, and that the spontaneous bias in using environmental landmarks is significantly related to the gray matter volume of the hippocampus across individuals (Bohbot et al., 2007). Very recently, Iaria and colleagues (2007) shed more light on the role of the hippocampus in spatial navigation: by using fMRI, the authors were able to demonstrate that the right and left hippocampi, together with the retrosplenial cortex, are critically involved during the formation and use of a mental representation of the environment respectively, namely a cognitive map (O’Keefe and Nadel, 1978), and that structural integrity of this brain region may affect the individual’s ability to orient within the environment (Iaria et al., 2008). In sum, altogether, these studies provide convergent functional and structural evidence suggesting that, in humans, the hippocampus is critically responsible for successful navigation and orientation within the environment, and that its role mainly subserves the formation and use of a cognitive map.

Despite of the growing evidence supporting the role of the hippocampus in human navigation, and at variance with studies in rodents, there are only few attempts trying to assess the relationships between sleep and spatial memory consolidation in humans. Recently, it has been shown that the hippocampal areas that are activated during route learning in a virtual town are then reactivated during subsequent REM and NREM sleep, the extent of reactivation during SWS being correlated with the improvement in route retrieval on the next day (Peigneux et al., 2004), which supports the theory that spatial memory traces are processed and strengthened during sleep in humans. A subsequent fMRI study by the same group suggests that sleep, compared to sleep deprivation, promotes the integration of recently acquired spatial memories into cerebral networks (Orban et al., 2006). However, the behavioral performance of sleep deprived and regularly sleeping subjects did not differ, casting some doubts on the real importance of sleep periods for spatial learning. Such discrepancy could be possibly related to the individual’s variability in adopting different cognitive strategies for orientating themselves in the virtual environment. Among different strategies, several studies showed that only the use of environmental landmarks relies on the hippocampal formation (e.g., Hartley et al., 2003; Iaria et al., 2003). Therefore, since sleep may be beneficial to the consolidation of hippocampus-dependent memories (e.g., Marshall and Born, 2007), in the present study we asked participants to use an hippocampus-dependent landmark-based strategy to orient and navigate within the environment. More specifically, we made use of a test that employs a virtual environment and requires the formation, first, and the use, then, of a cognitive map of the environment, which has been shown to rely on both proper functioning and structural integrity of the hippocampus (Iaria et al., 2007, 2008). We used this specific test in order to answer the question of whether spatial learning does really benefit from sleep. At the same time, we meant to control for some important confounding factors, such as the influence on navigation performance of a period of daytime wakefulness, of the circadian placement of the learning phase and of the length of the waking period interposed between learning and sleep.

**MATERIALS AND METHODS**

### Subjects

Sixty university students participated in this study (mean age: 22.3 yr, standard error of mean (SEM): 0.4). They were right-handed (score > 0.7), as assessed by a standard lateral preference questionnaire (Salsamo and Longoni, 1985). None of the subjects had history of medical, neurological, or psychiatric disorders. All of them had habitual sleep duration of 7–8 h/night, went to bed between 11 P.M. and midnight, and did not take naps during the day. Quality and quantity of participants’ usual sleep was assessed by a sleep log. Informed consent was obtained from all participants.

### Experimental Groups

The participants were randomly assigned to one of the six experimental groups (10 subjects in each group, 3 males-7 females). Test and retest sessions were always scheduled at 10 A.M., except when differently specified. A diagram of the complete experimental protocol is reported in Figure 1.

Subjects included in Group 1 (mean age = 24.1 yr, age range: 21–27) spent the night after the spatial learning session at home, sleeping as usual, and were retested 24 h after learning.

Subjects in Group 2 (mean age = 22 yr, age range: 20–25) stayed awake in the laboratory under the direct supervision of two experimenters during the whole night following the spatial learning session: they were allowed to briefly walk around, listen to music, read, watch the television or use a computer. Lying down, sleeping, and vigorous physical activity were not permitted. Light snacks were permitted, while caffeinated beverages, chocolate, alcohol, and medications that can induce/ reduce sleepiness were not allowed during the deprivation protocol. Time information was available to subjects. The laboratory was constantly illuminated by neon lamps.
Subjects in Group 3 (mean age 22.8 yr, age range: 19–28) were enrolled to control for the possible circadian effects on learning the navigation task, as well as for the effects of a different period of stabilization wakefulness interposed between learning and sleep. These subjects differed from those in Group 1 only because they learned the task at 8 P.M. instead of 10 A.M., and then they went home to sleep. Consequently, the sleeping Groups 1 and 3 differed as to the length of the retention interval (≈12 vs. 24 h).

On the other hand, the retention interval length was similar between Groups 3 and 4 (mean age = 21.9 yr, age range: 20–29). These subjects were enlisted to control for the effect of the mere passage of time on the consolidation processes involved in spatial learning. They spent the day following learning pursuing their usual student activities; however, they were not allowed to do any sports or to have stimulant drinks after 4 P.M. They were retested at 8 P.M., after 10 h of waking.

Subjects in Group 5 (mean age = 20.9 yr, age range: 19–25) were sleep deprived during the first night (monitored in the laboratory), and then retested after a full night of recovery sleep, 48 h after learning. On the other hand, subjects included in Group 6 (mean age = 21.9 yr, age range: 19–30) were also retested after a 48 h retention intervals including two consecutive nights of sleep.

Virtual Environment

The experimental paradigm consisted of a virtual city in which participants navigated by using three different key-buttons of a computer keyboard, each button corresponding to movement in one of three directions: left, forward, and right (for more details see Iaria et al., 2007). The virtual city, which was created by using the editor of a three-dimensional game software (Game Studio A6, LA Mesa, CA), was composed of several buildings of different size and shape, but the same texture. Thus, the buildings could not easily be distinguished from each other. However, the virtual city included six clearly identifiable landmarks: a bank, a church, a clinic, a police station, a post office, and a supermarket. Figure 2A provides a schematic outline of the city (top-view) and Figure 2B,C presents two examples of landmarks.

Procedure

The task has been designed to assess an individual’s ability to orient within the virtual environment, assessing two specific aspects of human topographical orientation: the ability to form a mental representation of the environment, and the ability to use that mental representation for the purpose of orientation. By presenting local properties (i.e., landmarks), the task requires that subjects, first, learn about the environment by forming a mental representation of it (i.e., cognitive map). Then, participants were required to rely on that mental representation to travel between different landmarks locations within the virtual environment.

The subjects first underwent a practice session to make sure they felt comfortable navigating within the virtual environment. In this session they were free to move in a virtual environment (different from the experimental one) by using three different key-

**FIGURE 1.** Experimental protocol. Sixty subjects were allocated into six experimental groups (n = 10 for each group) and either trained at 10 A.M. or 8 P.M. on day 1. They were then retested across the ensuing 10–48 h period with different retest schedules. Group 1: Trained at 10 A.M. and retested at 10 A.M., after 24 h including a night of sleep. Group 2: Identical to Group 1, except that subjects spent the night awake in the laboratory. Group 3: Trained at 8 P.M. and retested at 10 A.M., after 14 h including a night of sleep. Group 4: Trained at 10 A.M. and retested at 8 P.M. after 10 h of waking. Group 5: Trained at 10 A.M. and retested 48 h later, after one night of sleep deprivation in the laboratory followed by one night of recovery sleep. Group 6: Trained at 10 A.M. and retested 48 h later, following two consecutive nights of sleep.

**FIGURE 2.** Views of the virtual environment. (A) Top-view (survey map) of the virtual city including the locations of the six landmarks indicated by stars (not shown to the participants). (B and C) Views of two of the six landmarks as they appear to the participants while learning about the environment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
buttons (the upward, leftward, and rightward arrows available on the computer keyboard) to move in three different directions (left, forward, or right). The subject could move forward by keeping pressure on the forward button (upward arrow), and then, when necessary, could also press on either the left or the right directional button. There was no time constraint on this practice phase; that was stopped only when the subject told the experimenter s/he felt comfortable moving within the environment, and using the directional buttons. In the case when a subject felt dizzy, the experiment was stopped. Two subjects (not considered in the analyses) were discarded due to dizziness.

After the practice session, to ensure that the subjects had proficient motor skills, they were asked to perform three control tasks. These tasks required the subject to complete a predetermined route within the same virtual environment in which s/he performed the practice session. Along the route there were several black signs with white arrows visibly situated in the middle of the road. To complete the route, subject had simply to follow the directions indicated by the arrows. These arrow signs told the subject to turn right, left, or move forward until the next sign was reached. The number of times the control trials were repeated depended on how comfortable the subject felt when performing this task. The subject was considered ready to move onto the next stage (i.e., learning) only when she/he can follow the arrows without making any stops along the route, and was able to proceed along the pathway without wavering from side to side. At this time, the subjects were administered the experimental tasks, namely the learning and retrieval task, which were performed in a different virtual environment.

In the learning task, the subjects were required to form a mental representation of the virtual environment. They were first shown the pictures of the landmarks, named one by one by the experimenter. The subjects were informed that each landmark had an undisclosed location within the environment that she/he eventually be required to find and remember. They were informed that their task was to create a top-view mental representation of the environment. They were additionally told that this mental representation was critical in order to be able to solve the next task (i.e., the retrieval task). They were also informed that, at the end of the exploration of the city, they will be presented with a top-view outline of the city and asked to identify the landmark locations. During the learning task, the subject was free to course through the environment using whatever path and strategy she/he chose. After 7 min of free exploration, the accuracy of the cognitive map that was formed was assessed by asking the subjects to draw on a sheet of paper, that reported the 2D top-view outline of the city map, the respective location of each landmark as precisely as possible. If subjects were not perfectly accurate, they were allowed to explore the city for different sessions of 1 min each. The first assessment of the formation of the cognitive map occurred after 7 min based on a previous study showing that the cognitive map does not form earlier (Iaria et al., 2007). The learning phase ended when the subject correctly reported on the map the location of the six landmarks with an accuracy of 100%. All subjects were able to form a perfect mental representation of the environment (100% accuracy) in a mean time of 10.2 min of exploration (SEM = 0.2).

Immediately after the learning phase, the subjects were administered the retrieval task consisting of 18 trials. In each trial the subjects were started facing a landmark and a sign reporting the landmark they need to reach following the shortest pathway as quickly as possible. Nine trials out of 18 were included in the so called test phase, and nine trials were included in the retest phase. Test and retest phases were performed for each group at different times according to the experimental design. For all the 18 trials (9 test phase and 9 retest phase) the task was the same, but the starting and target locations varied across all trials. In other words, the 18 trials included different pathways which the subjects had to perform in order to reach each target location. This allowed us to control for the use of procedural memory while performing the task. Procedural memory, indeed, occurs during navigation when subjects perform the same pathway several times and are familiar with that specific pathway (Hartley et al., 2003; Iaria et al., 2003). In the present study, this is not the case, since none of the pathways are repeated during both test and retest phase of the experiment. Moreover, all participants are administered the cognitive map test only after they performed the three control trials (after training) in the most efficient way, that is moving within the environment and along a pathway without ever stopping. This procedure leaves little room (if any at all) for improvement, thus controlling for any procedural memory consolidation that may occur with sleep. Thus, as shown by a previous fMRI study investigating the neural mechanisms involved in performing this task (Iaria et al., 2007), subjects are required to make use of declarative memory (i.e., the cognitive map previously formed) in order to perform correctly each trial of the retrieval task. For each trial, an experimenter recorded the chronometer reading for the time the subject spent to reach the target location.

**Data Analysis**

The time (seconds) spent to reach the target locations (landmarks) during each of the nine test and retest trials was treated as dependent variable. Given the large interindividual differences and intraindividual fluctuations in this kind of performance, we used the median time as a more stable index of central tendency. Medians were log transformed and submitted to a mixed design Analysis of Variance (ANOVA) with State after Learning (Sleep vs. Wake) and Retention Interval [Short: ~12 h (Groups 4 and 5) vs. Middle: 24 h (Groups 1 and 2) vs. Long: 48 h (Groups 3 and 6)] as between factors, and Testing Session (Test vs. Retest) as a repeated measure. This analysis took into account the fact that some of the experimental conditions differed not only as to the presence of a period of sleep or wakefulness after learning, but also as to the length of respective retention intervals.

Then, sleep-wake comparisons between groups of equal retention interval length, i.e., Group 1 vs. 2, 3 vs. 6, and 4 vs. 5 were carried out by means of mixed-design ANOVAs with Group as between factor and Testing Session (test vs. retest) as a repeated measure. Significant interaction effects were tested by means of post hoc Fisher’s LSD test.
The Effect of Sleep/Waking as a Function of Length of Retention Intervals

The ANOVA showed a main effect of Testing Session ($F_{1,54}$: 10.96; $P = 0.002$) and a significant State after Learning by Testing Session interaction ($F_{1,54}$: 17.33; $P = 0.0001$; see Fig. 3). Post hoc comparisons indicated that a significant test-retest performance improvement is seen only when spatial learning is followed by a period of sleep ($P = 0.000002$), and that retrieval performance is largely better when learning is followed by sleep compared to waking ($P = 0.000002$), regardless of the retention interval length.

Further Evaluations of the Sleep Effect on Spatial Memory Consolidation

The ANOVA comparing Groups 1 and 2 showed a non significant main effect for Group ($F_{1,18}$: 1.35; $P = 0.25$), a significant main effect for Testing Session ($F_{1,18}$: 7.68; $P = 0.01$) and, more importantly, a significant Group × Testing Session interaction ($F_{1,18}$: 8.26; $P = 0.01$, see Fig. 4, panel A). Post hoc tests indicated that, although the two groups did not differ at test ($P = 0.69$), they do differ at retest ($P = 0.0003$), because of the fact that only those who slept significantly improved their performance from test to retest ($P = 0.0008$). Thus, this result supports the existence of a sleep effect on the consolidation of spatial memory, as indexed by the enhancement of the navigation performance only in the sleeping group.

Since Groups 3 and 4 have been retested after periods of time of equivalent length filled with sleep or wake, respectively, their performance have been compared to give further support to the differential effects of sleep and wakefulness on navigational performance. ANOVA showed only a significant Group × Testing Session interaction ($F_{1,18}$: 12.17; $P = 0.003$). Post hoc comparisons showed that only Group 3 significantly improved from test to retest ($P = 0.004$), outperforming Group 4 at retest ($P = 0.001$), further indicating that the enhancement of spatial memory performance needs sleep after learning (Fig. 4, panel B). Incidentally, the lack of any test-retest performance improvement shown by Group 4 contributes to rule out the nonspecific effects of the passage of time on spatial performance.

We then compared Groups 5 and 6 performance. These groups, both retested 48 h after learning, differ only for the presence of sleep during the first post-training night. In this case, ANOVA did not show any significant main effect (Group, $F_{1,18}$: 1.07; $P = 0.31$; Testing Session, $F_{1,18}$: 3.82; $P = 0.07$) or interaction (Group × Testing Session, $F_{1,18}$: 1.55; $P = 0.22$). Nevertheless, post hoc comparisons of interaction’s means indicated that only Group 6 showed a significant test-retest performance enhancement ($P = 0.036$; see Fig. 4 panel C), and that Group 6 outperformed Group 5 at retest ($P = 0.029$).

Control Analyses

Besides the main comparisons between groups matched for length of retention interval, it is possible to further contrast the six experimental groups in order to control for possible confounds such as circadian factors, arousal state at retest and strengthening of the spatial memory trace by a second sleep night. However, these results are to be taken with caution, because the groups hereafter compared were retested after retention intervals of different length.

Given the existence of a sleep effect on spatial learning, one may ask whether such effect could be modulated by the circadian placement of the learning phase and by the length of the waking period interposed between learning and sleep. To control for these factors, Group 3 was compared to Group 1. The ANOVA showed a significant main effect for Testing Session ($F_{1,18}$: 29.24; $P = 0.00004$), due to the large performance improvement of both groups from test to retest. However, the main effect for Group ($F_{1,18}$: 1.25; $P = 0.28$) and, most importantly, the Group × Testing Session interaction effect were not significant ($F_{1,18}$: 0.005; $P = 0.95$), the latter indicating that the circadian placement of learning and the length of the stabilization period preceding sleep exert negligible effects on the consolidation of spatial memory.

Another possible concern in every sleep-deprivation study is related to the sleep deprivation group being tested in a different vigilance state than the sleeping group. To control for this confound we compared the sleep deprived group (Group 2) and Group 5, retested after one recovery night that followed sleep.
deprivation to ensure similar vigilance parameters as in the sleep group. The ANOVA did not show any significant main (Group, \(F_{1,18}: 0.40; P = 0.54\); Testing Session, \(F_{1,18}: 0.11; P = 0.74\)) or interaction (Group \times Testing Session, \(F_{1,18}: 0.17; P = 0.69\)) effect. These results lend some support to the idea that the differences between Groups 1 and 2 were not because of nonspecific effects of the arousal state at retest.

Finally, a concern regarding the role of sleep in the consolidation of spatial memory is whether or not such process continues beyond the first postlearning night. To this aim, Group 1 was compared to Group 6, who slept for two consecutive nights after learning. The ANOVA showed a significant main effect for Testing Session (\(F_{1,18}: 21.74; P = 0.0002\)), again because of the large performance improvement of both groups from test to retest. On the other hand, the main effect for Group (\(F_{1,18}: 0.21; P = 0.65\)) and the Group \times Testing Session interaction were not significant (\(F_{1,18}: 0.18; P = 0.67\)), the latter suggesting that a second post-learning night of sleep does not add any benefit to the navigation performance.

**DISCUSSION**

In our study we report evidence that spatial memory for navigation and orientation related to the formation and use of cognitive maps in humans needs sleep to be consolidated. Indeed, we found that a performance improvement is seen only when spatial learning is followed by a period of sleep, regardless of the retention interval length. This finding is not trivial as the only two previous reports on behavioral performance in a 3D virtual town provided diverging evidence regarding the actual existence of a sleep-dependent spatial memory consolidation. In fact, while in a first study all of the six tested subjects improved their navigation performance after sleep (Peigneux et al., 2004), surprisingly in the second study the spatial performance did not change from test to retest either in the sleeping or in the sleep deprived group (Orban et al., 2006). It can be speculated that a possible explanation for these inconsistent results may rely on the individual’s variability in adopting different cognitive strategies useful for orientation, and helpful for solving the task. As the use of a set of environmental landmarks is related to the hippocampal formation (Hardley et al., 2003; Iaria et al., 2003), it is possible that sleep may affect the memory consolidation that is related to spatial learning mostly when individuals adopt a hippocampus-dependent landmark-based strategy to orient and navigate. If this is the case, the use of different strategies relying on different brain structures such as the caudate nucleus (Hardley et al., 2003; Iaria et al., 2003), rather than the hippocampus, may benefit from sleep differently. We can not disentangle this issue because we did not manipulate the strategies that individuals might have adopted to perform the learning task. Nevertheless, because of the specificity of our
task, the hippocampus-dependent instructions suggested for task solution and the previous findings reporting the critical contribution of the hippocampus for solving it (Iaria et al., 2007), we assume that any delay in reaching a target location should reflect selectively the strength of consolidation of the cognitive map and, consequently, the ability to make use of it (i.e., a hippocampus-dependent declarative memory).

In the present study, the beneficial effect of sleep was found in all groups of subjects sleeping for one or two nights after learning (Groups 1, 3, and 6). This confirms and extends the findings of a previous study in which we showed that sleep strengthens the memory traces of a newly learned route in a real-life unfamiliar environment (Ferrara et al., 2006). Altogether, our results suggest that spatial learning really benefits from sleep periods, as indicated by the general positive effects of sleep after learning.

On the other hand, one night of total sleep loss (Groups 2 and 5) does not allow the spatial performance enhancement as a night of sleep does. This negative effect may be attributed to the lack of consolidation during post-training sleep. The need of a period of sleep during the first post-training night is suggested also by the difference at retest between the group undergoing postlearning sleep deprivation followed by recovery sleep (Group 5) and the group sleeping for two consecutive nights (Group 6). Both these groups were retested 48 h after learning, allowing to control for circadian influences, arousal state at retest and length of retrieval interval, but differed as to the presence of sleep during the night immediately following training.

These findings imply that sleep deprivation exerts a kind of proactive disruption of spatial memory consolidation. Indeed, in our study the lack of spatial performance improvement in the subjects undergoing sleep deprivation (Group 2) was not reverted by one night of recovery sleep (Group 5), indicating that sleep deprivation on the first postacquisition night does not allow the strengthening of spatial memory traces. The results of this comparison (Groups 2 vs. 5), although to be considered cautiously given the different length of the retention intervals, also suggest that the lack of performance improvement after sleep deprivation cannot be attributed to reduced cognitive abilities and vigilance after extended wakefulness, a typical concern in sleep deprivation studies.

Altogether these results point to the need for a period of sleep during the first postacquisition night. This period cannot be substituted for by an equivalent period of wakefulness, since 10 h of wakefulness during the daytime (Group 4) did not exert similar beneficial effects. This control group was retested at 8 pm; consequently, circadian influences cannot be completely ruled out. However, such explanation seems unlikely, given that the comparison between the sleeping groups who learned the task in the morning or in the evening (Groups 1 and 3, respectively) already excluded a circadian effect, at least on test performance.

More generally, our findings do not suggest a facilitating role for waking on spatial memory performance improvement. In fact the two groups retested after about 12 h of sleep or wakefulness (Groups 3 and 4, respectively) did significantly differ at retest, further indicating the importance of a period of sleep to obtain a significant enhancement of spatial performance.

With regard to the control analyses carried out comparing groups retested after retention intervals of different length, although they should be considered cautiously, they however, give some hints of additional factors that in principle may influence the relationship between sleep and spatial memory consolidation. The comparison between the sleeping group who learnt the spatial task at 10 A.M. (Group 1) and the group who learned the task at 8 P.M. and then went to sleep (Group 3) suggest that sleep in the few hours after learning is not necessarily better than sleep obtained in the night, several hours after learning. The lack of difference between those groups also suggests that it does not matter whether learning occurs as first thing in the morning or later in the evening, night sleep can enhance performance regardless. Moreover, the comparison between subjects sleeping for one or two nights after learning (Groups 1 and 6, respectively) do not support the existence of a progressive consolidation of spatial memories across subsequent nights, in the absence of any further explicit recall of those memories. Altogether, these findings begin to answer the question of whether there is an important window of time for the consolidation of (spatial) learning. Given that a second night of sleep does not add any significant performance improvement, the results suggest that this time window is within 24 h.

In conclusion, in our study we presented evidence that new spatial memories acquired in a virtual environment are strengthened by a nocturnal sleep period, regardless of the retention interval length. In particular, navigation performance enhancement needs sleep in the first post-training night. On the other hand, sleep deprivation seems to hinder any improvement in performance accuracy. Moreover, spatial memory performance does not benefit from the simple passage of time, and a period of wakefulness between learning and sleep does not seem to exert a supportive role on the sleep-related spatial memory consolidation.

Acknowledgments

The authors thank Chiara Gioia, Pierangela Pellegini, and Valentina Petrangeli for their help in data collecting. They also thank two anonymous reviewers for their helpful comments on a previous version of the manuscript.

REFERENCES


