Effects of Sleep Deprivation on Consolidation of Spatial Memory in Rats after One-Day Training in a Morris Water Maze

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The effects of sleep deprivation produced using a carousel method on the consolidation of spatial memory were studied in rats (male Wistar rats) after one-day training using the Frick et al. protocol (2000) in a Morris water maze. Data were obtained providing evidence that the memory trace after rapid 3-h training was retained for one day. Sleep deprivation for 24 h after training prevented reinforcement (consolidation) of spatial memory. The results led to the conclusion that a model based on one-day training can be used to study the neurophysiological and neurochemical mechanisms of the effects of sleep deprivation on consolidation of spatial memory.

Keywords: consolidation of spatial memory, one-day training, Morris water test, sleep deprivation.

There is a strongly held opinion that one of the most important functions of sleep is its involvement in the formation of memories [8, 20, 21]. In the state of waking, learning occurs by means of encoding information followed by its consolidation [3, 8]. The term consolidation refers to the processes transforming new, labile memory traces into stable long-term memories. Sleep is believed to promote this process. Reports on the involvement of sleep in memory consolidation have a long history [11] and remain under active development [2, 8, 14, 20, 22, 26]. However, how the duration of sleep is related to its influences on memory remains unknown. Sleep consisting of eight hours overnight and short periods (1–2 h) has been shown to have positive influences [7, 17, 25], as has even transient (6–7 min) daytime sleep [12]; longer-lasting sleep appears to have a more marked effect on memory [8, 22]. As regards the time inter-val between training and sleep, data have been reported showing that shorter intervals increase the influence of sleep on the process of memory consolidation. For example, sleep 3 h after training was more effective for declarative memory than sleep at 10 h [10, 23]. However, the question of the nature of the effects of different phases of sleep (slow-wave and paradoxical sleep) on different types of memory (declarative and procedural) remains controversial [7, 13, 17, 25].

The standard test for studies of learning and memory processes in animals is the Morris water test [15]. This test allows use of types of learning with different levels of involvement of the hippocampus. Spatial hippocampus-dependent memory in animals is comparable with declarative memory in humans, while memory not dependent on the hippocampus is comparable with procedural memory. In the Morris water test, the animal learns to avoid forced swimming by finding a platform, which can be hidden beneath the surface or visible above the surface, placed in one of the four quadrants (termed the "target"). When the platform is under water, learning is associated with remembering spatial relationships in the surrounding environment,

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which actively involves the hippocampus; when the platform is above the water, memory for spatial relationships is not required [18]. Use of automation systems with the water test (for example, the Noldus system) yields accurate quantitative assessments of the dynamics of the formation of spatial skills and allows the animal's behavioral strategy during the experiment and minor differences in behavior to be detected [1, 5].

The classical type of learning in the Morris water test is based on training animals over a period of several days with testing for reproduction of memory one day after the last training procedure. According to current concepts, memory consolidation takes several hours [8, 22]. Fixation of a memory trace on training with a multi-day scheme is quite complex in nature, because of secondary processes: extraction of the trace from memory, consolidation, reconsolidation, etc. [22]. The use of one-day types of training [9, 24, 27], which allow sleep deprivation immediately after training of the animals to be introduced, with testing of the result at 24 h, significantly simplifies analysis and interpretation of the effects of sleep deprivation on memory consolidation.

A model of rapid, one-day training in the Morris test using the Frick et al. protocol [9] was used by Tartar et al. [24] to study the mechanisms of the effects of sleep fragmentation on spatial learning. Sleep fragmentation is a symptom of many clinical disorders (restless legs syndrome, depression, post-traumatic stress, narcolepsy, obstructive apnea, etc.). In the present study, one-day sleep fragmentation was produced by waking the animals 30 times per hour. In this procedure, episodes of slow-wave sleep become short, though there is virtually no change in its total duration; on the other hand, the paradoxical phase of sleep is essentially eliminated. The result of sleep fragmentation is an increased level of drowsiness [24]. The authors have shown that one-day sleep fragmentation used before the training procedure leads to deterioration of hippocampus-dependent spatial learning and disappearance of hippocampal long-term potentiation.

Another protocol of one-day spatial training in the Morris test [16], which takes even less time (about 2 h), was used in the studies reported by Ward et al. [27] from the same laboratory. The investigators were able to show that one-day sleep fragmentation before training did not affect the learning process itself, though it selectively degraded reproduction of the spatial water avoidance skill on testing one day after training.

Results reported in [24, 27] demonstrated that the effects of sleep disturbance (sleep fragmentation) on the consolidation of hippocampus-dependent memory could be studied using a model based on rapid one-day training in the Morris water test. In the laboratory where these studies were performed, two different rat strains were used, along with two different types of one-day training, because of the desire to find the optimum conditions for studying the effects of sleep fragmentation on memory consolidation.

The rapid one-day spatial training model is of interest for studies of the molecular-genetic mechanisms of the influences of sleep on the process of consolidation of hippocampus-dependent learning. In contrast to the studies cited above [24, 27], we investigated the effects of sleep on memory consolidation by sleep deprivation after the training procedure. The experimental paradigm used here combined the rapid training model of Frick et al. [9] with the stress-free sleep deprivation method (the "carousel" method), developed by Rechtschaffen [19] in a version modified by Lan et al. [13], which produces virtually total sleep deprivation. Preliminary results from this study have been published as a conference presentation [4].

METHODS

Training was performed using the Ethovision program (version 3.1) with an automatic track analyzer on a Noldus apparatus (Holland) placed in location with artificial illumination (100 Lx). The round basin (diameter 150 cm, height 60 cm) was surrounded by various objects to allow spatial orientation and filled with warm water ($t = 24 \pm 1$°C).

Sleep deprivation was produced using an apparatus with a rotating disk over water (a so-called "carousel") [19] as modified by Lan et al. [13]. This sleep deprivation method, without feedback, was tested in [6], where polysomnographic studies demonstrated the absence of the paradoxical phase of sleep and the almost complete disappearance of the slow-wave stage of sleep. Before training, rats were handled for five days and were adapted to the carousel (the disk was rotated in different directions in random order for periods of 5 min separated by 5-min pauses for a period of 60 min, completing half a rotation in 1 min [24]. The location containing the carousel had a 12:12 h light regime (electric illumination was switched on and off at 08:00 and 20:00, respectively), which corresponded to the light regime of the animal house. The moment at which the light was switched on was taken as the beginning of the new day, during which rats normally sleep, while the time of switching off was taken as the beginning of the night, when they wake. Air temperature at this location was kept within the range 21–24°C; access to water and food was unrestricted. Training was performed after daytime sleep, from 17:00 to 20:00, and testing for retention of memory traces was performed at the same time one day later.

Experiments were performed using 20 adult Wistar rats (male). The rats were presented with 12 trials (four in each of three series separated by 30-min intervals) consisting of attempts to find the platform (diameter 12 cm), which was positioned at the center of the "target" quadrant and was hidden beneath the water (at a depth of 2 cm). During the intervals between trials and series, rats were kept in their transfer boxes. Rats were launched into the basin from each quadrant in random order. If the rat failed to find the plat-

form in 2 min, the experimenter pushed it to touch the platform; the rat remained on the platform for 15 sec. After training, rats were immediately placed on the carousel. Nine of the 20 animals were subjected to sleep deprivation by rotating the disk for 30-sec with a 15-sec pause for 24 h. In the control group, consisting of 11 animals, the disk remained stationary. One day after training, animals were tested for reproduction of spatial learning: the rat was placed in the quadrant located diagonally opposite the target quadrant, but now without the platform, and was given the opportunity to swim (to find the platform) for 1 min.

The rats' behavior was analyzed using video recordings of their movement trajectories. Relative changes in the values of the following parameters were evaluated: 1) the duration of swimming before finding the platform; 2) the length of the path to this point; and 3) the ratio of the time spent by the rat in the target quadrant to the times spent in the other three quadrants. The mean swimming durations and path lengths for all 20 animals were calculated separately for each of the three series of trials. Training outcomes were evaluated using the data obtained in the test trials 24 h after training separately for deprived and non-deprived rats. The learning criterion was the ratio of the time spent by the rat in the target quadrant to the time spent in the other three quadrants.

Data were analyzed statistically on Statistica 6.0. Differences were regarded as statistically significant at $p \leq 0.05$. The presence of the memory trace was determined by comparing the mean time spent in the four quadrants one day after training by unifactorial analysis of variance (ANOVA) with repeat measures. The post hoc test for differences in mean values was the Newman–Keuls test. Mean times spent in the fourth quadrant in deprived and non-deprived rats were compared using Student's t test. Relative times spent in the fourth quadrant in the control group of rats were determined using the rank test (Mann–Whitney test).

RESULTS

Analysis of the overall data obtained with one-day training revealed changes in all the behavioral measures analyzed: 1) swimming duration; 2) path length before finding the platform; and 3) relative time spent by the rats in the target quadrant to that spent in the other three.

Individual changes in behavioral characteristics were quite variable. Figure 1, *A* shows examples of the paths taken by one of the rats launched into the water in the second quadrant during presentation of the first (*a*), second (*b*), and third (*c*) series of trials and test trials (*d*) presented one day after training. The paths taken by this rat on presentation of trials in the third (Fig. 1, *A*, *c*) series were significantly shorter than those on presentation of trials in the first (*a*) and second (*b*) series. In this case, the platform was found quickly in the target (fourth) quadrant in the first

series of trials, possibly due to random encounter with the platform, as the path taken by the rat during the second series was more than twice as long as the previous path. However, in the third series, the path was significantly shorter than in the two previous series. Path lengths in the 2nd, 5th, and 12th trials were 633.71, 1497.81, and 123.64 cm, respectively. In the trial for testing retention of the memory trace, performed one day after training, the path segment taken to the target quadrant was longer than the segments to the other three quadrants (Fig. 1, *A*, *d*). The relative time spent by the rats in the target quadrant was 33.24%, while the times spent in the first, second, and third quadrants were 24.53%, 24.40%, and 17.83%, respectively. Figure 1, *B* shows an example of the paths taken by another rat seeking the platform in the water, again launched in the second quadrant. On training, the path taken by this rat gradually shortened as trials were presented (*B*, *a*, *b*, *c*). Path length on presentation of the second (*B*, *a*), seventh (*B*, *b*), and ninth (*B*, *c*) trials in the three series were 1989.94, 289.15, and 297.71 cm, respectively. In the trial testing retention of the memory (*B*, *d*), in this case presented after one-day sleep deprivation, the longest path segment was not to the target quadrant, but to the third quadrant. The relative time spent by the rat in the target quadrant was 22.33%, while the times in the other three quadrants (first, second, third) were 11.10%, 14.57%, and 52.01% respectively. In other words, after sleep deprivation, the time spent by the rats in the target quadrant was not greater than the time spent in the other three quadrants.

Figure 2 shows overall data for all rats, providing evidence of changes in behavioral parameters on presentation of 12 trials organized as three series (first, second, third) each of four trials. Results for all parameters here and henceforth are given as mean ± standard error of the mean. Figure 2 shows first a gradual reduction in the duration of swimming by the rats (Fig. 2, *A*) and secondly, a shortening of the path followed by the rats to find the platform (Fig. 2, *B*). The mean durations of swimming were 58.95 ± 5.18, 33.95 ± 4.23, and 25.59 ± 3.83 sec in the first, second, and third series of trials respectively. The mean paths followed by the rats to find the platform were 1177.03 ± 101.99, 660.60 ± 79.74,

Fig. 1. Changes in the nature of paths taken by rats during training and one day after presentation of trials. *A*) The path taken by a rat (No. 29) on launching into the water in quadrant 2 in the second, fifth, and twelfth trials, respectively, in the first (*a*), second (*b*), and third (*c*) series of trial presentations and one day after training in the memory test (*d*). *B*) Path taken by a rat (No. 12) on launching into the water in quadrant 2 in the second, seventh, and ninth trials, respectively, in the first (*a*), second (*b*), and third (*c*) series of trial presentations and after one-day sleep deprivation (*d*). The large circle shows the circumference of the basin whose surface was divided by the vertical and horizontal lines into four quadrants designated *1–4*. Black rectangles show the points at which the rats were launched into the water; circles show the locations of the platform hidden beneath the water (*a*, *b*, *c*) and previous platform location (*d*).
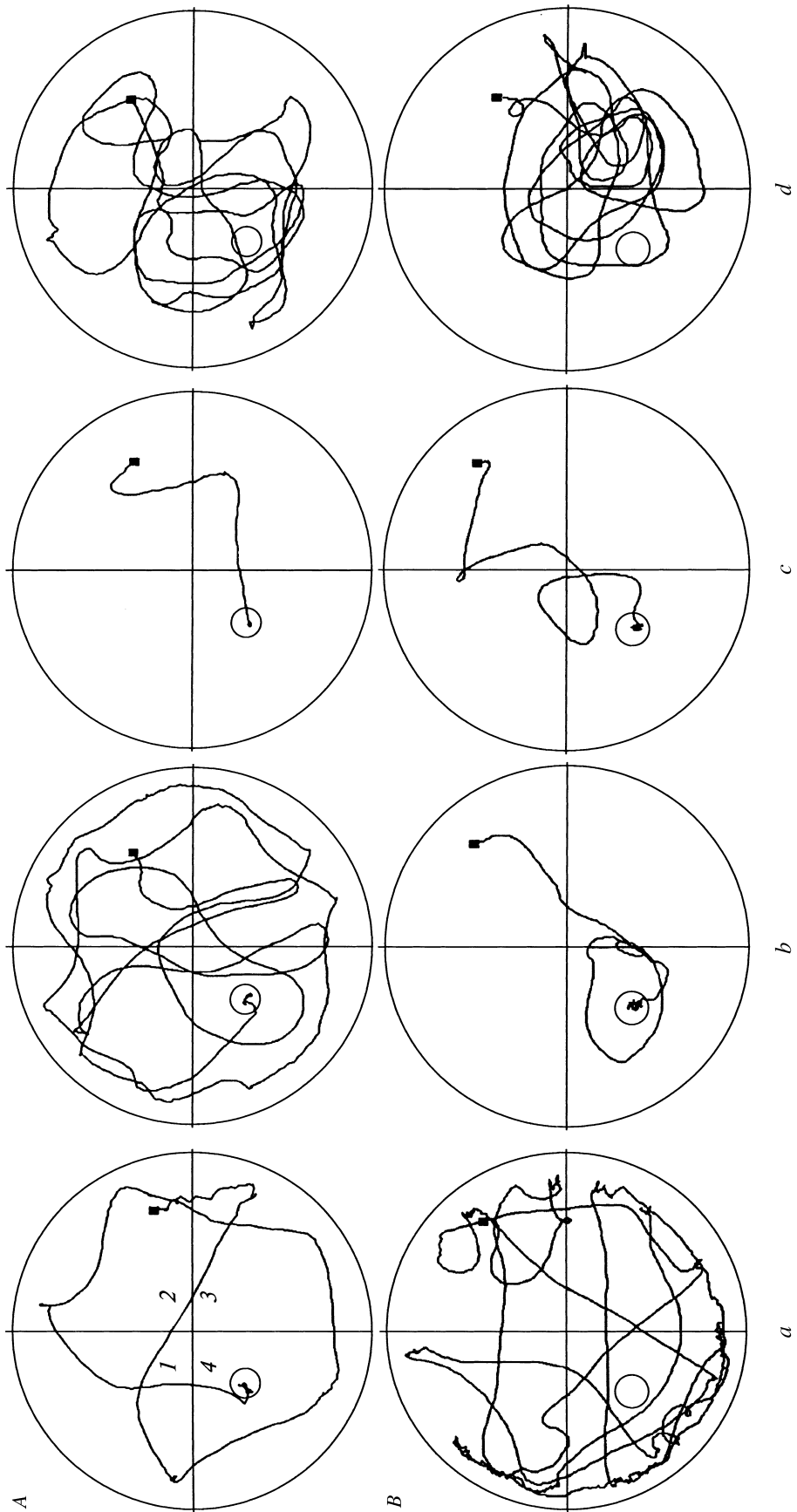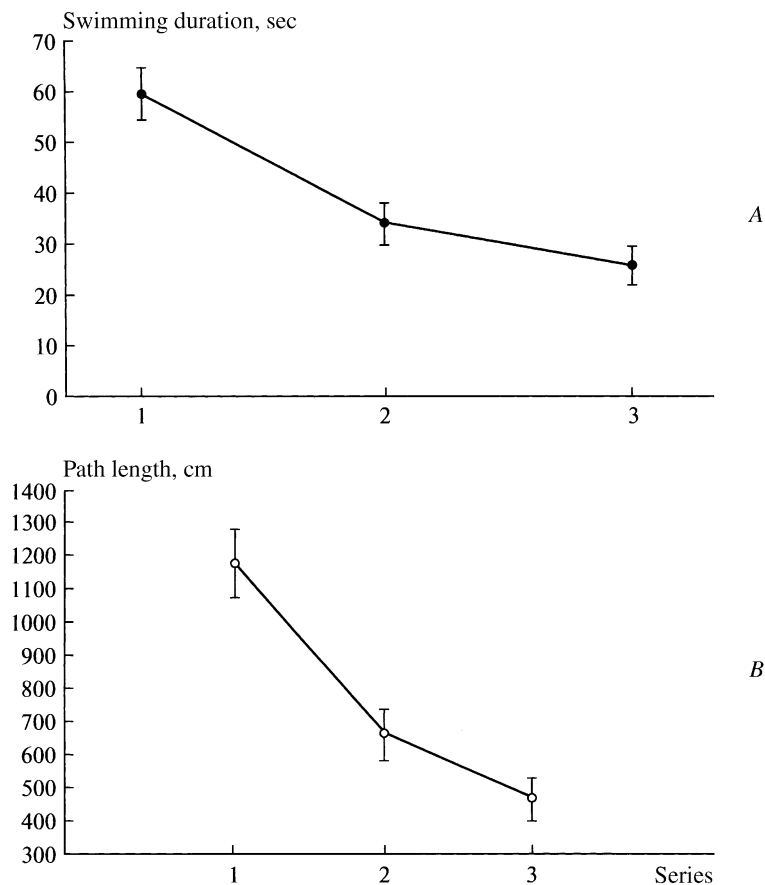
Fig. 2. Dynamics of behavioral measures after one-day training. *A*) Decrease in the duration of swimming by rats during presentation of 12 trials organized into three series (four trials in each series). The vertical axis shows the mean (all rats) duration (sec) of swimming; the horizontal axis shows the first, second, and third series. Vertical lines through points show standard errors of the mean. *B*) Decrease in the path taken by rats during presentation of trials in three sequential series. The vertical axis shows the mean (for all rats) path (cm). For further details see caption to *A*.

and 464.79 ± 65.13 cm in the first, second, and third series of trials, respectively.

Analysis of changes in the relative time spent in the target (fourth) quadrant during the second and third series of trial presentations as compared with values in the first series, for each rat separately, showed significant changes in 30% of cases (Wilcoxon–Mann–Whitney test).

On presentation of test trials one day after training, the mean swimming time of rats without sleep deprivation was 59.53 ± 1.27 sec, compared with 61.89 ± 1.96 sec in deprived rats. The mean path length in rats of the first group was 1349.11 ± 64.14 cm, compared with 1560.16 ± 100.88 cm in the second. Values for both of these parameters in non-deprived rats were smaller than those in deprived rats, though the differences between groups were not significant.

Analysis of variance showed that the mean relative time spent in the target (fourth) quadrant on presentation of memory test trials one day after training in non-deprived rats was significantly greater than the times spent in each of

the other three quadrants ($F_{3,30} = 3.16$; $p(F) = 0.04$). Application of the Newman–Keuls test showed that the probability of a random difference between the mean relative time spent in the target quadrant and the times spent in each of the other quadrants was greater than $p < 0.05$. For deprived rats, the difference between the mean relative times spent in each quadrant were not statistically significant ($F_{3,24} = 3.24$; $p(F) = 0.24$), i.e., memory traces were statistically unidentifiable. Comparison of the mean times spent in the fourth quadrant by deprived and non-deprived rats revealed no significant difference ($p(t) = 0.17$). However, the absence of a difference was explained by the atypical behavior of one rat in the deprived group, which justified application of the Mann–Whitney test, which showed that the sum of the rankings of relative times spent in the target quadrant (a behavioral characteristic of the control group of animals) was significantly greater than that in deprived rats ($p(U) = 0.05$). Thus, there is evidence that firstly, there was a significant increase in the mean relative

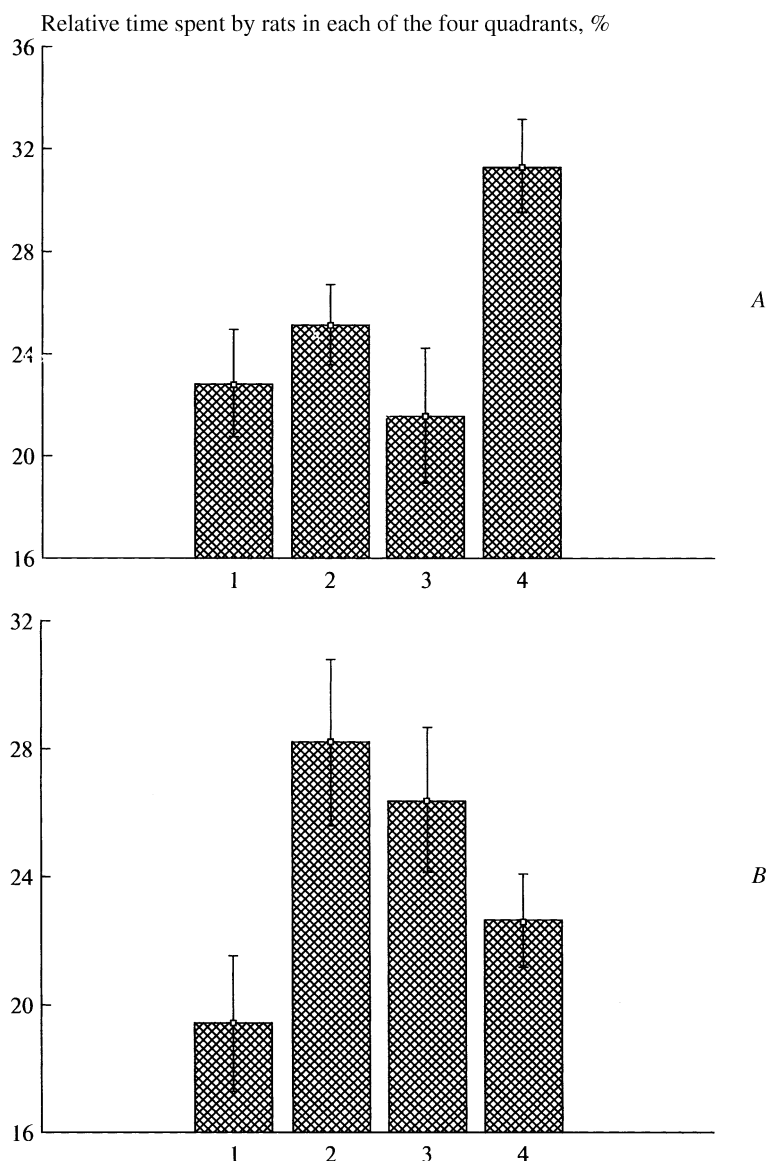Relative time spent by rats in each of the four quadrants, %

Fig. 3. Results of memory testing in control and deprived rats one day after one-day training. *A*) Greater relative time spent by non-deprived rats in the target (fourth) quadrant over times spent in the other quadrants one day after training. The vertical axis shows the mean (for all rats) relative times spent by the rats in the four quadrants; the horizontal axis shows quadrant No. The vertical lines on columns show standard errors of the mean. *B*) Absence of predominance of relative time spent by deprived rats in the fourth quadrant one day after one-day training. For further details see caption to *A*.

time spent in the target quadrant one day after one-day training in non-deprived rats. This change in deprived rats could be due to random causes. Secondly, use of the Mann–Whitney test indicated that the typical time spent in the target quadrant in the non-deprived group of rats was significantly greater than that in deprived rats.

The data presented in Fig. 3 show that in the test trial presented one day after training, the relative time spent by rats of the control group in the target (fourth) quadrant was greater than the times spent in the other quadrants (Fig. 3, *A*).

This difference was not seen in deprived rats (Fig. 3, *B*) in the test trial presented after one-day deprivation. The mean time spent by non-deprived rats in the target quadrant was 31.32 ± 1.82 sec, while the mean times spent in the others (first, second, and third) quadrants were 22.80 ± 2.10, 25.10 ± 1.82, and 21.60 ± 2.63 sec, respectively. In deprived rats, the mean time spent in the target quadrant was 22.61 ± 1.46 sec and the times spent in the other quadrants (first, second, and third) were 19.40 ± 2.13, 28.17 ± 2.60, and 26.37 ± 2.27 sec, respectively.

DISCUSSION

These data show that 24-h sleep deprivation immediately after one-day training induced impairment to the reproduction of spatial learning. This result indicates that sleep deprivation affects consolidation of spatial memory.

Comparison of our results with previous studies [24, 27] supported the negative influences of impairment of sleep processes on consolidation of spatial memory in the one-day form of spatial training in the Morris water test. The one-day training protocols in our study and in that of Tartar et al. [24] were identical, while another study from the same laboratory [27] used faster training, taking about 2 h [16].

There are two significant differences between our experiments and the experimental procedures used in [24, 27]. Firstly, we produced almost complete sleep deprivation, while these studies used sleep fragmentation in which the paradoxical phase of sleep almost disappeared, though the total duration of slow-wave sleep was almost unaltered, leading to an increased level of drowsiness. Secondly, spatial training in our studies was performed on the background of a normal sleep-waking cycle with subsequent sleep deprivation, while in the experiments reported in [24, 27], this cycle was degraded by 24-h sleep fragmentation. Considering the data reported by Ward et al. [27], it can be concluded that sleep impairment for one day had adverse influences on consolidation of spatial memory in both cases – when used before as well as after training.

The large spread in values for individual learning measures may be due to the decreased spatial learning ability of the Wistar rats used here using the one-day training procedure [9]. Different levels of spatial learning ability in the Morris water test with one-day training have been demonstrated in rats and mice [9], and in rats of different strains [24, 27]. While the sleep fragmentation used by Tartar et al. [24] influenced spatial learning in Sprague–Dawley rats, it had no effect on either spatial or visuomotor learning in Fischer/Brown Norway F1 rats, which have better learning ability [27]. In this latter study, sleep fragmentation had an adverse effect on memory consolidation after spatial training [27].

CONCLUSIONS

Thus, the data obtained here show that our experimental model can be used for studies of the mechanisms of the effects of sleep on spatial learning and memory. However, given the wide spread in values for individual learning parameters and the effectiveness of the influences of sleep deprivation, our further studies on the effects of sleep on memory consolidation will be performed on rats with better spatial learning ability than Wistar rats.

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