THE ROLE OF THE SUPRACHIASMATIC NUCLEUS IN TEMPORAL GATING OF PERFORMANCE ON A REWARD-BASED LEARNING AND MEMORY TASK

by

Caroline Hee-Jeung Ko

A thesis submitted in conformity with the requirements for the Degree of Masters of Arts
Graduate Department of Psychology
University of Toronto

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Abstract

In the present experiment, the role of the biological clock in the suprachiasmatic nucleus (SCN) is investigated as a provider of temporal information underlying time dependent changes in cognitive performance. Hamsters were tested on the conditioned place preference task before, and after ablation of the SCN. Animals were conditioned at Zeitgeber time 15 (ZT15), then tested at two different times of day: ZT15 and ZT6. All animals expressed time dependent preference in test phase - they spent significantly greater amount of time in the paired chamber when tested at ZT15 but not when tested at ZT6. This supports the notion that circadian information is an essential component of context representation (Antoniadis et al, 1999). Furthermore, there were no differences in temporal modulation of context preference expression between the experimental and sham groups. An oscillator, outside of the SCN, may be responsible for time discrimination in reward-based learning.
Introduction

Different types of associations between cognitive performance and daily or circadian rhythmicity have been described. In humans and animal models, cognitive processing varies with the time of day or phase of the circadian cycle. Cognitive performance is impaired in situations where circadian rhythms have been disrupted, such as during shiftwork, transmeridian flight, and in aging. In many organisms, the ability to learn and remember the specific timing of events has an enormous adaptive significance.

In most studies that have examined the relationship between time of day and cognitive processing, the assumption or implication has been that the SCN is somehow involved, and may be essential to providing the internal representation of time of day. However, recent reports have indicated that some of these tasks may be completed successfully by animals that do not have an intact SCN. This brings into question what the function of the SCN might be in the temporal regulation of cognitive performance, and if the SCN is not a necessity, what other brain regions might be involved.

The present experiment has tested the hypothesis that the biological clock in the SCN is responsible for providing time of day information that results in the temporal modulation of context learning in hamsters. Specifically, whether the expression of context learning changes with time of day following ablation of the SCN.

Circadian Rhythms: An Overview

The earth, spinning on its axis approximately once every 24 hours, exposes organisms to highly predictable physical oscillations in environment. The daily cycle of
light and dark is the most important and expected physical oscillation (Moore-Ede et al., 1982). Rhythms in biological functions of organisms closely match the periodicity in the environment (Pittendrigh, 1960). These biological rhythms persist in the absence of the external cues (Bunning, 1977; Pittendrigh, 1993), and maintain "freerunning" periods that are close to 24 hr in length (Moore-Ede et al., 1982). The term "circadian" (Latin: circa = about; dies = day) was first used by Halberg (1959) to describe a biological system that generates a period that is close to but not exactly 24 hr in length.

In the laboratory or in natural environmental conditions, biological rhythms are synchronized to environmental time cues referred to as zeitgebers. A difference between an organism's freerunning circadian period and 24 hr period is thought to be a factor in stabilizing the phase relationship between the endogenous circadian oscillations and their zeitgebers.

The intrinsic circadian period has been observed to be remarkably stable at all levels of phylogeny from single cell organisms to mammals. This attests to the general adaptive advantage for organisms to possess a biological clock. In general, circadian systems are relatively unaffected by environmental cues other than light, and can compensate for environmental changes (i.e., temperature). Most biochemical processes have $Q_{10}$ values greater than 2, which means that the rate of a process increases by greater than twofold when temperature is raised by 10 degrees Celsius (see Appendix 1). The periodicity of endogenous circadian rhythms has $Q_{10}$ values near or even less than 1 (Pittendrigh, 1993; Aschoff, 1984). This protects organisms from being affected inappropriately by acute environmental changes (Hastings and Sweeney, 1957).
The main benefits of circadian rhythms are to allow organisms to be synchronized to local environmental cycles. Appropriate temporal organization of an organism's physiology and behavior are fundamental for its survival. The ways in which the biological systems respond to zeitgebers determine whether and how entrainment will occur. In constant external conditions, a circadian cycle is divided into subjective day and subjective night. These are portions of the cycle in which an organism exhibits physiology and behavior that are characteristic of daytime or nighttime. For nocturnal animals, the onset of nighttime behavior (e.g., locomotor activity) is defined as circadian time 12 (CT 12). Hence, CT 12 indicates the beginning of subjective night.

**A Circadian Clock: The Suprachiasmatic Nucleus**

A primary function of circadian pacemakers is to coordinate behavior with important environmental cycles. To achieve this, coupling pathways must exist between the environment and the pacemaker, and between the pacemaker and effector systems for behavior. In mammals, a circadian pacemaker has been identified in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Moore and Eichler, 1972; Stephan and Zucker, 1972; Moore, 1983; Ralph et al., 1990; Inouye and Shibata, 1994). The SCN are two clusters of small, densely packed cells (eight to ten-thousand cells per nucleus) located just dorsal to the optic chiasm (Moore and Eichler, 1972).

Evidence implicating the SCN as the neural substrate responsible for the generation and regulation of 24 hr rhythms in mammal is diverse. Anatomical tracing studies have shown that the SCN is a target site of a direct retinal projection (Haymaker et al., 1969) known as the retinohypothalamic tract (RHT) (Moore and Lenn, 1972). The
SCN is responsible for coordinating behavioral rhythms with daily light-dark (LD) cycles, and is entrained to LD by the RHT (Moore and Lenn, 1972). The RHT leaves the retina with the optic nerve and terminates in the ventrolateral portion of the SCN (Moore, 1973; Meijer and Reitveld, 1989). Sectioning of the RHT abolishes entrainment to light-dark cycles in many species of mammals, however, sectioning of the optic tract beyond the RHT does not disrupt entrainment (Klein and Moore, 1979).

*In vitro* studies have shown that isolation from efferent and afferent projections does not abolish rhythmic electrical activity of the SCN. Electrical activity remains high during the subjective day and relatively low during the night (Green and Gillette, 1982; Groos and Hendriks, 1982; Gillette and Reppert, 1987; Prosser and Gillette, 1989). Inouye and Kawamura (1979) were among the first to test the hypothesis that the rhythm of electrical activity is an output of the time keeping mechanism, and possibly, that it is this rhythm that drives rhythms of multi-unit activity in other brain areas.

The integrity of the SCN is critical for the normal display of circadian rhythmicity in several vertebrate species. This is demonstrated by a number of ablation studies. For example, bilateral lesions of the SCN in rats and hamsters abolish the circadian rhythms of adrenal corticosterone, locomotor activity, and drinking behavior (Moore and Eichler, 1972; Stephan and Zucker, 1972, Ralph et al., 1990). Furthermore, transplanting SCN fetal tissue into animals with lesioned SCN re-establishes circadian rhythmicity (Sawaki et al., 1984; Lehman et al., 1987). To date, the discovery of the *tau* mutation in the Golden hamster has provided the strongest evidence that the SCN is indeed a circadian clock in mammals. Transplanting fetal SCN tissue from a *tau* mutant donor, which exhibits a circadian period of 20 hours, into an SCN lesioned host that previously
displayed a circadian period of 24 hours results in a recovery of circadian rhythmicity that reflects the circadian period of the donor (Ralph et al., 1990).

**Other Mammalian Circadian Clocks**

The mammalian circadian system consists of different types of pacemakers. While the role of the SCN as the primary coordinator of circadian rhythmicity is not in dispute, there is evidence implying that the SCN is not the only competent circadian oscillator in the mammalian brain. In particular, mammalian retina holds a circadian oscillator that displays all the requirements of an independently functioning clock (Tosini and Menaker, 1996, 1998). Cultured neural retinas of the golden hamster synthesize melatonin via a mechanism that is regulated by a genetically programmed circadian oscillator. This oscillator is entrained by light cycles, and is affected by the *tau* mutation in a similar way as is the SCN (Tosini and Menaker, 1996).

It has been demonstrated that SCN lesioned rats can restore their circadian rhythmicity when they are chronically exposed to amphetamine derivatives (Honma et al., 1987; Honma et al., 1989). Using chronic d-amphetamine can also induce circadian rhythms of locomotor behavior unaffected by a mutation known as *clock* in mice (Coward et al., in press). However, these induced oscillations are not self-sustaining. A chemically inducible oscillator is speculated, which may involve a neurochemical mechanism that is different from that underlying the generation of self-sustaining SCN rhythms (Coward et al., in press).

Cycles of eating and fasting have been shown to entrain certain circadian rhythms in rats (Sulzman et al., 1977; Stephan, 1984; Mistlberger, 1992), and rats are still able to
anticipate cycles of food availability following the SCN lesions (Boulos et al., 1980; Stephan et al., 1979; Stephan, 1989). Daily social interactions, induced locomotor activity, and maternal rhythmicity are also capable of entraining activity rhythms (Mrosovsky et al., 1989; Davis and Gorski, 1985). The results from these studies strongly suggest the presence of other circadian oscillatory centers in mammals.

In addition, the molecular components that are thought to define the mammalian circadian pacemaker are widely found in tissues other than the SCN, both within and outside of the central nervous system. The circadian expression of rPER2, BMAL1, mPER1, mTIM and CLOCK has been observed in brain areas outside of the SCN and in peripheral tissues such as eye, heart, kidney, and lung (Sakamoto et al., 1998; Oishi et al., 1998; Zylka et al., 1998). It is plausible to note, however, that the pacemaker cells in the SCN may be at the top of hierarchy in circadian systems (Ralph et al., 1990). The "peripheral" circadian genes, and their proteins, abolish circadian expression in SCN lesioned animals that show behavioral arrhythmicity (Sakamoto et al., 1998).

**The SCN, Temporal Information and Behavior**

The mechanisms by which the circadian clocks are coupled to behavior are unknown. However, it would be intuitively advantageous to have a clock that can be continuously consulted for recognizing and recording time of day (Enright, 1975). According to this conception, the pacemaker may provide temporal-phase information that can be accessed in subsequent occasions, i.e., the information can be accessed by a cognitive apparatus that would link phase with events and places, and thereby regulate the timing of specific behaviors in accordance with prior experience. In principle, such a
mechanism could contribute powerfully to behavioral plasticity and optimal timing of behavior (Enright, 1975; Gallistel, 1990). For example, different mammals, although entrained to the same environmental LD conditions, may present different activity level at given times. In a situation of predator versus prey, it would be an advantage for the prey to be able to predict when the predator may try to attack. Upon making this prediction, the prey may be able to altogether avoid an encounter with the predator.

Evidence that the circadian system can function as a continuously consulted clock is available in some organisms. Studies of sun-compass orientation have shown that bees use circadian phase to compensate for a continuously moving celestial directional cue (Wallraff, H.G., 1981). Both birds and bees use circadian phase as a discriminative cue for choosing among two or more food locations exhibiting time-place learning (Biebach et al., 1991; Gould, 1987). These tasks require that subjects have access to time-of-day information not necessarily available in their environment, and that they perform cognitive operations on this information to direct behavior optimally (Mistlberger et al., 1996).

Disrupted Circadian Rhythms and Behavior

Circadian periodicity is deemed to be influential on memory processes (Holloway and Wansley, 1973a, 1973b; Wansley and Holloway, 1975). Experimental data supports that impaired cognitive performance is observed in situations where circadian rhythms are disrupted. Natural breakdown of circadian rhythms in physiology and behavior is observed with aging in many species. In mammals, the age-dependent disorganization of overt rhythmicity appears to be due to a functional decay of the SCN (Rosenberg et al.,
1991; Weilard and Wise, 1990; Wise et al., 1988). Changes in amplitude in circadian rhythms, and altered responsiveness of the circadian system to environmental stimulation are observed with aging (Turek et al., 1995; Edgar, 1994; Pittendrigh and Daan, 1974). This is concurrent with reductions in cell number and volume in the SCN (Swaab et al., 1985), and it has been shown that age-related rhythm fragmentation can be reversed by transplantation of fetal or culture SCN cells (Hurd and Ralph, 1998; Ralph, 1991).

Age-related circadian differences are tied to the cognitive performance differences between young and old animals. It is reported that memory-related cognitive function, in particular inhibitory control, is affected by time of testing in old but not in young animals (Winocur and Hasher, 2000). A study by Antoniadis et al. (2000) supports the notion that age-related rhythm fragmentation contributes to the age-related memory decline. In this study, context conditioning is intact in hamsters with consolidated activity rhythms but impaired in hamsters with fragmented activity rhythms of the same chronological age. Furthermore, Cain et al. (2000) report that context learning is impaired in aged hamsters with decreased circadian amplitude in plasma corticosterone levels. A high amplitude rhythm in plasma corticosterone levels is seen in young hamsters. This high circadian fluctuation becomes blunted with aging, rendering low amplitude, almost constant level of corticosterone in old hamsters. Animals with high amplitude rhythms showed intact context conditioning; however, ones with low amplitude (no circadian fluctuation) in corticosterone level were impaired in learning.

Disrupting circadian rhythms in young healthy animals can also result in memory impairment. Phase shifting the LD cycle has been shown to impair performance on active and passive avoidance tasks (Fekete et al., 1985; Tapp & Holloway, 1981; Davies
et al., 1974), and retention of place information in the water maze, a spatial task sensitive to hippocampal dysfunction (Devan et al., 2001).

**Time Dependent Learning and Memory**

To examine whether the mammalian circadian system can function as a consulted clock, Mistlberger et al. (1996) trained food-restricted rats to press a lever for food two times each day in a T-maze, with the correct arm conditional on time of day. Animals were trained to press levers for food in which the left arm was correct in a morning feeding session, and the right arm in an afternoon session (7 hour interval). All the animals (n=6) learned the task and exhibited anticipatory wheel running prior to most sessions. To rule out simple alternation strategy, as opposed to true time-of-day discrimination, T-maze sessions were omitted intermittently. To examine the role of the light-entrainable pacemaker as the consulted clock mediating time-of-day discrimination, the LD cycle was inverted on two occasions in one group, and the SCN was ablated in a second group. The results showed that rats can discriminate between at least two times of day, that this was not based on an alternation strategy, was not generally disrupted by LD shifts, and can be accomplished by consulting a pacemaker (possibly food-entrainable pacemaker) that is outside of the SCN.

Antoniadis et al. (1999) also provided evidence that the circadian pacemaker can function as a consulted clock mediating time-of-day discrimination in mammals. They used the conditioned place preference (CPP) paradigm and showed that animals are able to concurrently learn the environmental context, the internal rewarding effects of the suspected reward stimulus and the temporal-phase information of the event. Wheel-
running served as the reward property to induce preference (McDonald et al., 1997), and as a result, animals expressed preference when tested at the same time of day as they were trained but did not show preference when tested at another time of day showing a “time-stamp” effect in learning.

An extension to the Antoniadis et al. (1999) study was conducted by Ko et al. (1999) to investigate whether the time-specific learning in the CPP paradigm can be generalizable to other forms of reward. It has been repeatedly shown that peripheral administration of addictive drugs like amphetamine, cocaine and morphine can induce conditional place preference (Hiroi and White, 1991; Carr and White, 1983; Mackey and van der Kooy, 1985; Mucha et al., 1982). Ko et al. (1999) used peripheral administration of D-amphetamine as the rewarding property in the CPP paradigm and supported the idea that a consulted clock is able to provide very specific temporal-phase information that is linked to particular events and experiences. Furthermore, Ko et al. (1999) extended the Antoniadis et al. (1999) findings by demonstrating that the time-stamp effect in reward-based learning (i.e., in CPP paradigm) can be generalizable to different types of reward.

The Conditioned Place Preference (CPP) Paradigm

The CPP paradigm has proven to be a powerful tool in assessing the two fundamental aspects of rewarding stimuli: their approach-eliciting property and their ability to maintain the organism’s presence. In the CPP task, animals are simultaneously exposed to environmentally neutral stimuli and the internal rewarding effects of the suspected reward stimulus. If the stimulus or event is successful in activating the neural substrates of reward the animal will approach and maintain contact with the environment
or context in which the stimulus was experienced, in the absence of reward (White, 1989, Carr et al., 1989).

The expression of preference in CPP is informative with respect to the learning and memory capacities and its organization within circadian systems. In order to express such a preference (or lack of preference) on test day, animals must be able to form and retain an association between the properties of the rewarding stimulus and the environmental context and, as shown in the Antoniadis et al. (1999) study, the temporal information about the event. These studies provide clear evidence that animals can consult a circadian clock to discriminate the time of day in expectation of a reward, i.e., food, drug, or wheel running.

**The Present Experiment**

Mistlberger et al. (1996), as mentioned above, indicate that time-of-day discrimination may be accomplished by consulting a clock that is outside of the SCN and possibly this is a food-entrainable oscillator. Indeed, this is a plausible hypothesis within their experimental parameters. However, the equivalent time-of-day discrimination has been shown in the CPP experiments using different types of reward other than food, i.e., wheel-running and amphetamine. It is possible that an oscillator that is responsible for time discrimination in reward-based learning is generally associated with the mammalian reward system (i.e., activating the same neural substrates indifferent to the types of reward).

The present study was designed to further investigate the involvement of a circadian clock, in particular the suprachiasmatic nucleus, in reward-based learning in
hamsters. The purpose was to clearly understand a role of the SCN in associating temporal information with reward-based learning. Hamsters were used in this experiment because they are the species of choice for studying circadian rhythms in mammals, due to the high accuracy and precision in their locomotor rhythm (Morin, 1985). Hamsters are nocturnal rodents that show maximal amplitude of locomotion, as expressed in wheel running activity, at circadian hour 13 (CT13). Circadian hours for nocturnal animals are defined by the onset of the animal's locomotor activity, which is equated to be circadian time 12 (CT12). The peak of inactivity in hamsters is circadian time 4 (CT4). To assess the effect that circadian time has on context conditioning, training time was chosen at CT13 because the animals are highly motivated to use a wheel during this time period (Davis and Menaker, 1980).

The subjects were kept in 14:10 LD cycle with lights-off at Zeitgeber time 14. Onset of nocturnal wheel running occurred at lights-off. Hamsters were trained daily at ZT15 (one hour after lights off) then tested at two different times of day, ZT15 and ZT6. All animals expressed time dependent learning consistent with the Anontoniadis et al. (1999) study suggesting that circadian information is an essential component of context representation. Hamsters spent significantly more time in the paired than in the unpaired context when they were trained and tested at the same time of day (i.e., trained at ZT15 and tested at ZT15). However, when testing occurs at an unpredicted time (i.e., trained at ZT15 and tested at ZT6), animals did not show significant preference for either of the chambers. Before the second phase of the experiment, about a half of the subjects received SCN lesion. The control group consisted of the remaining hamsters and received sham surgeries. A new pair of context chambers was used to condition at CT13.
and to test the animals again at CT13 versus CT4. No apparent differences in context preference were observed between the experimental and control groups. Both the SCN lesioned group and the control group showed the time-stamp effect. This suggests existence of an oscillator, outside of the SCN, that is responsible for time discrimination in reward-based learning.
Method

Subjects

Fifty-three male Golden hamsters (*Mesocricetus auratus*) were obtained from Charles River Canada (Montreal, PQ) and the breeding facility in University of Toronto Zoology Department. Subjects were approximately 80 to 90 days old at the beginning of the experiment. Animals were housed individually in a polypropylene cage (22cm x 24cm x 20cm) equipped with a stainless steel running-wheel (17cm in diameter). Wheel running activity was monitored continuously using VitalView (Mini Mitter Co., Inc., Sunriver, Oregon). Food and water were available *ad libitum*. All cages were kept inside a light-tight ventilated box (6 cages per box) for the duration of the experiment. Illumination was provided from an overhead GE Cool White fluorescent tube emitting 700-800 lux. The hamsters were kept in a light-dark cycle of 14-hr light and 10-hr dark (LD 14:10).

Apparatus

The design of the apparatus is depicted in Figure 1. Two pairs of context chambers were used: triangle-octagon pair and square-pentagon pair. Each context differed in three dimensions: color, shape and odor. Context A was a triangular chamber painted with black-and-white horizontal stripes. It measures 60cm long x 60cm wide x 30cm high (floor measure of 1800cm$^2$). Context B was a black octagonal chamber (measuring 42cm long x 42cm wide x 30cm high (floor measure of 1086cm$^2$). Context C was a white square with length of 40cm and 30cm in height (floor measure of 1600cm$^2$).
Context D was in the shape of a pentagon (40cm long x 40cm wide x 30cm high) with floor measure of 1530cm². All four boxes contained a small plastic cylinder (pill bottle) that was mounted on one of the walls of the chamber. On each pre-training, training and test day a drop of odorant, serving as the olfactory cue, was placed on a cotton ball and inserted within one of the bottles. Isoamyl acetate served as the olfactory cue in Context A and Context C. Eucalyptus served as the olfactory cue in Context B and Context D. Two chambers of each pair were connected by an alley (16.5cm x 11cm x 11cm) into which animals were placed during pre-exposure and testing. The entire structure was placed on a Plexiglas table. A mirror, inclined by 45 degrees, was placed on the floor of the testing room providing experimenter with a non-intrusive view of the chambers. A video camera was placed in front of the mirror to allow the experimenter to videotape all phases of the experiment for future reference.

**Experimental Procedures**

Experimental groups

Animals were randomly assigned to one of eight groups. Groups 1-4 were trained using Contexts A and B prior to SCN lesioning. Groups 5-8 were trained using Contexts C and D. Experimental groups and timeline are outlined in Table 1. All hamsters were subjected to the CPP paradigm in the phase 1 with appropriate counterbalancing of context chambers and testing time. Following SCN lesioning, each group was retrained using the alternate pair of contexts.

For the surgical procedure, half of the groups were given SCN lesions, and half were sham operated. The running activities of the SCN lesioned animals were closely
monitored to observe returning of overt behavioral circadian rhythms. Animals whose rhythmicity returned post-surgery were removed from the experiment since rhythmic behavior is indicative of incomplete SCN lesion.

Conditioned Place Preference (CPP) Paradigm

Animals were entrained to an LD (14:10) cycle prior to beginning the CPP paradigm. All parts of the experiment were conducted in the light condition and Figure 2 outlines the paradigm schedule. Temporary exposure to light did not significantly affect the animals' entrainment to the given LD cycle (see Figure 3). On Day 0, the experimenter handled animals individually for 5 minutes. On Day 1, animals were individually pre-exposed to the entire apparatus. They were placed in the alley and given free access to both chambers for a total period of 10 minutes.

Day 2 to Day 9 were conditioning days. Wheel-running served as the reward to induce preference of one chamber over the other. Conditioning involved four training sessions and each session was consisted of two days. During conditioning, animals were individually confined in one of the chambers for 30 minutes. The running-wheel is present in the “paired” context. The order in which the animals experienced each context was counterbalanced so that half the animals are confined in the paired context on Day 1 of each session and confined in the unpaired context on Day 2. The other half of the group is confined in the unpaired context on Day 1 and confined to the paired context on Day 2 of each training session. The chamber that served as the paired context was also counterbalanced so that the wheel was present for a half of the animals in Context A (or C) and the other half in Context B (or D).
On the test day, animals were individually placed into the alley and given free access to the entire apparatus for 20 minutes. Animals were considered to be dwelling inside of a chamber when both forepaws were past the threshold of the doorway into the chamber. When both forepaws were past the threshold of the doorway into the alley, animal was considered as being outside of the chamber. Time spent dwelling in the paired context was noted to represent a measure of appetitive context learning and thus, an expression of context preference.

_Surgery_

SCN lesions were performed under pentobarbital anesthesia using an electrolytic lesion maker (Grass LM-5 DC). Stainless-steel electrode insulated with epoxylite except at the tip (0.2 mm) was used. Stereotaxic coordinates were +6.0 mm anterior to bregma and -8.2 mm below the skull surface directly on the midline. Tooth bar was set at -2.0 mm. A 4 mA DC current was delivered for 14 seconds and the electrode was kept stationary for additional 5 minutes before it was removed from the animal's head. Sham surgeries were also performed under pentobarbital anesthesia. Some of the sham animals received an incision on top of the head that was sewn back together.

_Data Analysis_

The results are statistically evaluated using a 2 x 2 ANOVA (testing time and chamber context) on the dwelling time in both chambers. Planned comparisons were used to determine the difference in the amount of time spent in each chamber. The accepted level of significance (p) used was less than 0.05.
Histology

The SCN-lesioned animals were perfused intracardially with saline (exsanguination) and 4% paraformaldehyde (fixation). Brains were removed from calvarium and stored for 2-3 days in a 4% paraformaldehyde-30% sucrose solution. They were then frozen and sectioned at 40 μm. Sections were mounted on gelatin-coated glass slides, dried for at least one day, hydrated in distilled water (4 min), and gradually dehydrated in 95 and 100% ethanol. To reveal the anatomical landmarks, sections were stained using 0.1% cresyl violet. Slides were cleared in Hemo-De and coverslipped with Permoun.
Results

**Conditioned Place Preference (CPP)**

**Phase 1:**

The mean amount of time spent in the initial pairs of context chambers (either triangle-octagon pair or square-pentagon pair) during pre-exposure phase was same for all subjects during the phase 1 (Figure 6). The $2 \times 2$ ANOVA (time and context) on the time spent in both chambers revealed a non-significant time effect [$F(1, 65) = 0.79, p > 0.05$], a non-significant context effect [$F(1, 65) = 0.75, p > 0.05$], and a non-significant time x context interaction [$F(1, 65) = 2.23, p > 0.05$]. Planned comparisons revealed that there was no significant difference in the amount of time spent in each context chamber [time ZT15: $F(1, 65) = 2.82; p > 0.05$]; [time ZT6: $F(1, 65) = 0.20; p > 0.05$]. This lack of preference for any intrinsic features of the chambers demonstrates that this is unbiased CPP procedure for the subjects (Carr et al., 1989).

On test day, hamsters tested at ZT15 spent considerably more time in the paired context compared to the unpaired context, while hamsters tested at ZT6 did not show preference for a particular context (Figure 6). The $2 \times 2$ ANOVA (time and context) on the time spent in a given pair of chambers revealed a non-significant time effect [$F(1, 65) = 2.35, p > 0.05$], a significant context effect [$F(1, 65) = 10.84, p < 0.05$], and a non-significant time x context interaction [$F(1, 65) = 1.30, p > 0.05$]. Planned comparisons revealed that there was significant difference in the amount of time spent in each context chamber at ZT15 [$F(1, 65) = 9.98; p < 0.05$]. There was no significant difference in the
time spent in each context at ZT6 \( F(1, 65) = 2.28; p > 0.05 \). This confirms the notion that circadian temporal information is an essential component of context representation.

After the subjects were randomly assigned to control versus experimental groups, data from the phase 1 were divided according to the groups. There was no preference for a particular chamber during pre-exposure for both groups (see Table 2A). Time-dependent expression of preference was maintained within each group (see Figure 7 for control group, Figure 9 for experimental group, and Table 2B). It should be noted that the control group tested at ZT6 spent greater amount of time in the paired chamber than in the unpaired chamber. However, the difference in time spent in the paired versus unpaired chambers was not statistically significant for this group \( F(1,30) = 2.21; p > 0.05 \).

**Phase 2:**

The mean amount of time spent in the second pairs of context chambers (either square-pentagon pair or triangle-octagon pair) during pre-exposure phase was statistically indifferent for both control and experimental groups during phase 2 (Figure 8 and 10, respectively; Table 3A). Both the control and the experimental groups, however, express time-dependent preference for the paired chamber when tested at ZT15. This preference is not displayed at ZT6 testing in neither of the groups (Table 3B).

**Conditioning Days:**

The total amount of time spent running during conditioning phase for the SCN lesioned and the control group were noted. When the total amount of running time was
compared between the groups as the percent of total time in the paired chamber, the
difference between the groups was not significant (p > 0.05). The amount of time spent
running on the wheel for each animal was also examined in relation to the individual
amount of preference exhibited on the test day. There was no correlation between the
amount of time spent running and the amount of preference shown on the test day (p >
0.05).

Histology

Light microscopic examination revealed that twenty-one of thirty two hamsters
sustained complete ablations of the SCN, with minor damage to adjacent hypothalamus
(Figure 5).

Pre- and Post-lesion behavioral rhythms

Prior to the lesions, all hamsters had free-running rhythms in activity. The period
of the activity rhythm was between 23.9 and 24.2 hr. The 21 hamsters with complete
SCN lesions showed the typical disruption of activity (Figure 4) while the control group
maintained the free-running rhythms (24-24.2 hr). No statistically significant rhythms
appeared in the periodogram over a range from 20.5 to 26.5 hr in the SCN lesioned
animals. Only these 21 subjects were included in the experimental group for data
analysis (discussed above; Figures 6-10 and Tables 2-3).
Discussion

Circadian rhythms allow organisms to coordinate various physiological and behavioral activities with daily fluctuations in the environment. This, in turn, prepares organisms for predictable events. Timing is crucial. The ability to anticipate risks and opportunities that occur in the environment can considerably improve an animal’s chances of survival.

Studies have indicated that many organisms use their circadian clocks to retain the time-of-day information when a significant event occurs. Results from the present experiment confirm previous findings by demonstrating that animals express a context-preference only when the temporal-phase information coincided with their previous rewarding experiences. However, a question of the location of this clock mechanism still remains.

The vast majority of studies of circadian rhythms in mammals are conducted under the assumption that the SCN is the location of a central circadian pacemaker. There is no dispute over the SCN’s primacy as the mammalian circadian clock. However, it is clear from the results of this experiment and a few others that the SCN is not the only location of oscillators that link time-of-day information to an event.

There is a growing body of literature that demonstrates the existence of circadian oscillators outside the SCN in mammals. A competent, light-entrainable clock has already been described in the retina (Tosini & Menaker, 1996). Of a particular interest in relation to the present study is the food-entrainable oscillator (FEO). Animals with SCN lesions are able to anticipate mealtime when they are on a restricted feeding schedule.
consisting of one daily meal. Food is accessible to these animals approximately every 24 hr. Anticipation is manifested as increases in locomotor activity and body temperature several hours before feeding (Mistlberger, 1994). Food-anticipatory activity is believed to be mediated by a circadian oscillator because it has limits of entrainment in the circadian range, free runs during food deprivation (Stephan, 1981), and displays transients in response to phase shifts of food access (Stephan, 1992). Thus, the FEO is an example of a biological clock that is functionally and anatomically distinct from the light-entrainable clock located in the SCN.

An extensive effort has been directed at identifying the location of FEO. However, despite many attempts to identify the biological substrate for the FEO, its location has not been established. Lesion studies targeting other brain structures, such as paraventricular and lateral hypothalamic regions, fail to abolish food anticipatory activities (Mistlberger & Rusak, 1988). Lesions of hippocampus, amygdala, and nucleus accumbens yielded the same result (Mistlberger & Mumby, 1992). Although ventromedial hypothalamic lesions transiently eliminate food-anticipatory behavior (Mistlberger & Rechtschaffen, 1984), the eventual recovery of the anticipatory behavior implies that these nuclei are not the locus of the FEO. Nonetheless, it is suggested that the ventromedial nuclei are involved in food anticipation and that they may be involved in the input or output pathways of the FEO (Davidson and Stephan, 1999). The theoretical and methodological approaches taken in search of the FEO can help guide research for locating oscillators that are of importance in reward-based time-of-day learning.
In pursuit of identifying the circadian oscillators responsible for time-of-day learning, a few considerations must be kept in mind. Many functions that influence memory such as brain protein synthesis, neural activity, synaptic excitability, neurotransmitter synthesis and hormone secretion display circadian oscillations (Tapp and Holloway, 1981). Animals subjected to disorganization of these rhythms by phase shifting LD cycles perform poorly on given cognitive tasks (Devan et al., 2001; Stone et al., 1992; Fekete et al., 1985; Holloway & Wansley, 1973a, 1973b). Most of these rhythms are blunted in absence of the SCN. However, there is evidence suggesting that learning-induced brain activity rhythms are unaffected by, and independent from circadian rhythms (Chen & Wolpaw, 1995). The extent to which these functions are able to self-sustain their rhythmicity should be explored. In addition, it should be noted that these functions are distributed across many anatomical brain regions. Their functional and periodical variability on each region should be considered.

Secondly, it seems reasonable to confine the search to a specific type of learning. Although this idea of confining research may be limiting, this is necessary due to the complex mammalian physiology that underlies and differentiates behavioral systems. In addition, one should be careful not to generalize different learning and memory systems. Most of the evidence that demonstrate time-dependent learning has employed reward-based learning tasks. Under an aversive condition, animals may exhibit different patterns of behavior. This can be supposed given that different physiological mechanisms are involved in approach versus withdrawal behaviors. Intuitively, it would be advantageous for animals to require a minimal amount of information to associate certain environmental contexts with danger. The temporal-phase information may be
unnecessarily required information and the circadian systems may exert different effects. It is possible that adaptive advantage of having a circadian clock to retain information about time of an event may not apply to all aspects of learning.

One approach to identifying the locus of circadian oscillators has been to trace the inputs and outputs of the system. To locate the oscillators that are accountable for temporal-phase information in reward-based learning, particularly for contextual learning displayed in CPP, one can begin by investigating the hippocampal system, and the rhythmic nature of its neuronal activity (e.g., its neuroelectrical activities).

The hippocampus is essential for the acquisition, encoding, and retrieval of complex representations of the elements that define a specific event and the environment (O'Keefe & Nadel, 1978). Several limbic structures, including hippocampus, receive both direct and indirect inputs from the SCN 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 (Watts et al., 1987, Wyss et al., 1979). It is possible that some component of these connections may modulate hippocampal function and influence the consolidation of hippocampal-dependent place memory (Devan et al., 2001). The study by Devan et al. (2001) also speculates that the mechanism by which the circadian system influences hippocampal consolidation processes may be a direct input from the pacemaker cells. However, a direct influence from the SCN to hippocampus is now questionable since the present experiment shows that context representation was intact, and time-dependent representation persistent, in SCN lesioned animals.

It seems peculiar that animals with disrupted circadian rhythms due to phase-shifting LD cycles, or aging, showed learning impairment, but animals with completely
arrhythmic rhythms due to SCN lesion did not. One possible explanation comes from the fact that SCN lesioned animals in the present experiment displayed entrainment or daily masking of activity when they were subjected to CPP paradigm (Figure 4, phase 2). It should also be noted that these animals immediately returned to their arrhythmic behavior once the CPP task ceased.

In most of retention memory studies that report learning impairment, the testing occurred shortly after phase-shifting the subjects. The observed deficits during retention testing, which probably occurred before re-entrainment, could have resulted from other physiological effects of desynchronous rhythms. SCN lesioned animals in this study, however, displayed a masked behavioral, and possibly physiological, entrainment. However, a closer look at the timing of the entrained rhythm suggests that wheel-running activity does not precede but follows the daily task trials. Upon being returned to their home cages, animals run on their wheels. Subsequently, this activity seems to induce a physiological arousal that entrains their behavioral activity. This entrainment could correspond to a number of physiological oscillators synchronizing their rhythms. The overall phase relationship among different physiological oscillators may be an important factor of circadian periodicity and its effect on learning and memory. This notion of importance of phase relationship among oscillators could also be applied in further investigation of the food-entrainable oscillator.

Recent study by Devan et al. (2001) demonstrated that phase shifting LD cycle appears to disrupt the long-term consolidation of spatial information when animals were tested after re-entrainment. However, acquisition or short-term consolidation was not
affected. It would be interesting to see if SCN lesioned animals could retain the context representation after returning to their arrhythmic behavior.

Circadian rhythms research in conjunction with learning and memory provides important means to study cognitive performances in human population. It is known that disrupted sleep-wake cycles in older adults are related to their age-related cognitive decline (Nesca & Koulack, 1994; Mirmiran et al., 1992; Bliwise, 1989). A drastic cognitive impairments and circadian dysfunction have also been noted in the cases of Alzheimer's disease and other neurodegenerative dementia (Stopa et al., 1999). Furthermore, it has been indicated that impaired cognitive performance is a risk for people on shift-work schedules and during re-adjustment to new time zones due to temporary and/or long-term disruptions of circadian rhythms (Cruz et al., 2000; Harma & Ilmarinen, 1999; Marquie & Foret, 1999). This emphasizes a clinical importance for identification of mechanisms with which circadian systems are influential in cognitive processing.

In conclusion, while disrupted circadian rhythms are an important factor in learning impairment, the suprachiasmatic nucleus (SCN) does not seem to be involved in associating temporal information, at least with reward-based context learning. The current results demonstrate that SCN lesioned animals are able to retain the time information for cognitive processing even when their behavioral and physiological oscillations are arrhythmic. Unknown circadian oscillators therefore must be accountable for time discrimination. A reasonable hypothesis is that the mechanisms responsible for time-of-day learning on the CPP task are the same as those that underlie conditioning to periodic food availability. As discussed, the neural substrate for the FEO is not known.
However, circadian oscillations in the absence of an SCN are also inducible by chronic administration of amphetamine and derivatives. This suggests that a likely place to look for an oscillator outside the SCN is the ascending dopamine system. It is significant in this regard that dopaminergic pathways are critical for the central processing of reward information. Manipulations of dopamine transmission during learning and retrieval may shed some light on this issue.
References


Coward, D.J., Cain, S.W. & Ralph, M.R. (In press) A circadian rhythm in mice that is unaffected by the period mutation at *CLOCK*. *Biological Rhythms Research*.


information in the water maze. *Neurobiology of Learning and Memory*, 75(1), 51-62.


Prosser, R.A. & Gillette, M.U. (1989) The mammalian circadian clock in the suprachiasmatic nuclei is reset in vitro by cAMP. *Journal of Neuroscience*, 9(3), 1073-81


circadian clock, the suprachiasmatic nucleus in the brain. *Journal of Biological Chemistry*, 273, 27039-27042.


Appendix 1

Q_{10}: A concept that describes the temperature sensitivity of a reaction or process. It is a quotient calculated by dividing the rate of a process or reaction at a certain temperature, R_T, by the rate of that process or reaction at a temperature 10 degrees lower, R_{T-10}. \[ Q_{10} = \frac{R_T}{R_{T-10}} \]
Figure 1. Experimental set-up for conditioned place preference (CPP) paradigm.
Figure 2. CPP paradigm: Experimental Schedule. Hamsters were trained at ZT15 from Day 0 to Day 8.

Day 0: Handling (5 min)
Day 1: Pre-Exposure (10 min)
CD 1: Paired Chamber
CD 2: Unpaired Chamber
CD 3: Paired Chamber
CD 4: Unpaired Chamber
CD 5: Paired Chamber
CD 6: Unpaired Chamber
CD 7: Paired Chamber
CD 8: Unpaired Chamber
Day 10: Preference ZT15 (20 min)
Day 11: Preference ZT16 (20 min)
Figure 3. Locomotor activity record of a hamster in control group. Starting and ending days for Phase 1 and Phase 2 are indicated to the right. Actogram is double-plotted so that each line presents 48 hr of continuous recording, with the second 24 hr repeated on the first half of the subsequent line.
Figure 4. Locomotor activity record of a hamster in experimental group. Starting and ending days for Phase 1 and Phase 2, along with day of SCN lesion are indicated to the right. Actogram is double-plotted so that each line presents 48 hr of continuous recording, with the second 24 hr repeated on the first half of the subsequent line.
Figure 5. A picture of SCN lesion.
Figure 6. Mean amount of time spent in each chamber by all animals in Phase 1. Both groups A and B were pre-exposed and trained at ZT15. Group A was tested at ZT6 and Group B was tested at ZT15.

* Indicates significant difference.

Phase 1 - All subjects
Figure 7. Mean amount of time spent in each chamber by control animals in Phase 1. Both groups were pre-exposed and trained at ZT15. Group A was tested at ZT6 and Group B was tested at ZT15.
* Indicates significant difference.

Control - Phase 1
Figure 8. Mean amount of time spent in each chamber by control animals in Phase 2. Both groups were pre-exposed and trained at ZT15. Group A was tested at ZT6 and Group B was tested at ZT15. * Indicates significant difference.
Figure 9. Mean amount of time spent in each chamber by experimental animals during Phase 1. Both groups were pre-exposed and trained at ZT15. Group A was tested at ZT6 and Group B was tested at ZT15. * Indicates significant difference.

Experimental - Phase 1

![Graph showing dwell time in each chamber by Group A and Group B during Phase 1](image-url)

- Group A pre-exp (ZT15)
- Group B pre-exp (ZT15)
- Group A pref (ZT6)
- Group B pref (ZT15)

- Bar color legend: paired (hatched), unpaired (solid)

- Axes: Dwell time (sec)
Figure 10. Mean amount of time spent in each chamber by experimental (SCN lesioned) animals in Phase 2. Both groups were pre-exposed and trained at ZT15. Group A was tested at ZT6 and Group B was tested at ZT15.

* Indicates significant difference.

Experimental - Phase 2
Table 1. Experimental Groups and Timeline. ZT0 = lights on. ZT14 = lights off. Onset of nocturnal wheel running occurred at lights-off. Training was performed daily at ZT15 (one hour after lights off). Lesioned animals showing residual circadian locomotor rhythmicity were omitted from the analysis. Final n = 53.

<table>
<thead>
<tr>
<th>Phase 1: All animals trained at ZT 15 (n = 53)</th>
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<tbody>
<tr>
<td>Train using Context A vs B (n = 24)</td>
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<tr>
<td>Test @ ZT6 n = 4</td>
</tr>
<tr>
<td>Test @ ZT6 n = 7</td>
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</table>

| Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 |
Table 2. Statistics (ANOVA) for Phase 1 by experimental groups: both the control and the experimental groups do not show preference for a particular context (A). Both groups express preference for the paired chamber when tested at ZT15, but not at ZT6 testing (B).

A) Pre-Exposure

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B) Preference

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Table 3. Statistics (ANOVA) for Phase 2 by experimental groups: both the control and the experimental groups do not show preference for a particular context during pre-exposure (A). However, both groups express preference for the paired chamber when tested at ZT15, but not at ZT6 (B).

### A) Pre-Exposure

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### B) Preference

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