Circadian Phase-Shifted Rats Show Normal Acquisition but Impaired Long-Term Retention of Place Information in the Water Task

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It is thought that circadian rhythms may influence learning and memory processes. However, research supporting this view does not dissociate a mnemonic impairment from other performance deficits. Furthermore, published reports do not specify the type of memory system influenced by the circadian system. The present study assessed the effects of phase shifting on acquisition and expression of place navigation in the water maze, a task sensitive to hippocampal dysfunction. The results showed that phase-shifting circadian rhythms in rats impaired the expression of place information on a retention test but not initial acquisition or encoding of place information. These results suggest that disruption of circadian rhythms may impair consolidation of previously encoded hippocampal place information.

Key Words: memory; consolidation; circadian rhythms; hippocampus; spatial; relational.

INTRODUCTION

Memory processes may be influenced by circadian periodicity (Holloway & Wansley, 1973a, 1973b; Wansley & Holloway, 1975). Disrupting circadian rhythms by phase shifting the light:dark (LD) cycle has been shown to impair retention of active and passive avoidance tasks (Davies, Navaratnam, & Redfern, 1974; Tapp & Holloway, 1981; Fekete, Research supported by NSERC grants awarded to RJM and MRR. We are indebted to Mike Child for his intensive monitoring of the circadian recording system during the execution of this experiment. We also thank Andy Gristock for excellent animal care and Dr. Nicholas Mrosovsky for the use of the rat running wheels.

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VanRee, Niesink, & DeWied, 1985; Fekete, VanRee, & DeWied, 1986; Stone, Rudd, Ragozzino, & Gold, 1992). Although these findings show that mnemonic performance can be influenced by phase shifting the LD cycle, it is not clear whether a specific type of memory is particularly susceptible to circadian disruption. For example, it would be useful to know whether circadian timing modulates hippocampal-based learning and memory processes given that aged human and nonhuman animals experience both circadian decline (Stone, 1989) and impairments of hippocampal memory processes (Rapp, Rosenberg, & Gallagher, 1987; Gallagher & Pelley, 1988; Gallagher, Gill, Baxter, & Bucci, 1994).

The hippocampus is a complex learning and memory system which seems essential for the acquisition, encoding, and retrieval of complex representations of the elements that define a specific event and the environment it occurred in (O'Keefe & Nadel, 1978). In humans, the hippocampal learning and memory system has been referred to as the episodic memory system and is deemed critical for an “... individual’s awareness of personal experiences in subjective time” (Schacter & Tulving, 1994).

To address the issue of whether circadian rhythms influence hippocampal-based memory processes, the present study sought to determine whether phase shifting the LD cycle, which causes temporary rhythm disruption, would specifically impair spatial learning and/or memory in the water-maze place task (Morris, 1981). This task is mediated by a neural circuit in which the hippocampus is a central module (Sutherland & Rudy, 1989) and is sensitive to age-related memory decline in rodents (Gallagher et al., 1987, 1994; Rapp et al., 1987).

In several studies that have manipulated the LD cycle, retention testing was conducted shortly after phase shifting, before adequate time for reentrainment had occurred. Therefore performance deficits may have been the result of disrupted rhythms, a specific mnemonic impairment, or both (Takamure, Murakami, Takahashi, Kuroda, & Etoh, 1991). To dissociate a mnemonic impairment from other performance deficits, the animals in the present study were given two probe tests. The first probe test was performed 7 days after the last day of training, before all of the animals had reentrained to the LD cycle. This probe was performed to rule out state dependency effects of training and testing in different circadian contexts. Because the shifted light cycle would alter the animals’ activity cycles, it was clear that the first retention probe, which occurred 7 days following reinstatement of the final zeitgeber, would be performed at a different circadian time compared to the nonshifted group. For this reason, the second probe test occurred 10 days after the first probe, which allowed sufficient time for all of the animals to reentrain before retention testing.

**METHOD**

**Subjects**

Eighteen male Long–Evans hooded rats, approximately 6–8 weeks of age (Charles River Laboratories, Quebec), were used. The animals were housed individually in wire-mesh cages with ad lib access to food (Purina laboratory chow) and water. Each cage was individually attached to a large running wheel (36 cm in diameter and 12 cm wide) to which the rats had continuous access. Each running wheel was connected to an activity-recording device through which wheel running activity was continually monitored. The
housing environment was maintained on a 12:12 LD cycle completely independent from the LD cycle in the rest of the laboratory. The room temperature was kept constant at 21°C.

**Apparatus**

A circular pool (184 cm in diameter and 60 cm deep) was used. The water maze was placed in the center of an experimental room that measures 430 × 262 cm. During training, the pool was filled with 20–22°C water to a level of 36 cm, and the water was made opaque by adding nontoxic white paint. The submerged (hidden) platform was made of clear Plexiglas and measured 12 × 12 cm. This platform was mounted on a Plexiglas column held in place on the bottom of the pool by weights. Several salient cues were located at fixed positions in the extramaze environment including several posters affixed on the walls, the computer tracking system, and the experimenter.

**Procedure**

**Illumination schedule.** All animals were put on a 12:12-h light:dark cycle for 10 days beginning immediately after their arrival at the laboratory. Daily inspection of the running records revealed that this 10-day period was necessary for animals to entrain to the LD cycle. Prior to acquisition training, animals were randomly assigned to a control group \((n = 9)\) or an experimental (phase-shifted) group \((n = 9)\). The control group remained under the original LD cycle throughout the experiment and all training and test trials began 1 h before the dark phase. The phase-shifted group was exposed to a 3-h phase advance of the LD cycle on 6 consecutive days, beginning on the day prior to acquisition (see Table 1). Training trials for the phase-shifted group began every 23 h (see Table 1) in order to closely approximate the 24-h delay between the daily training sessions for the control group. Following place acquisition training, the phase-shifted animals were kept on a constant 12:12-h LD cycle until reentrainment to the LD cycle was attained. Reentrainment for all of the rats in the experimental group occurred after 17 days of exposure to the LD cycle. Testing occurred at approximately the same time of day as the last day of training for the phase-shifted group.

**Place acquisition.** All rats were trained on the place navigation task described by

| **TABLE 1** Schedule of Phase Shifting and Training/Testing Times for the Experimental Group |
|-----------------------------|-----------------------------|-----------------------------|
| Day                         | Light phase                 | Train/test time             |
| Entrainment (10 days)       | 15:00–03:00                 | —                           |
| Day prior to acquisition    | 12:00–00:00                 | —                           |
| Day 1—acquisition          | 09:00–21:00                 | 1000                        |
| Day 2—acquisition          | 06:00–18:00                 | 0900                        |
| Day 3—acquisition          | 03:00–15:00                 | 0800                        |
| Day 4—acquisition          | 00:00–12:00                 | 0700                        |
| Day 5—acquisition          | 21:00–09:00                 | 0600                        |
| Reentrainment              | 18:00–06:00                 | —                           |
| Retention probes           | 18:00–06:00                 | 0500                        |
Morris (1981). Prior to training, the hidden platform was positioned at a fixed location in the center of the southeast quadrant of the pool (not a true compass heading). On each trial the animal was placed in the water maze facing the wall and released from one of the four randomly chosen start positions (N, E, W, and S). The order of the start positions was the same for each animal on a block of four trials. On each trial the rat was allowed to swim until it located the platform or until a 60-s trial termination interval had elapsed. If a rat had not found the platform by the end of the termination interval, the experimenter placed the animal onto the platform. Following escape or aided placement onto the platform, the animal was left there for an additional 10 s, after which the animal was placed back into its holding cage and the next animal was trained. Each animal received 40 trials of place training over a 5-day period (2 blocks of 4 trials/day). The measures of navigation assessed during training were (1) latency to locate the platform, (2) path length, and (3) quadrant preference. Retention tests occurred 7 days (probe 1) and 17 days (probe 2) after the completion of acquisition, the hidden platform was removed from the water maze and each animal was given one swim from the north start point and allowed to swim for a period of 60 s. This start point was selected pseudorandomly from the subset of start points that were furthest away from the platform position (north and west). The measure of spatial bias recorded on these probes was quadrant preference.

Data analysis. As mentioned above, we assessed multiple parameters and a separate analysis was performed on each parameter. For the acquisition data, ANOVAs with repeated measures were performed on the shifted and nonshifted groups. For the retention probes, we performed one-tail t tests because our a priori assumption for quadrant preference was that the animals would spend more time in the target quadrant versus the others.

RESULTS

Circadian Rhythms

For the circadian manipulations, in this experiment, the state of the circadian system in our subjects was assessed in the pattern of locomotor rhythmicity expressed. Specifically, home-cage wheel-running activity was recorded throughout, to provide a complete, continuous record of rhythmic behavior. Actograms for control (nonshifted; Fig. 1) and experimental (shifted, Fig. 2) demonstrate typical entrainment and temporal activity patterns for the two groups. Rats performed most of their daily wheel-running activity following light-offset when entrained to a 24-h LD cycle. However, when the LD cycle was phased advanced by 3 h/day, the animals’ activity was also initially advanced (1–2 days) before becoming temporally scattered and reduced in intensity (Fig. 2).

Entrainment is defined as a stable phase relationship existing between zeitgeber cycle (lights off) and activity (activity onset). A stable phase relationship exists when the average period of the animal’s rhythm (assessed from regression analysis of onset times) matches that of the LD cycle to within 0.5%. Reentrainment to a shifted LD cycle has occurred when the original phase relationship is attained with the shifted cycle.

Place Acquisition

Figure 3 shows the mean escape latencies (top left panel), path length (top right panel), and the quadrant preference (bottom panel) for each group across 4 days of training. The
FIG. 1. The actograms from two representative rats in the nonshifted group. Light offset is indicated by the vertical black line. As can be seen, there was a clear relationship between light-offset and wheel-running activity.

data from day 3 were unusable because of a technical failure associated with the tracking system on that day. Overall group × day ANOVAs on the latency and path length indicated, in both cases, a significant effect of day \([F_3, 48 = 60.37, P < .05; F_3, 48 = 83.13, P < .05]\) but no significant effects of group or a group × day interaction. An overall group × day × location ANOVA on the quadrant preference data revealed a significant effect of day \([F_3, 48 = 29.21, P < .05]\) and location \([F_1, 16 = 174.82, P < .05]\), but no significant difference of group. The day × location interaction was the only two-way interaction that was significant \([F_3, 48 = 29.18, P < .05]\), indicating that both groups of rats showed an increased preference for the target quadrant over days. The results indicated that significant learning occurred in both the control and phase-shifted groups during acquisition. Moreover, these results suggest that phase shifting the LD cycle, as
FIG. 2. The actograms from two representative rats in the shifted group. The top portion indicates the initial entrainment to a 12:12 LD cycle. The middle portions show the effects of phase advancing 3 h per day for 5 days on wheel-running activity (indicated by stepped black line). The bottom portions of the actograms show the effects of reentrainment to a 12:12 LD cycle.

described above, does not produce any significant performance deficits on the spatial version of the water task.

Retention Probes

The mean time spent in the training quadrant was weighted against the mean time spent in the other three nontraining quadrants combined on probe trials 1 and 2 for the shifted (top panel) and nonshifted animals; these data are shown in Fig. 4.

One-tailed $t$ tests comparing the amount of time spent in the target versus other quadrants on both probes for the nonshifted group were significantly different ($T_8 = 3.25, P < .05; T_8 = 2.14, P < .05$). The tests for the shifted group were not significantly different on either probe ($T_8 = .30, P > .05; T_8 = .05, P > .05$). This pattern of results shows that the shifted animals exhibit a significant retention deficit for place information regardless of whether their circadian rhythms had reentrained (probe 2) or not (probe 1). This is an important demonstration because it suggests that the reason for the retention deficits in the shifted animals is not due to state-dependent learning. That is, shifted animals were impaired on the retention tests regardless of whether they were trained and tested while
DISCUSSION

The present data show that disrupting circadian organization in rats by phase shifting the LD cycle during place training in the water maze selectively impairs retention of the hidden platform location following photic reentrainment. However, even though the phase-shifting procedure in the present study was much more extensive (+3 h/day × 6 days) than past studies that have used single phase-shifts, our data suggest that this does not disrupt acquisition of normal escape behavior. Moreover, the fact that place acquisition performance was not influenced in the phase-shifted group, while receiving daily training sessions at different times within the altered 12:12 LD cycle (compared to controls who always received their training 1 h prior to light offset), strongly suggest that the selective retention impairment observed in the phase-shifted group resulted from a specific mnemonic deficiency.

In a previous study of avoidance behavior by Fekete et al. (1985) impairments were consistently observed on retention testing when phase shifting occurred within a 24-h period prior to the test, but not when the phase-shift occurred before that time period. This finding suggested that retrieval processes were disrupted by the phase-shifts. Consistent with this view, the results of the present experiments could also be interpreted as an
instance of retrieval failure because the phase-shifted rats were probably trained and tested in different circadian states. That is, the phase-shifted rats could be showing a state-dependent circadian retrieval effect when they were retested after reentrainment procedures. However, we have recently completed a series of studies investigating the effects of different training/testing circadian times on the spatial version of the water task and found no effect of these manipulations on expression of this task (unpublished data). These data, combined with the results of the present study showing disrupted retention performance even when phase-shifting had ended 17 days prior to retention testing, support a learning/consolidation view rather than a retrieval interpretation.

The posttraining paradigm frequently used in pharmacological studies may provide the strongest evidence that a manipulation selectively acts upon a time-dependent memory consolidation process (McGaugh, 1966; McGaugh & Herz, 1972; Alvarez & Squire, 1994). Treatments such as these are unlikely to produce nonspecific sensory, motor, or motivational effects because they occur after the training experience and before the
retention test. Although the manipulation (phase-shift) in the present study occurred during training, the evidence still implicates some type of consolidation process as the affected mechanism. First, a nonspecific influence on performance is unlikely because phase shifting did not impair place acquisition. Second, based on this same finding, it may be argued that sufficient learning occurred in the phase-shifted group. Third, as described above, it is unlikely that retention performance was influenced by altered activity rhythms or state dependency. Therefore, the retention impairment may represent incomplete consolidation of spatial information. However, it is important to make a distinction between two different types of memory consolidation. One type of memory consolidation is a set of processes that take place over a relatively short duration (minutes to hours) and another type that takes place over days and weeks. The former is based on a theory (McGaugh, 1966) suggesting that during the period immediately after information is first encoded in the brain, these neural representations undergo some type of physiological change that renders them more permanent. With the passage of time, these memories become more resistant to change. The other type of memory consolidation is of a longer duration and is based on recent ideas that the hippocampus consolidates recently acquired memories by reactivating these representations during slow-wave sleep (Wilson & McNaughton, 1994; Houston, Stevenson, McNaughton, & Barnes, 1999). It is this type of memory consolidation that we seem to be manipulating in the present experiment.

Although the task used in the present study has been shown to be sensitive to hippocampal damage (Morris, 1982; Sutherland, Whishaw, & Kolb, 1983), lesions of other brain structures (Whishaw, Mittleman, Bunch, & Dunnett, 1987; Sutherland, Whishaw, & Kolb, 1988; Sutherland & Rodriguez, 1989; Kolb, Buhrmann, McDonald, & Sutherland 1994; Devan, Goad, & Petri, 1996; Devan, McDonald, & White, 1999; Devan & White, 1999) also impair place acquisition. However, rats who are pretrained on the water task before receiving damage directed at these structures (nucleus accumbens, anterior thalamus, mammillary bodies, medial septum, and prefrontal cortex) show normal place memory. All of these structures are major efferents of the hippocampus and are part of a neural circuit mediating initial encoding during place learning. This anatomical organization and these behavioral findings suggest that hippocampal system damage in particular disrupts retention performance on the water-maze place task. Because the phase-shifting impairment in the present study was restricted to the retention test, it is likely that the mnemonic disruption was due to some influence on the hippocampal system rather than on these other brain structures that produce acquisition deficits in the water task.

The suggestion that the retention impairment resulting from phase shifting in the present study may have involved a disruption of normal hippocampal function assumes a direct or indirect influence of structures involved in photic entrainment. In mammals, the biological clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Ralph, Foster, Davis, & Menaker, 1990). Several limbic structures, including the hippocampus, amygdala, and nucleus accumbens, receive both direct and secondary inputs from the suprachiasmatic nucleus and subparaventricular zone via the lateral and medial septal nuclei, the parataenial and paraventricular nuclei of the thalamus, and the bed nucleus of the stria terminalis (Wyss, Swanson, & Cowan, 1979; Watts, Swanson, & Sanchez-Watts, 1987). It is possible that some component of these connections may modulate hippocampal function and influence the consolidation of hippocampal-dependent place memory. This view suggests that the mechanism by which the circadian system influences consolidation
processes in the hippocampus could be through direct input from the pacemaker to the learning and memory system.

Alternatively, an intriguing possibility is that pacemaker influences on hippocampal consolidation processes are a secondary consequence of the pacemaker’s influence on sleep patterns (Stone, 1989). Damage to the pacemaker in rats disrupts amount, continuity, and organization of sleep cycles. These effects of the pacemaker on sleep are relevant to the interpretation of the results reported in the present article because some researchers (Wilson & McNaughton, 1994) have provided evidence for a role of slow-wave sleep processes in the hippocampal-based consolidation processes. These investigators recorded from large ensembles of place cells in the hippocampus while the rats were acquiring place information. These same cells were recorded while the animals were in slow-wave sleep, either before or after place acquisition. The ensembles of cells that showed spatially selective firing during place learning also showed an increased tendency to fire together during slow-wave sleep. This data suggests that slow-wave sleep is important for memory consolidation processes in the hippocampus. Consistent with this view, Buzsaki (1989) argued, based on various types of electrophysiological evidence, that sharp wave activity during slow-wave sleep might be an important component of memory consolidation in the hippocampus. Thus, disruption of circadian rhythms alters the amount and patterns of sleep which may disrupt normal consolidation processes in the hippocampus during sleep. According to this view, place information would be encoded and stored normally in animals under drastic phase-shifting conditions but memory consolidation processes that strengthen these representations would be weakened. The net result of this lack of consolidation during sleep is that the place memories will show a more rapid rate of decay over time, as was demonstrated in the present experiments. Empirical support for the direct or secondary consequence views of the influence of the circadian system on hippocampal-based learning and memory processes awaits further investigation.

In conclusion, the present findings demonstrate that phase-shifting circadian rhythms can disrupt cognitive retention independent of its effects on nonmnemonic performance. These findings may further provide a useful animal model of circadian-dependent hippocampal disruption in humans.

REFERENCES


