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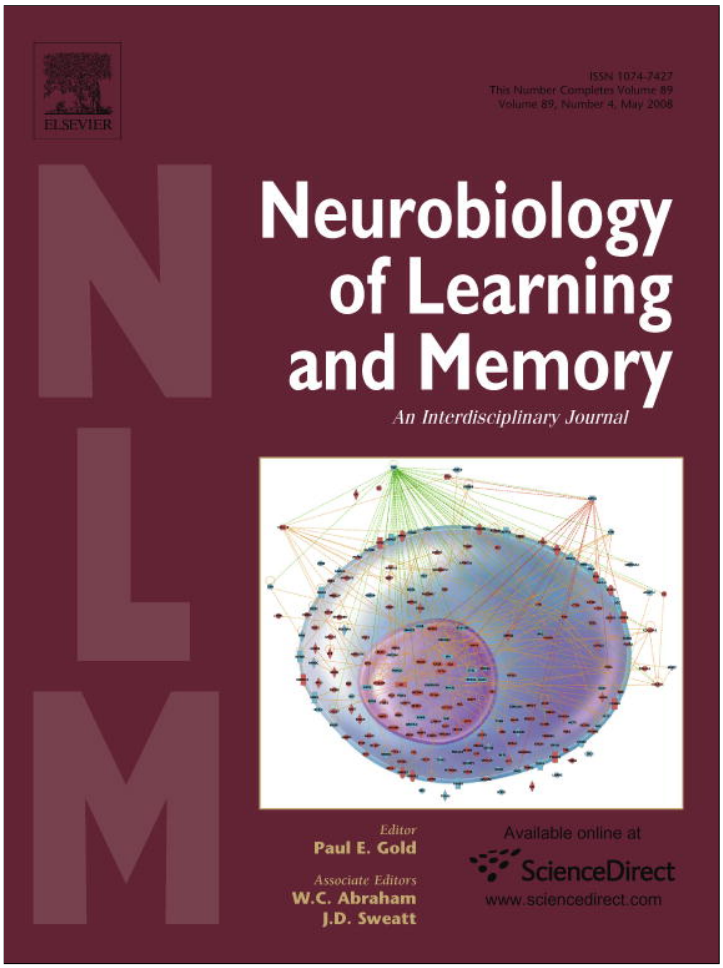

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Brief Report

Memory for time of training modulates performance on a place conditioning task in marmosets ☆

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Abstract

In rodents, the expression of a reward-conditioned place preference (CPP) is regulated in a circadian pattern such that the preference is exhibited strongly at the circadian time of prior training but not at other circadian times. Because each animal is trained only at a single circadian phase, the concept of time as a context cue is derived from a rhythmic internal state rather than learned explicitly from the external cues. We now report that the same ''time memory'' is expressed following context conditioning in the common marmoset (*Callithrix jacchus*). Animals were trained at a specific time to discriminate between an unpaired context and a context paired with food reward. Marmosets were then tested for preference at circadian times that were either the same or different from the training time. Preference was expressed only when training and testing times matched. The results show that time of day learning can be generalized to this new world primate implying that a similar circadian mechanism might regulate craving for reward in diverse mammals including human beings.
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Time plays an important role in essentially every biological process. Complex functions such as learning and memory exhibit diverse types of temporal modulation ranging from the ability to predict an event based upon the time following a cue (interval timing), to the regular modulations imposed by biological clocks. One important behavior with clear survival significance is time–place learning, or the ability to predict both the location and time of important environmental factors such as food availability or the likelihood of predation.

When time–place learning involves remembering particular times of day, circadian timekeeping can be a critical factor (e.g. Aragona, Curtis, Davidson, Wang, & Stephan, 2002; Crystal, 2006; Pizzo & Crystal, 2002; Thorpe, Wilkie, & Bates, 2003). The nature of the system underlying the ability to remember the time of day is not well understood. The issue is complicated by the fact that a variety of cues can indicate time of day under different experimental tasks or natural situations. Thus, animals use their hypothalamic circadian clock in the suprachiasmatic nucleus (SCN) to prepare for regular changes during the 24 h day, and perhaps an interval timer to register the time elapsed since a regular daily cue (such as dawn or dusk). Nonetheless, some data suggest that animals may be able to learn and remember the time of day even when the SCN has been ablated and when daily cues are absent. This is the case

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with time–place learning where lesioned animals continue to use time as a discriminative cue after SCN lesions (Gooley, Schoner, & Saper, 2006; Mistlberger, de Groot, Marchant, & Bossert, 1996), and may be employed in food entrainment where anticipatory behavior prior to daily food availability could reflect a memory for time (Stephan, 1989).

In food entrainment, and most time–place learning paradigms, the subjects discriminate between one time and another—e.g. one time when a reward is present and another when it is not. However, one of the simplest forms of time of day learning occurs when animals are given experience with the training apparatus only at a single training time. In this case performance is best when testing (retrieval) occurs at the same time of day as the prior training (acquisition), and performance may be completely absent when testing occurs at a different time. The codification of time, or phase of the circadian cycle, therefore is a feature that is learned together with all the other salient features present during a learning task, but as time is not a discriminative cue in this case, the behavior reflects strictly a memory for time or "time memory". The phenomenon (originally termed multiple retention deficits) was first reported in rats trained in active avoidance (Holloway & Wansley, 1973a), passive avoidance (Holloway & Wansley, 1973a; Holloway & Wansley, 1973b; Wansley & Holloway, 1976) and appetitive learning tasks (Wansley & Holloway, 1975). Recently, this type of time effect in learning has been observed in a reward-conditioned place preference (CPP) task in hamsters (Ralph et al., 2002) and rats (Cain, Ko, Chalmers, & Ralph, 2004), as well as in conditioned place avoidance (CPA) (Cain, Chou, & Ralph, 2004). In these experiments the CPP and CPA are regulated in a circadian pattern such that the learned preference or avoidance is exhibited strongly at the circadian time of prior training but not at other circadian times.

Because conditioning tasks provide part of the foundation for studies of learning, memory, reward, and addiction, it is essential to understand how factors such as circadian rhythms and time memory affect performance, and whether the phenomenon is generalizable to other species, including human beings. Whereas the phenomenon has now been demonstrated in hamsters, rats and mice (manuscripts in preparation), there is no clear demonstration of implicit time memory in a primate model. Due to its easy maintenance in captivity, the common marmoset, a small diurnal primate native of northeastern Brazil, is largely used in research concerning behavior, ecology and physiology. We used 20 adult marmosets (*Callithrix jacchus*), between 4 and 6 years of age, weighing 350–450 g, from the Primate Center of the University of Rio Grande do Norte (Natal, Brazil, 05°46′S and 35°12′W). The center has authorization from the Brazilian Environmental Institute (*Instituto Brasileiro de Meio Ambiente-IBAMA*), *Registration No. 1/24/92/0039-0.* Approximately half were females and half were males distributed equally in all groups. Animals were kept in semi-natural conditions in individual cages (2.20 m high, 0.90 m wide and 2.10 m deep; inside measures). The two lateral sides of the cages were brick walls which obscured visual contact with the neighboring animals. The front and back walls were made of wire mesh. Approximately half of the cage was roofed, allowing protection from rain and sun. Cages had two metal plates for food, water bottles and a wooden nest box. There were also items that enriched the animal's environment such as a few tree branches, two to three wooden platforms distributed in the lateral walls and a concave wooden nest hanging from the ceiling. Feeding occurred two times per day. In the morning, between 8:30 and 9:30 a mixture consisting of a combination of milk, boiled eggs, bread, vitamins A, B and C and amino acids was given. In the afternoon, between 14:00 and 15:00, food consisted of a variety of small pieces of tropical fruits.

Two different conditioning chambers were used. These were made of wood and were painted to provide distinctiveness and waterproofing. Context A had the format of a triangle (38 cm × 31 cm wide and 28 cm deep) with a white circle painted in the inside right wall. Context B was a square chamber (21 cm high, 28 cm wide and 25 cm deep) with three black horizontal lines painted in the inside and outside back wall and a horizontal groove in the left wall. Both chambers had a circular opening (10 cm diameter) on the front wall for marmoset's entrance and exit. A door used for cleaning the inside of the chambers between sessions was located on the right wall of the square chamber and left wall of the triangular chamber.

The experiment comprised three phases. One day of pre-exposure confirmed the pre-training neutrality of the contexts. Both chambers were placed in the home cage and left there for 10 min. Access to both chambers was free and no reward was present in either of the chambers during this phase. For the next eight consecutive days, each animal experienced only one of the experimental chambers per day for 10 min. One of the contexts contained several food treats. The rewarded context (square or triangle) was assigned in an unbiased fashion such that the same number of animals received rewards in each context. Gummy candies, cookies, raisins and condensed milk were used as rewards. The use of a combination of treats tried to accommodate the fact that each animal had different food preferences. The order of presentation of the chambers (Context A and B) and consequently the availability of the reward were counterbalanced throughout the eight days of training. The only condition was that each animal was exposed four times to each of the contexts, i.e., four times to the rewarded context and four to the non-rewarded context. On day number 10, testing for context preference consisted of placing both chambers for 10 min within the home cage without the reward.

Behavioral recording consisted of quantifying any direct interaction with the chambers (physical contact, climbing on chamber, chamber entrance or scent marking). The total time engaged in all of these behaviors for each 10-min session (frequency) was considered for data analysis.

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Two methods were used simultaneously to register behaviors during the sessions. Each time the animals exhibited any of these behaviors one observer would voice record with a tape recorder and a second observer would enter the data in an Excel worksheet using a computer. Also, latency to make the first contact with each of the chambers was registered. The experimental chambers were cleaned after each session with abundant water to eliminate odor marks, and were dried out before using the chamber with other animals.

Animals were assigned to two groups: half of the animals were trained between 06:00 and 07:00 and the other half between 15:00 and 16:00 named Morning (M) and Afternoon (A) group, respectively. These time points were selected based on the bimodal pattern of activity of this species, characterized by two daily peaks of activity (Menezes, Moreira, Azevedo, Costa, & Castro, 1993). During the test sessions each of these groups was further divided. Half of each was tested at the same time of prior training (M–M and A–A) and the other half at a different time point relative to training (M–A and A–M). Normal daily food was provided only after the sessions. In this way we assumed that the marmosets had a certain degree of hunger and consequently motivation for the food reward.

A two-way analysis of variance was performed for each variable (frequency and latency) with one grouping factor and one repeated measure. The repeated measure was rewarded context and non-rewarded context. For the pre-exposure phase the grouping factor was time of day (morning or afternoon) while for the testing sessions it was time coincidence between training and testing (Same time or different time). The latency values were further analyzed calculating for each animal the difference between the non-rewarded and rewarded contexts. Since *a priori* we predict that conditioning will drive animals toward high preference and that a preference at one time of day will be reduced at another time, a one-tailed *t*-test for unequal variance was also used here to compare the non-training time group with the training time group.

Pre-exposure sessions for both the morning and afternoon groups demonstrated that there was no pre-existing preference or bias for either of the two chambers (data not shown). Following conditioning, a preference for the rewarded context developed but was present only in the animals tested at the same time of training (SAME group) (Fig. 1). This preference is indicated by both a lower latency to make the first contact (top panel), and higher frequency of interactions (bottom panel) with the rewarded context. The ANOVA revealed a significant two-way interaction, $F_{(1,15)} = 6.75$; $p < .02$ for frequency values. When analyzing latency values we found a significant context effect $F_{(1,15)} = 5.42$; $p < .03$ and a trend in the interaction effect, $F_{(1,15)} = 3.82$; $p < .07$. The latency data are important because they give an idea of the animal's first reaction which may reflect more reliably the learned information. The frequency data are likely affected by extinction in that trained animals may be less likely to make subsequent visits

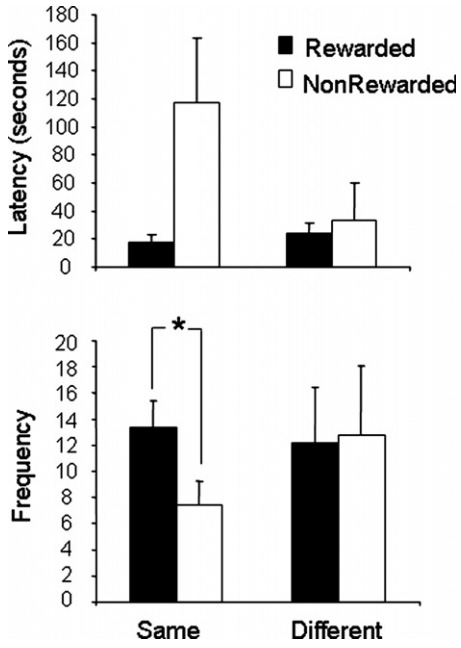

Fig. 1. Conditioned place preference at test times that are same or different from the circadian time of training expressed in latency to make the first contact and frequency of interactions with the contexts. Significant preference is seen only when training and testing are the same. Error bars indicate standard error.

or remain engaged with the chamber if food is not present in the rewarded context on test day. Fig. 2 shows the latency data expressed as difference between non-rewarded and rewarded context. Here, the *t*-test revealed a significant difference between groups tested either at the same or different time relative to training ($t = 1.986$, $p = .04$), with a very large effect size ($d = 1.0$; Cohen, 1992).

The combination of the two measures, frequency of interactions with the context and latency to make the first contact with each context, reveals that the food rewards used here were clearly rewarding and can condition a place preference in marmosets. Marmosets are capable of making associations and remembering the distinct differences

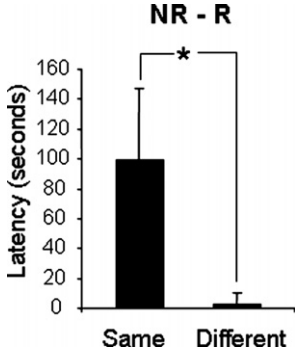

Fig. 2. Latency values to make the first contact expressed as the difference between the non-rewarded and rewarded context. Animals take much longer to make the first contact with the non-rewarded context relative to the rewarded context only when training and testing times are the same. Error bars indicate standard error.

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between the two context used in these experiments. However, the expression of this kind of association depends on the training–testing time coincidence. In other words, a place preference was expressed only when the testing time was the same as the training time suggesting the occurrence of time stamp in this type of task.

In nature it may be adaptive for an animal to restrict its activity or presence in a given context to times when rewarding features have been encountered before. The potential ecological value of the acquisition of a place preference is obvious. It may be important for survival and long term maintenance, for an animal to learn to approach and dwell in environments that are related to increased probability of finding food. In this sense, motivational change related to time of day will then be reflected in the animal's performance in the place preference task, and it is a reasonable prediction that the positive emotional valence of the context would be reduced at other times.

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