Research report

The significance of circadian phase for performance on a reward-based learning task in hamsters

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Abstract

In humans and animal models, circadian modulation of learning has been demonstrated on numerous tests. However, it is unclear which aspects of the cognitive process are rhythmically regulated. In these experiments, we used a conditioned place preference task in hamsters to ask whether memory acquisition (hypothesis 1) or memory recall and performance (hypothesis 2) were subject to circadian modulation. In golden hamsters, access to a running wheel has been used as a reward to condition a place preference, but when given unrestricted access to a wheel, animals perform most of their spontaneous running within a few hours each day or circadian cycle. This suggested that either the perceived reward value of the wheel changes through the day or that the response to this reward is temporally restricted. Contrary to the hypotheses, we found that learning was not tied to the time of training nor to the time of testing, but rather animals showed a preference for a reward-paired context only at the circadian time that training had taken place. Timing is not an explicit discriminative cue in these experiments. Hence, the learning mechanism must be predisposed to register circadian time as an attribute during context learning.

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1. Introduction

A growing body of evidence demonstrates that circadian timekeeping can influence the processes of learning and memory. This comes from studies of both humans and animal models (see Ref. [12]). The ability to learn, remember, and then to modify current responses according to prior experience are fundamental attributes of animal behavior. In mammals, learning involves a complex set of steps that comprise sensory processing, acquisition, memory formation, memory retention (and extinction), and memory retrieval. Recent studies have begun to demonstrate that these processes may be differentially influenced by the animal’s circadian rhythms or by the time of day.

An association between rhythmicity and learning has been demonstrated in three general ways. First, situations where circadian rhythms are disturbed are invariably linked with cognitive impairment. In humans, these include the aging process, shift work, transmeridian travel (jet lag), and disease. In animal models, the circadian disruptions produced by phase shifting the light–dark cycle result in impaired performance on passive avoidance tasks [6,10,27]. Age-related rhythm disturbances impair performance on a conditioned place preference (CPP) task in hamsters [1], and on a test of spatial learning in rats, memory retention is selectively impaired while the initial acquisition and recall are intact [7].

Second, processes underlying cognitive performance may be modulated over the day or a circadian cycle. Numerous studies have shown that optimal times of day exist for learning different tasks in humans and in animals [11,15,16,20,21,29,30]. In addition, retention deficits on numerous tasks recur at periodic intervals in rodents, suggesting that memory recall is subject to temporal modulation separately from acquisition and memory formation [18,15–17,29].
Finally, in some species and situations, the time of day or the phase of the circadian cycle may be an attribute of the environment that is learned. The ability to learn, remember, and respond according to the specific timing of events has an enormous adaptive significance [2,9,12,13,28]. Animal’s ability to learn the timing of significant events is indicated by the antecipatory behavior of various animal species that are presented with timed rewards, e.g. temporally restricted feeding schedules [3,8,13,23,26].

Taken together, these studies show that essentially all steps in the learning process may be subject to temporal regulation. The degree to which each step is affected appears to depend on the species and the learning paradigm that is used. In addition, the time of day may be a feature that is learned along with other attributes such as location, context and value of the presented stimulus [12].

In our experiments, we have begun to address the extent to which the circadian rhythms influence each of these aspects of learning. Because reward is a strong motivator of learning and performance, we have focused on reward-associated learning. Using a CPP task, we have shown previously that the ability to learn associations between reward and context is impaired in hamsters with circadian rhythm disruption [1]. In rodents, and especially in hamsters, running wheel activity is highly rewarding. However, when given free access to a running wheel in constant environmental conditions, running activity is strongly restricted to the animal’s subjective nighttime. This suggested two alternative hypotheses: (1) that the perceived reward value of the wheel changes through the day; or (2) that the response to reward is temporally restricted. Using brain stimulation reward (BSR), Yanofski et al. [31] showed that perceived reward value does not vary with circadian time in rats. However, we have found significant effects of BSR on hamster locomotor rhythms that are not detectable in rats (S.W. Cain et al., manuscript in preparation). Therefore, there may be important species differences in the connections among circadian and brain reward mechanisms.

Based on the first hypothesis, we predicted that the strength of the CPP that is developed by using timed wheel reward will vary with the animals’ spontaneous patterns of behavior and wheel use. This was complicated by the fact that hamsters may be induced to use a wheel at times when spontaneous activity is low, i.e. a novel wheel can be potentially rewarding when the home cage wheel is not. This issue was addressed in the test of the second hypothesis from which we predicted that the acquisition of a place preference could occur any time that the relative reward value of the stimulus were high, but that the expression of the preference would still depend on the animal’s natural pattern of locomotor activity.

Neither of the two hypotheses was supported by the results of these experiments. Instead, CPP expression was restricted to the temporal match between training and testing times. This indicates that the internal representation of time is a significant attribute of context even when time is not a discriminative cue.

2. Materials and methods

2.1. Animals and activity recording

Forty-eight male Golden hamsters (Mesocricetus auratus) were obtained from Charles River Canada (Montreal, Quebec), and were between 80 and 90 days old at the beginning of the experiment. Animals were housed individually in polypyrrole cages (22 × 20 cm) with free access to food, water, and a stainless steel running-wheel (17 cm in diameter). Wheel running activity was monitored continuously throughout the experiment using VitalView (MiniMitter Co., Inc., Sunriver, Oregon). Cages were kept inside light–tight ventilated boxes (six cages per box).

2.2. Lighting conditions

Illumination was provided by two GE Cool White fluorescent tubes that provided 180–200 lux at the floor of each cage. Two experiments were performed. In experiment 1, the animals were held on a light–dark cycle of 14 h of light and 10 h of dark. In this situation, time is reported relative to the light–dark cycle. Dark onset is defined as Zeitgeber Time 12 (ZT12). To determine whether animals used internal versus external time cues, we repeated part of the experiment in constant light (experiment 2). To reduce the potential deleterious effects of bright light, we covered each bulb with a broadband acrylic sheet that reduced illuminance to 40–50 lux red light. In this case, the onset of wheel running behavior was defined as Circadian Time 12 (CT12), and all other times were defined relative to this point. In both experiments 1 and 2, the CPP apparatus was illuminated by 40-lux dim red light.

2.3. Experimental procedures

The CPP requires that an animal learn an association between a reward stimulus and a context in which the reward is found. The test of learning is that the animal will show a preference for a rewarded context over a non-rewarded context in the absence of reward. The two contexts otherwise will remain neutral (see Ref. [4]). The contexts in this study differed in three ways. Context A was a three-sided chamber painted with horizontal black and white stripes, and contained a distinct odorant (isoamyl acetate). Context B was a black, eight-sided...
chamber that contained a second distinct odorant (eucalyptus). Tests for context preference were conducted by placing an animal in an alley that connected the two context chambers, then recording the amount of time (dwell time) that was spent in each chamber. An animal was considered to be within a chamber when both forepaws were past the threshold into the chamber.

The floors of each chamber and the alley were made of clear Plexiglas. A mirror placed beneath the apparatus was inclined at 45° so that a videotape recording of each animal’s activity could be recorded unobtrusively for later analysis.

2.3.1. The CPP paradigm

The two experiments were conducted in three steps.

2.3.1.1. Phase 1: Pre-exposure. This confirms the pre-training neutrality of the contexts. The two chambers were connected by an alley with open access to both ends. Each animal was placed in the alley and allowed 20 min to explore the two contexts. Dwell time in each chamber was recorded.

2.3.1.2. Phase 2: Conditioning. For 8 consecutive days, each animal was allowed to experience one of the two contexts each day for 30 min. For each animal, one context contained the running wheel. Animals experience alternately Context A (or B) on day 1, Context B (or A) on day 2, and so on, hence each context was experienced four times. The order of presentation and location of the running wheel reward (Context A or B) were counterbalanced. In addition, the experiments were conducted with 50% of the animals trained at ZT13 and 50% at ZT04. For experiment 2, animals were trained at either CT13 or CT04.

2.3.1.3. Phase 3: Testing. On the day following the last conditioning day, each animal was tested for context preference by placing it in the connecting alley with free access to both context chambers. In experiment 1, 50% of the animals that were trained at ZT13 were also tested at ZT13 while the others were tested at ZT04. Similarly, the animals trained at ZT04 were tested at ZT04 or ZT13. The same approach was taken for experiment 2 but conducted in constant light. In this case, animals were trained at CT13 and tested at either CT13 or CT04. A 2 × 2 (group × location) ANOVA was applied to the data from each experiment. This was followed by planned comparisons of groups as indicated in Figs. 1–3.

3. Results

The results from both experiments confirm the previously reported findings that the running wheel is

Fig. 1. Place preference conditioned by reward-pairing at ZT13. The left pair of histograms indicates the absence of a context preference prior to conditioning (n = 16). The middle pair indicates the preference for the paired context when tested at ZT13 (n = 8). The right pair indicates a lack of preference when tested at ZT04 (n = 8) (significance tested by ANOVA and planned comparisons).

Fig. 2. Place preference conditioned by reward-pairing at ZT04. The left pair of histograms indicates the absence of a context preference prior to conditioning (n = 16). The middle pair indicates a lack of preference for the paired context when tested at ZT13 (n = 8). The right pair indicates the preference when tested at ZT04 (n = 8) (significance tested by ANOVA and planned comparisons).

Fig. 3. Place preference conditioned by reward-pairing at CT13. The left pair of histograms indicates the absence of a context preference prior to conditioning (n = 16). The middle pair indicates the preference for the paired context when tested at CT13 (n = 8). The right pair indicates a lack of preference when tested at CT04 (n = 8) (significance tested by ANOVA and planned comparisons).
rewarding and can condition a place preference in hamsters. In addition, they demonstrate that the hamster is capable of making associations and remembering the distinct differences between the two contexts used in these experiments.

3.1. Experiment 1

Results are shown in Fig. 1 (training at ZT13) and Fig. 2 (training at ZT04). Prior to conditioning, animals showed no significant difference in dwell time at either ZT13 or ZT04 when presented with a choice of contexts (Figs. 1 and 2, left histograms). On training days, all animals interacted with the running wheel by exploring as well as running. The amount of running has been shown previously to be uncorrelated with the measurement of preference [1]. Following conditioning, animals that were tested at ZT13 showed a significant preference for the rewarded context when tested at ZT13 (Fig. 1, middle histogram), but showed no preference if tested at ZT04 (Fig. 1, right histogram). Similarly, for the group that was trained at ZT04, a preference was expressed when tested at ZT04 (Fig. 2, right histogram), but not at ZT13 (Fig. 2, middle histogram).

The results of experiment 2 (Fig. 3) essentially duplicated those of experiment 1 except that they were obtained in constant lighting conditions. All animals in experiment 2 were trained at CT13. A significant place preference was found when animals were tested at CT13; however, animals did not express the preference when tested at CT04. There were no significant main effects of training time, testing time, or context in any of the experiments.

4. Discussion

The predominant message that is communicated through these results is that the expression of a preference for a reward-associated context depends on whether the preference test is conducted at the same time of day (or circadian cycle) that the training occurred. Because all subjects were trained in the same manner at either time point, the results suggest that animals had learned during training, but did not behave as though they had learned unless the timing of the test approximated the time of training. How close these two times must be has yet to be determined. The clear conclusion from this is that the animals encoded the time of day, or phase of the circadian cycle, even though these were not discriminative cues in this task.

These experiments were based on the notion that the spontaneous locomotor behavior of the hamster reflects a natural motivation to move about. We had predicted that because this was rhythmic, then either the value of the running wheel as a reward (hypothesis 1) or performance in reward-associated contexts (hypothesis 2) would be modulated with similar timing. Neither of these was supported by the results.

First, contrary to the prediction from hypothesis 1, the wheel reward induced a place preference at times of both low and high spontaneous activity, hence conditioning in these experiments was not influenced significantly by the inherent drive to run on the wheel. Hypothesis 1 is not disproved, however, since the reward associated with the wheel in the home cage may still vary rhythmically, whereas the novelty of the wheel in the CPP apparatus might be rewarding at all times.

Second, contrary to the prediction from hypothesis 2, the CPP was not restricted to the time of highest spontaneous wheel running. The endogenous drive to run on the home cage wheel did not alter the degree to which the wheel-paired context was preferred. The most obvious indication of this was the lack of preference shown at ZT13 by animals that were trained at ZT04. While it can be argued that CPP may not be sensitive enough to detect influences of the endogenous rhythmic drive to run, it is certain that this is much less important to the organism than the temporal coincidence of training and testing times.

Perhaps the most interesting and puzzling of the results reported here is that CPP was not expressed when testing and training times were different. Certainly in the combinations tested, neither time of day nor circadian time would be expected to predict reward location. However, the animals were quite capable of using three other context features for discrimination, and it seems unlikely that the animals were unable to make these distinctions at one time that they made so readily at the other. Therefore, the best explanation of the results is that it was the motivation to obtain the wheel reward (indicated by dwell time) that became linked to the time that the reward had been encountered.

Superficially, these results appear to be similar to the time of day learning that occurs with scheduled food availability. However, unlike the anticipatory behavior that is evoked in food entrainment experiments, a discrete reward/time pairing is not required for the development of CPP time-stamp. The CPP paradigm does not explicitly state that reward will not be forthcoming at non-training times. This is an implied statement that is derived from the animal’s own attention to circadian time. For these animals, the internal representation of time is a feature of the context that in learned along with the external features. This suggests that the systems of learning and memory that are affected by CPP are predisposed to associate reward with circadian time of occurrence.

The neural mechanisms responsible for time of day learning are not known. A reasonable place to look is the hypothalamic suprachiasmatic nucleus (SCN), the
site of circadian rhythm generation in mammals. However, in experiments where timing is a discriminative cue, learning still occurs in the absence of the SCN [24]. Thus oscillators outside the SCN are responsible for registering the time of day, and for associating this internal state with the occurrence of an external stimulus. We have found that the time stamp induced in the CPP paradigm also does not require an intact SCN [19]. If the central circadian clock is involved in time of day learning, it appears to be involved mainly with temporal adjustments of learning and performance rather than learning itself.

These findings could have profound implications for the design of learning and memory experiments in other species, including humans. Already there exists evidence for time of day modulation of learning and performance, and recently, it has been shown that explicit and implicit recall in humans are rhythmic and out of phase with each other [14]. It may be the case that humans are also predisposed to register the time of day that certain information is acquired or events occur, without the need for explicit instruction to do so. If so, these results may also be relevant to the way in which cognitive performance in humans is evaluated, not only in experimental but also in educational or job related situations. If learning occurs at a particular time of the day and test performance depends on a coincidence of training and test time, then test results may not always be a truly accurate measure of learning. In this regard, it is important to point out that in our hands, the time stamp result reported here is not exhibited in rats [22]; nor to our knowledge has it been reported previously in rats using the place preference paradigm. The time stamp phenomenon, therefore, may be species specific and task dependent (see Refs. [5,25]). Its expression may depend on the degree to which each species has come to rely on a memory for time to predict the availability of reward stimuli in the natural environment. For some species, depending on their ecological history, it may be adaptive to restrict an animal’s activity or presence in a given context to times when rewarding features have been encountered before. If so, it is a reasonable prediction that the positive emotional valence of the context would be reduced at other times. It remains to be seen whether such a phenomenon can be demonstrated in humans.

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