ELSEVIER

Behavioural Brain Research 133 (2002) 95–108

BEHAVIOURAL BRAIN RESEARCH

www.elsevier.com/locate/bbr

Research report

Circadian modulation of learning and memory in fear-conditioned mice

Dipesh Chaudhury *, Christopher S. Colwell

Department of Psychiatry and Biobehavioral Sciences, Mental Retardation Research Center, University of California – Los Angeles, 760 Westwood Plaza, Los Angeles, CA 90024-1759, USA

Received 16 August 2001; received in revised form 28 October 2001; accepted 28 October 2001

Abstract

Endogenous processes referred to as circadian oscillators generate many of the daily rhythms in physiology and behavior of a variety of animals including humans. We investigated the possible circadian regulation of acquisition, recall and extinction in two strains of mice (C-57/6J and C-3H). Mice were trained in either the day or night with a tone and context fear conditioning protocol. The mice were then tested over the course of several days for their ability to recall the training. When comparing the performance of animals in the day and night, the mice acquired the conditioning faster in the day than in the night. Furthermore, the recall for context and tone consistently peaked during the day for at least 3 days after training, irrespective of the time of training. Finally, the loss of this training (or extinction) exhibited a rhythm in that mice trained in night exhibited a greater degree of extinction than mice trained in the day. For all of these rhythms in acquisition, recall, and extinction the phase of the rhythm was controlled by the prior light–dark (LD) cycle. When we reversed the phase of the LD cycle, the phase of the rhythm also reversed. Importantly, all three of the rhythms also continued in constant darkness demonstrating the endogenous, and presumably circadian nature, of the rhythms.

Keywords: Learning; Memory; Circadian; Fear-conditioning; Acquisition; Recall; Extinction

1. Introduction

Most organisms, including humans, exhibit daily rhythms in their behavior and physiology. The physiological system responsible for these rhythms is known as the circadian system and, in mammals, the core of this rhythm generating system can be localized to a site in the hypothalamus known as the suprachiasmatic nucleus (SCN; [50,59]). The endogenous rhythms generated by cells in the SCN repeat with a frequency close to, but not quite equal to, the 24-h period [2,23,64]. In general, the circadian timing system is thought to function to allow the temporal coordination of various physiological processes within an organism as well as allow the temporal coordination of the organism with the external world. In order to fulfill these functions, these rhythms must be synchronized to the exact 24-h cycle of the physical world. The daily cycle of light and dark is the dominant cue used by organisms to synchronize their biological clocks to the environment. Within an organism, the circadian system modulates many physiological processes and behaviors [39,65].

Though not extensively studied, there is evidence that diurnal variation may be a general feature of performance on learning and memory tasks. Certainly animals can form associations between the time of day and food availability. In an early study, Beling [5] noted that if bees were offered sugar water at a particular time of day, they quickly learned to arrive at the feeder in anticipation of the food reward. If the sugar water was omitted, the trained bees still arrived at the feeder at the correct time. This type of time–place association

* Corresponding author. Tel.: +1-310-825-0589; fax: +1-310-206-5060.

E-mail address: dchaudhury@mednet.ucla.edu (D. Chaudhury).

0166-4328/02/$ - see front matter
PII: S0166-4328(01)00471-5

has been described in many species including birds, insects, fish and mammals [8,12,32,46–49,55,62]. Other early studies on circadian phase dependence of learning have shown that animals can somehow 'time-stamp' information such that it is processed better at certain times of the day, where for example the performance of rats peaked at 24-h intervals following training on an avoidance task (e.g. [34]). Besides this diurnal periodicity, at least two other pieces of evidence link these rhythms in performance with the circadian system described above. First, lesions of the rat SCN [58] eliminate the 24-h rhythm in performance on a passive avoidance task. Second, Devan et al. [21] reported that rats subjected to desynchronization of the circadian system by rapidly changing the phase of the light–dark (LD) cycle experienced impaired recall of a spatial task. In addition to behavioral studies, electrophysiological studies using long-term potentiation (LTP), an electrophysiological analogue of learning and memory, has also been shown to undergo diurnal variation. For example, Barnes et al. [3] showed that synaptic responses in hippocampal granule cells following stimulation of afferent fibers from the entorhinal cortex fluctuates with a 24-h period. This group reported that synaptic activity for rats was highest in the middle of the dark phase and lowest in the middle of the light phase while the converse was true for diurnal squirrel monkeys. In another study, the magnitude of LTP, as a percentage of pretetanus basal response, was shown to vary in CA1 and dentate gyrus of hippocampal slices prepared from rats in the day or night [31]. More recently, Raghavan et al. [54] also reported diurnal variations in the magnitude of LTP in CA1 region of hippocampal slices prepared from the hamster.

In light of these studies, we designed a series of experiments to explore circadian modulation of learning and memory using a fear-conditioning paradigm. Fear conditioning is an associative-learning task that has become one of the leading behavioral models for investigating the neurobiological basis of learning and memory. Animals can learn to associate an initially neutral or conditioning stimulus (CS) such as a tone or context with a biologically significant event such as an unconditioned stimulus (US) like a footshock. In this assay, fear is measured as inactivity or 'freezing' after the stimulus. Freezing is a typical defensive response in rodents following exposure to aversive stimuli. The circuitry in fear-conditioning learning involves transmission of information about the CS (tone and context) and US (footshock) to the amygdala and the subsequent fear-response is linked to output projections from the amygdala to autonomic and behavioral responses in the brainstem [1,37]. In some experimental conditions, context, but not tone, learning requires normal hippocampus function [36,41,63].

In the present study, we investigated the possible circadian regulation of acquisition, recall and extinction in two strains of mice (C-57/6J and C-3H) following fear-conditioning training. The use of these two strains allows a comparison between the C-3H strain that secrete melatonin rhythmically and the C-57 strain that does not [22,30]. Animals were trained either during the day or at night. Mice were subsequently tested at least 24 h after training for context and tone learning every 6 h for 3 days. The degree of acquisition was greater in animals trained during the day then in animals trained at night. On tests for context and tone both strains of mice showed daily rhythms in recall, where greatest recall was usually observed when animals were tested during the day. Finally, the degree of long-term extinction of memories for context and tone were also shown to vary from day to night. Each of these diurnal rhythms persisted when animals were maintained in constant darkness, a finding that demonstrates the endogenous nature of these rhythms.

2. Methods

2.1. Subjects

Two-month old male mice (C-57/6J and C-3H) were purchased from Charles Rivers Laboratories. The UCLA Animal Research Committee approved the experimental protocols used in this study. Animals were housed in cages, which were placed in light-tight chambers where the light cycle could be controlled. For each experiment 8 mice were trained in the day and another 8 mice were trained at night.

2.2. Experimental procedure

Mice were allowed to entrain to the required LD cycle for at least 1 week prior to training on the fear-conditioning protocol. Mice were individually handled for approximately 1 min a day, a week prior to the start of the experiment to reduce the arousal associated with handling. Each day animals were handled at different times of the day or night to ensure that they did not entrain to handling by the experimenter at a specific time. Animals were handled by taking individuals out of their home cages and placing them on the experimenter's arm. Following entrainment, the animals were trained in separate contextual conditioning cages ($28 \times 21 \times 22$ cm^3: Lafayette Instruments). The chambers were constructed from aluminum (sidewalls) and Plexiglas (rear wall, ceiling, and hinged front door). A total of four identical conditioning cages were used that allowed 4 mice to be trained and tested per session. The floor of each cage consisted of 33 stainless steel rods (4 mm diameter, 4 mm apart) connected to a shock scram-

bler and generator (Master Shock, Lafayette Instruments). A speaker located at the roof of each cage permitted the delivery of tone (CS). To remove any variability in olfactory learning the inside of each cage was wiped with 0.01% benzaldehyde before the start of each experiment. On the day of training, mice were placed individually into cages and allowed to acclimatize to the new environment for 3 min after which time animals received a 30 s tone (CS; 80 dB, 2.8 kHz) followed by a 2 s footshock (US).

The number of CS–US pairing and US intensity was varied according to the strain of mice used. Preliminary studies showed that C-57 mice were better at learning the fear-conditioning paradigm compared to C-3H mice, so a stronger training protocol was used for C-3H animals (data not shown). The training protocol for C-57 mice consisted of 2 CS-US pairings with a 0.2 mA US. For the C-3H mice the training protocol consisted of 6 CS–US pairings with a 1 mA US. The inter-trial interval was 64 s in all protocols. At the end of the last tone-shock pairing the mice were left in the cage for a further 64 s after which time they were returned to the home cages. For the context test, animals were placed, individually, back into the same conditioning chamber and left there for 8 min. The behavior of the mice, whether it was freezing or mobile was noted. The first context test was carried out 24 h after training and animals were repeatedly tested for the context every 6 h for 3 days. Upon completion of the context test, animals were put through tone tests on day 4 and repeatedly tone tested every 6 h for another 3 days. For the tone testing (cued conditioning), 4 identical cages were used allowing 4 animals to be tested per session. Tone testing took place in a separate room. The cages used for tone testing were different from the context cages. The whole cage ($26 \times 31 \times 21$ cm^3) was made of Perspex (dark sides and clear back and ceiling) and the floor consisted of a removable wire mesh grid. The cages were cleaned with 0.01% diacetyl. For tone testing, baseline freezing of mice was measured for the first 2 min followed by the tone (80 dB, 2.8 kHz) which was activated for a further 6 min. Background noise (70 dB) during acquisition, context and tone testing was provided by a 'white' noise generator. The training and tone-testing procedures were automatically controlled by a computer using the ABET behavioral software (ABET systems, Lafayette Instruments).

Freezing during acquisition, context and tone recall was defined as the complete absence of somatic and motility movements with the exception of respiratory movements. For acquisition, context and tone recall an 8 s time sampling procedure was used in which each animal was observed 8 times per min interval (in this case a min refers to a 64 s block) and these were averaged to yield an estimate of percentage time freezing. Earlier studies had shown that this measure is amenable to parametric analysis [24]. During training, freezing was measured 64 s before the first CS–US pairing (baseline) and during the 64 s inter-trial interval immediately after each CS–US pairing, giving 8 observations per mice for baseline and for each subsequent CS–US pairing respectively. For context and tone testing each animal was observed a total of 64 times. To determine the degree of learning during training percent freezing was calculated as the number of times each animal was observed to be immobile over 8 observations. For context testing, percent freezing was calculated as the number of times each animal was observed to be immobile over 64 observations and for tone testing, percent freezing was calculated as the number of times each animal was observed to be immobile over 16 (2 min baseline) and 48 (6 min tone) observations. For the tone experiment's, freezing was normalized by subtracting the baseline, pre-tone, freezing of individual animals during the first tone test from freezing after tone activation in all-subsequent tests.

In light–dark (LD) and dark–light (DL) experiments the rooms were illuminated with a small 65 W incandescent light bulb during the animal's day. Experiments during the animal's night were done in complete darkness with the aid of infrared goggles. All dark–dark (DD) experiments were performed in complete darkness. Freezing was recorded with the aid of a video camera that had an in-built infrared system, which enabled us to record the behavior of animals in both light and dark conditions.

Control experiments were initially carried out to determine if training followed by repeated testing for context or tone affected the locomotor activity in both strains of mice. Animals were initially entrained to a LD cycle for 1 week then kept in DD 2 days prior to training. Mice were divided into 2 groups, one trained in the subjective day and the other trained in the subjective night. Twenty-four hours after training, animals were repeatedly tested for contextual learning every 12 h for 3 days in the subjective day and subjective night followed by tone testing every 12 h for another 3 days in the subjective day and subjective night. The findings from these control experiments showed that training and repeated testing did not affect the animal's circadian rhythm.

2.3. Light–dark cycle

LD signifies 'normal' light–dark cycle. By definition, the time of light onset is zeitgeber time 0 ([ZT 0], 06:00), while time of lights off is ZT 12 (18:00).

Mice were trained during the day (09:00, ZT 3) or night (21:00, ZT 15). DL signifies 'reverse' light–dark cycle. By definition, the time of light onset is ZT 0 (18:00), while time of lights off is ZT 12 (06:00). Mice were trained during the day (21:00, ZT 3) or night (09:00, ZT 15). DD signifies constant darkness. Animals were initially entrained to the LD cycle, then 2 days prior to training they were kept in constant darkness for the rest of the experiment. Animals were trained at subjective day (09:00, circadian time 3 [CT 3]) or subjective night (21:00, CT 15).

2.4. Data analysis

To compare acquisition following each CS–US pairing in animals trained in the day or night the Student's t-test was used. Recall and the degree of extinction were analyzed using a 1 way repeated measure ANOVA. Data for recall was divided into 24-h intervals. Where significance was seen, the post-hoc Tukey's test was done. Values were considered significantly different at $P < 0.05$.

3. Results

3.1. Rhythm in acquisition of C-3H mice

Our first experiment was designed to determine whether the ability of C-3H mice to learn the fear conditioning protocol varied between day and night. Mice trained in the day were trained 3 h after lights-on (ZT 3; Fig. 1a) while mice trained at night were trained 3 h after lights-off (ZT 15; Fig. 1b). Other than the time that the animals were trained and tested, all other conditions between the day and night groups remained constant. The degree of acquisition (Fig. 2a) was greater in mice trained during the day (ZT 3) compared to animals trained at night (ZT 15). There was also a significant difference in fear conditioning (measured as percent freezing) in animals trained at ZT 3 compared to animals trained at ZT 15 ($t = 3.54$ df $= 14$, $P = 0.003$). Freezing after the last CS–US pairing was $92.2 \pm 3\%$ ($n = 8$) in mice trained at ZT 3 and $48.9 \pm 12\%$ ($n = 8$) in mice trained at ZT 15. In order to demonstrate that the phase of the rhythm was determined by the prior LD cycle, a group of mice were

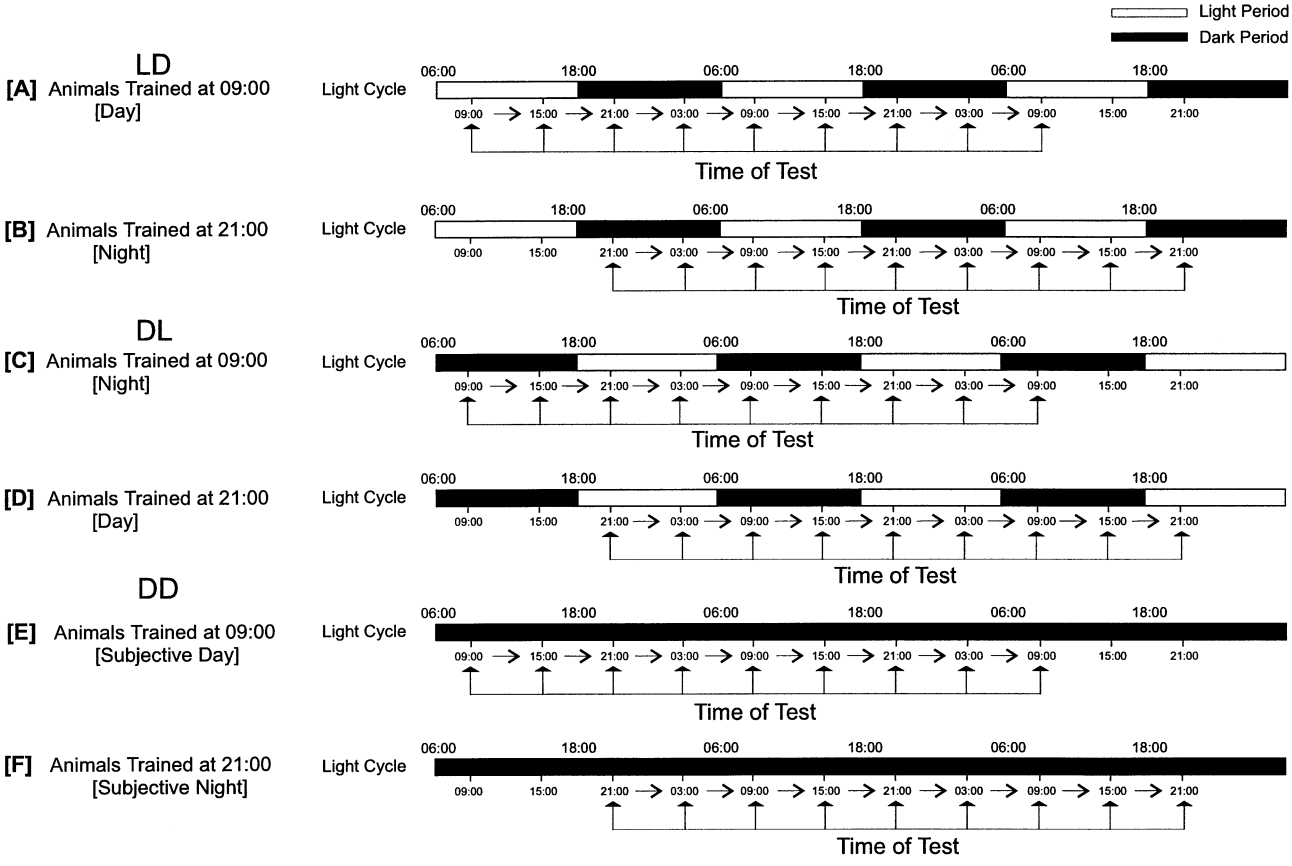

Fig. 1. Diagram showing the light cycle for testing in experiments carried out in normal light–dark cycle (LD), reverse light–dark cycle (DL) and constant darkness (DD). The numbers above each bar denotes the time light was turned on or off for the LD and DL experiments. DD experiments were conducted in complete darkness although the times of the prior LD cycle is shown as a way of standardizing the graphical representation. The numbers and arrows below each bar denotes the times and sequence that animals were tested for context and tone.

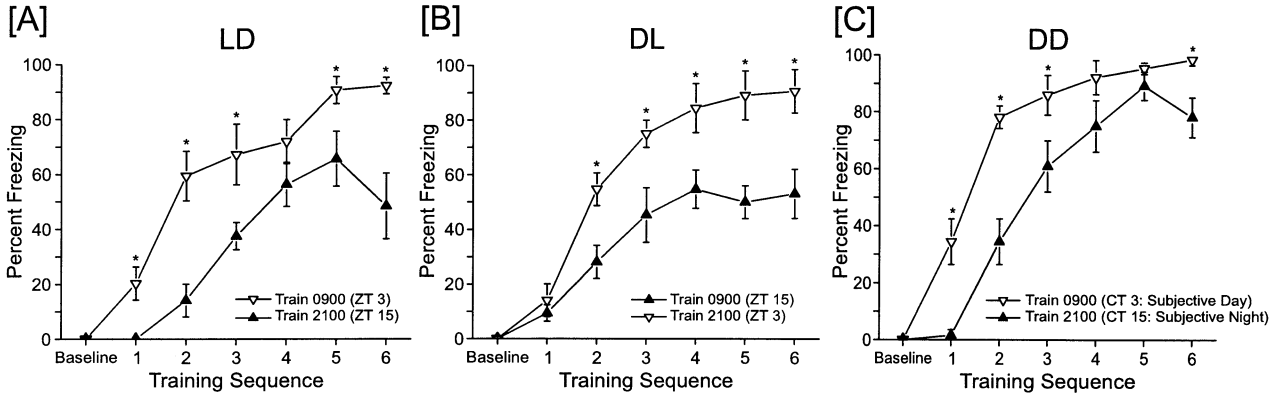

Fig. 2. Rhythms in acquisition in C-3H mice. Animals were trained either in the day (ZT/CT 3) or the night (ZT/CT 15). Conditioning consisted of six tone (CS) and footshock (US) pairings. Percent freezing following each tone-shock pairing in animals trained in the day was compared with percent freezing following the equivalent tone-shock pairing in animals trained at night. Animals trained in the day acquired the conditioning better than mice trained at night. (A) Mice were maintained on a LD cycle. (B) Mice were maintained on a DL cycle. (C) Mice were maintained in DD. Times of prior LD cycle indicated. Each group contained 8 animals. Statistical comparison made using T-test, * denotes $P < 0.05$.

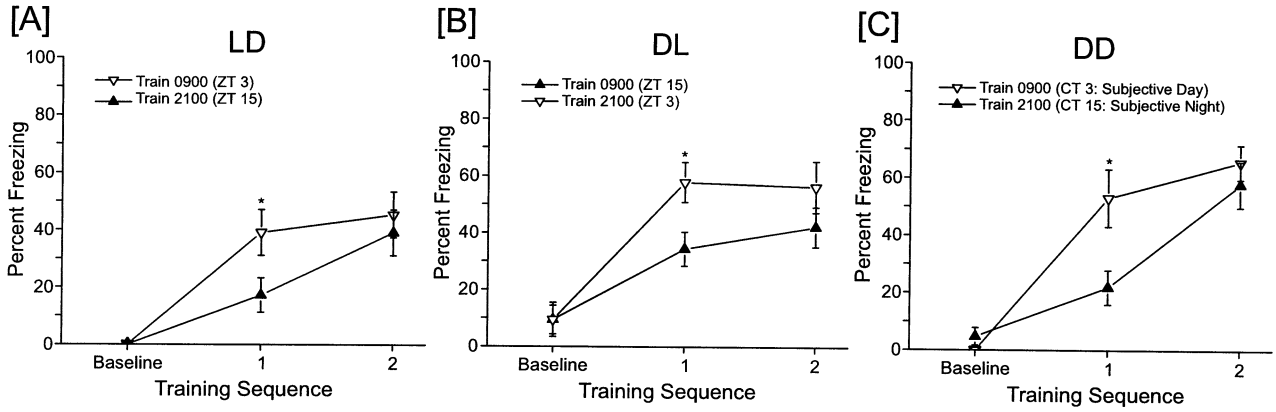

Fig. 3. Rhythms in acquisition in C-57 mice. Animals were trained either in the day (ZT/CT 3) or the night (ZT/CT 15). Conditioning consisted of two tones (CS) and footshock (US) pairings. Percent freezing following each tone-shock pairing in animals trained in the day was compared with percent freezing following the equivalent tone-shock pairing in animals trained at night. Mice trained in the day acquired the conditioning better than mice trained at night. (A) Mice were maintained on a LD cycle. (B) Mice were maintained on a DL cycle. (C) Mice were maintained in DD. Times of prior LD cycle indicated. Each group contained 8 animals. Statistical comparison made using t-test, * denotes $P < 0.05$.

housed in a 'reversed' LD cycle (Fig. 1c and d). Again, the degree of acquisition and peak freezing were significantly higher ($t = -3.14$, $\mathrm{df} = 14$, $P = 0.007$) during the day ($90.6 \pm 8\%$, $n = 8$) then during the night ($53.1 \pm 9\%$, $n = 8$) suggesting that the observed daily variation is determined by the phase of the LD cycle and not some other unknown variable (Fig. 2b). Finally, in order to demonstrate that any diurnal rhythm is circadian, it is necessary to show that the rhythm continues in DD. For these experiments, animals were placed in DD for 2 days prior to training. The data was collected at circadian time (CT) 3 and CT 15 to form a 'subjective day' and 'subjective night' group (Fig. 1e and f). Again, there was a daily rhythm in the degree and level of training as measured by percent freezing with measured values after the last CS-US pairing being higher during the subjective day ($98.4 \pm 2\%$, $n = 8$) compared to subjective night (78.1 ± 7, $n = 8$). Statistical analysis showed the difference to be significantly higher ($t = 2.69$, $\mathrm{df} = 14$, $P < 0.02$; Fig. 2c). Together, this data suggests that there is a circadian rhythm in the ability of C-3H mice to acquire the fear conditioning.

3.2. Rhythm in acquisition of C-57 mice

Next, we determined whether C-57 mice also exhibited a diurnal difference in their ability to learn the fear conditioning protocol. In a normal LD cycle, the degree of acquisition (Fig. 3a) was greater in mice trained during the day (ZT 3) compared to animals trained at night (ZT 15). There was a significant difference in fear conditioning (measured as percent freezing) after the first training stimulus in animals trained at ZT 3 ($39.1 \pm 8\%$, $n = 8$) compared to animals trained at ZT 15 (17.2 ± 6, $n = 8$; $t = 2.22$, $\mathrm{df} = 14$, $P = 0.04$). However, by the second training period, there was no longer

a significant difference between the day and night groups. Similarly, if C-57 mice were exposed to a strong training protocol consisting of 3 CS–US pairings, there was no rhythm in acquisition as learning was equally strong in mice trained in the day or night. Consequently, the rest of the experiments were carried out with a weaker training protocol. When the phase of the LD cycle to which the animals were exposed was reversed, so did the resulting rhythm (Fig. 3b). The percent freezing after the first training stimulus was significantly greater in animals trained at ZT 3 ($57.8 \pm 7\%$, $n = 8$) then in animals trained at ZT 15 ($34.4 \pm 6\%$, $n = 8$; $t = -2.59$, $\mathrm{df} = 14$, $P = 0.02$). Finally, to determine if the degree of acquisition was affected by the circadian system, the experiments were run in mice in DD (Fig. 3c). The degree of acquisition is greater in mice trained at 09:00 (CT 3) compared to mice trained at 21:00 (CT 15). Percent freezing after the first training stimulus was significantly greater in mice trained at CT 3 ($53.1 \pm 6\%$, $n = 8$) compared to mice trained at CT 15 ($21.9 \pm 6\%$, $n = 8$; $t = 2.73$, $\mathrm{df} = 14$, $P = 0.02$). Overall, the data demonstrate that there is a circadian modulation of the acquisition of fear conditioning in C-57 mice with faster learning occurring during the day. The magnitude of this modulatory effect was smaller than that seen with the C-3H mice and could be overcome by stronger training protocols.

3.3. Rhythm in recall in C-3H mice trained at day or night

Previous studies suggest that an animal's ability to recall a learned task may vary as a function of the time of day in which the training occurred. In order to investigate this possibility, C-3H mice were trained and then tested for their recall of the context and tone fear conditioning every 6 h for 3 days starting 24 h after training (Fig. 4). Mice trained during the day (ZT 3) exhibited a striking rhythm in recall (Fig. 4a), which peaked each cycle at ZT 3. Statistical analysis showed a significant time of test effect for the first ($F(3, 4764) = 28$, $P < 0.001$) and second ($F(4, 1729) = 27$, $P < 0.001$) 24-h period for context recall and the first ($F(3, 831) = 38$, $P < 0.001$) and second ($F(4, 412) = 17$, $P < 0.001$) 24-h period for tone recall. Post-hoc test showed that percent freezing was significantly greater when mice were tested at ZT 3 for both context ($P < 0.01$) and tone ($P < 0.001$). Reversing the phase of the LD cycle reversed the peak of the rhythm (Fig. 4b), where a significant time of test effect was seen for recall of both context and tone (Context: first 24-h period ($F(3, 4804) = 27$, $P < 0.001$) and second 24-h period ($F(4, 1279) = 16$, $P < 0.001$); Tone: first 24-h period ($F(3, 578) = 14$, $P < 0.001$) and second 24-h period ($F(4, 344) = 15$, $P < 0.001$)). Post-hoc analysis showed that percent freezing was significantly greater when mice were tested during the day for context ($P < 0.002$) and tone ($P < 0.03$), respectively. Finally, when animals were trained and tested in DD, the rhythms in the recall of context and tone conditioning continued to persist (Fig. 4c), where a significant time of test effect was seen for recall of context and tone (Context: first 24-h period ($F(3, 1182) = 7$, $P = 0.002$) and second 24-h period ($F(4, 1124) = 11$, $P < 0.001$); Tone: first 24-h period ($F(3, 754) = 16$, $P < 0.001$) and second 24-h period ($F(4, 3004) = 5$, $P = 0.003$)). In each of these cases, post-hoc analysis showed that mice trained at CT 3 exhibited peak in their recall when tested during the

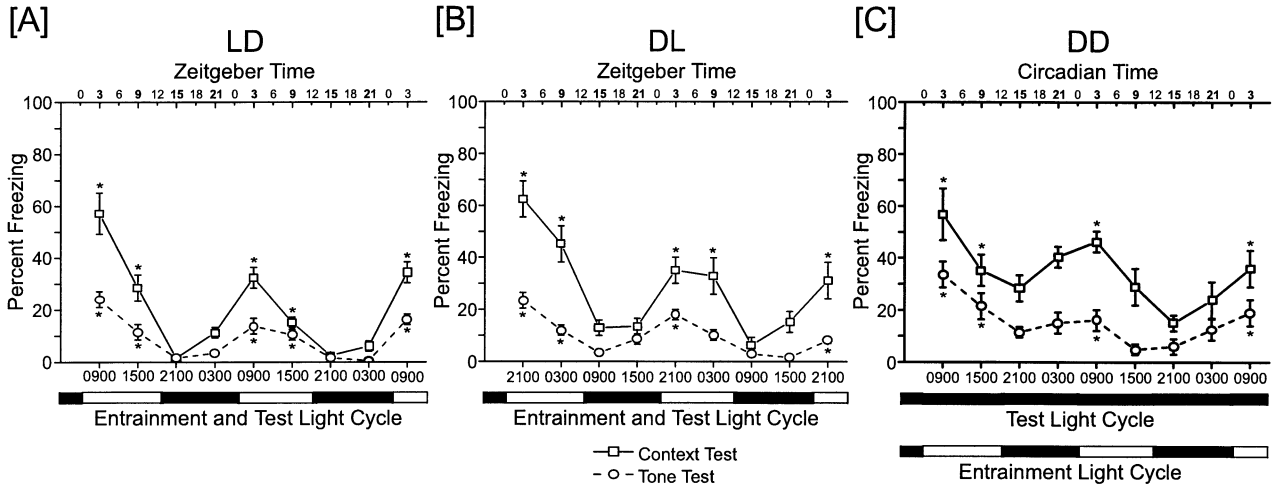

Fig. 4. Rhythms in recall in C-3H mice trained in the day (ZT/CT 3). In all experiments animals were first tested for context 24 h post-training then repeatedly tested every 6 h, for 3 days. On day 4, animals were tested for tone every 6 h for another 3 days. Testing was done at ZT/CT 3, 9, 15 and 21. (A) Mice were maintained on a LD cycle. (B) Mice were maintained on a DL cycle. (C) Mice were maintained in DD. Times of prior LD cycle indicated. Each group contained 8 animals. Within population one way RM ANOVA at the first and second 24-h periods for both the context and tone showed significant differences in recall at different times of test, where * denotes $P < 0.05$.

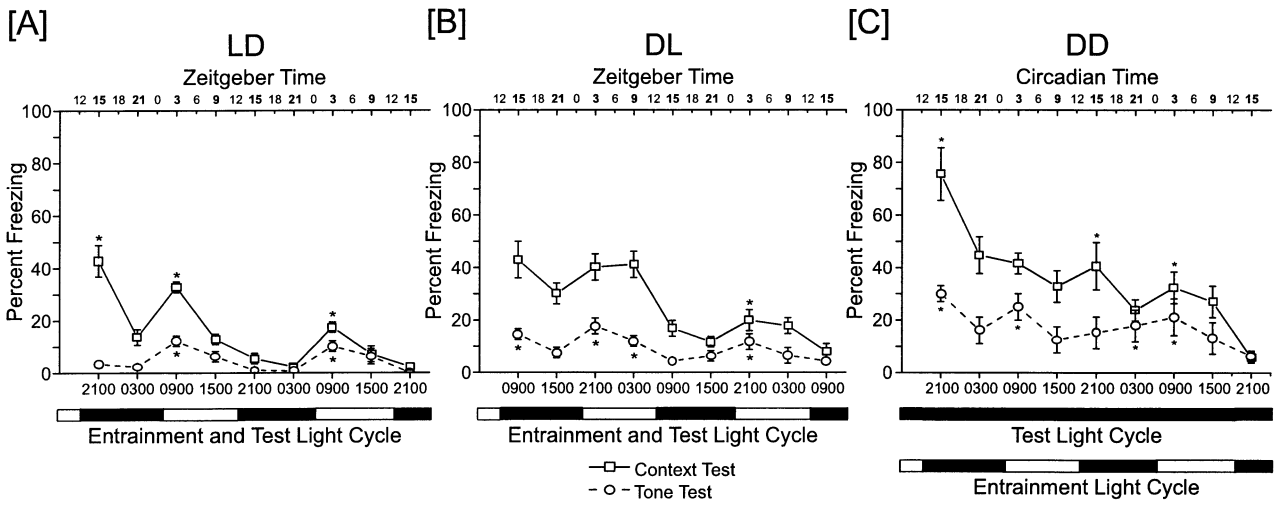

Fig. 5. Rhythms in recall in C-3H mice trained in the night (ZT/CT 15). In all experiments animals were first tested for context 24 h post-training then repeatedly tested every 6 h, for 3 days. On day 4 animals were tested for tone every 6 h for another 3 days. Testing was done at ZT/CT 3, 9, 15 and 21. (A) Mice were maintained on a LD cycle. (B) Mice were maintained on a DL cycle. (C) Mice were maintained in DD. Times of prior LD cycle indicated. Each group contained 8 animals. Within population one way RM ANOVA at the first and second 24-h periods for both the context and tone showed significant differences in recall at different times of test, where * denotes $P < 0.05$.

subjective day in subsequent days (CT 3 and CT 9, $P < 0.01$). Importantly, when similar experiments were performed on mice trained during the night (ZT 15), similar results were obtained in that there were rhythms in recall that peaked during the day, not the night (Fig. 5). For LD experiments there was time of test effect for context and tone (Context: first 24-h period ($F(3, 1735) = 21$, $P < 0.001$ and second 24-h period ($F(4, 307) = 13$, $P < 0.001$); Tone: first 24-h period ($F(3, 156) = 11$, $P < 0.001$) and second 24-h period ($F(4, 155) = 7$, $P < 0.001$)). Post-hoc analysis showed that recall for context and tone was generally better in animals tested in the day ($P < 0.05$). For DL experiments a significant time of test effect was seen in animals tested for context in the second but not first 24-h period and in both periods of the tone test (Context: ($F(3, 269) = 2$, $P = 0.1$) and second 24-h period ($F(4, 189) = 5$, $P = 0.01$); Tone: first 24-h period ($F(3, 148) = 6$, $P = 0.003$) and second 24-h period ($F(4, 71) = 4$, $P = 0.02$)). Post-hoc analysis showed that recall was significantly better at certain times of test ($P < 0.05$). For DD experiments a time of test effect was again observed for context and tone (Context: first 24-h period ($F(3, 2775) = 18$, $P < 0.001$) and second 24-h period ($F(4, 1306) = 6$, $P = 0.001$); Tone: first 24-h period ($F(3, 454) = 8$, $P = 0.001$) and second 24-h period ($F(4, 219) = 4$, $P = 0.01$)). Post-hoc analysis showed recall was significantly better at certain times of test ($P < 0.05$). These results demonstrate that the recall of the fear conditioning in C-3H mice is modulated by the circadian system. In addition, our data indicate that the peak in fear conditioning performance occurred during the day regardless of the time of training.

3.4. Rhythm in recall in C-57 mice trained at day or night

Next C-57 mice were trained and then tested for their recall of the context and tone fear conditioning every 6 h for 3 days starting 24 h after training as described above. C-57 mice trained in the day (ZT 3) show diurnal rhythm in recall for both context and tone in LD and DL light cycles (Fig. 6a and b). For both the LD and DL experiments a time of test effect was observed (LD Context: first 24-h period ($F(3, 1037) = 7$, $P = 0.002$ and second 24-h period ($F(4, 3139) = 75$, $P < 0.001$); LD Tone: first 24-h period ($F(3, 1063) = 11$, $P < 0.001$) and second 24-h period ($F(4, 963) = 13$, $P < 0.001$); DL Context: first 24-h period ($F(3, 2531) = 37$, $P < 0.001$) and second 24-h period ($F(4, 1884) = 38$, $P < 0.001$); DL Tone: first 24-h period ($F(2, 3487) = 50$, $P < 0.001$) and second 24-h period ($F(4, 1546) = 7$, $P < 0.001$)). Post-hoc analysis showed that recall was generally significantly greater when animals were tested during the day (ZT 3 and ZT 9, $P < 0.01$). A time of test effect was seen in animals trained and tested in DD for both periods of the context test and for the second period of the tone test (Context: first 24-h period ($F(3, 1234) = 6$, $P = 0.006$) and second 24-h period ($F(4, 1281) = 7$, $P < 0.001$); Tone: first 24-h period ($F(3, 314) = 1$, $P = 0.1$) and second 24-h period ($F(4, 851) = 7$, $P < 0.001$)). Post-hoc analysis revealed that mice expressed significantly greater freezing at subjective day for the first and second 24-h periods (CT 3; $P < 0.01$) thus showing that C-57 mice show a circadian rhythm in their ability to recall conditioning for both context and tone (Fig. 6c). The same rhythm in recall was also seen in mice trained at night (ZT 15, Fig. 7). Mice

trained at night, in both the LD and DL experiments, also show a time of test effect (LD Context: first 24-h period ($F(3, 860) = 15$, $P < 0.001$) and second 24-h period ($F(4, 1086) = 10$, $P < 0.001$); LD Tone: first 24-h period ($F(3, 444) = 4$, $P = 0.02$) and second 24-h period ($F(4, 1593) = 28$, $P < 0.001$); DL Context: first 24-h period ($F(3, 630) = 11$, $P < 0.001$) and second 24-h period ($F(4, 574) = 4$, $P = 0.01$); DL Tone: first 24-h period ($F(3, 1083) = 7$, $P = 0.002$ and second 24-h period ($F(4, 573) = 9$, $P < 0.001$)). Post-hoc analysis showed that mice expressed greater recall for both context and tone at ZT 3 and ZT 9 when compared to tests at ZT 15 and ZT 21 ($P < 0.04$). The rhythms in contextual and tone recall was maintained in animals trained and tested in DD (Context: first 24-h period ($F(3, 1737) = 15$, $P < 0.001$) and second 24-h period ($F(4, 663) = 8$, $P < 0.001$); Tone: first 24-h period ($F(3, 1053) = 7$, $P = 0.002$) and second 24-h period ($F(4, 588) = 7$, $P < 0.001$)). Post-hoc analysis showed that recall was greater when animals were tested during the subjective day (CT 3; $P < 0.02$).

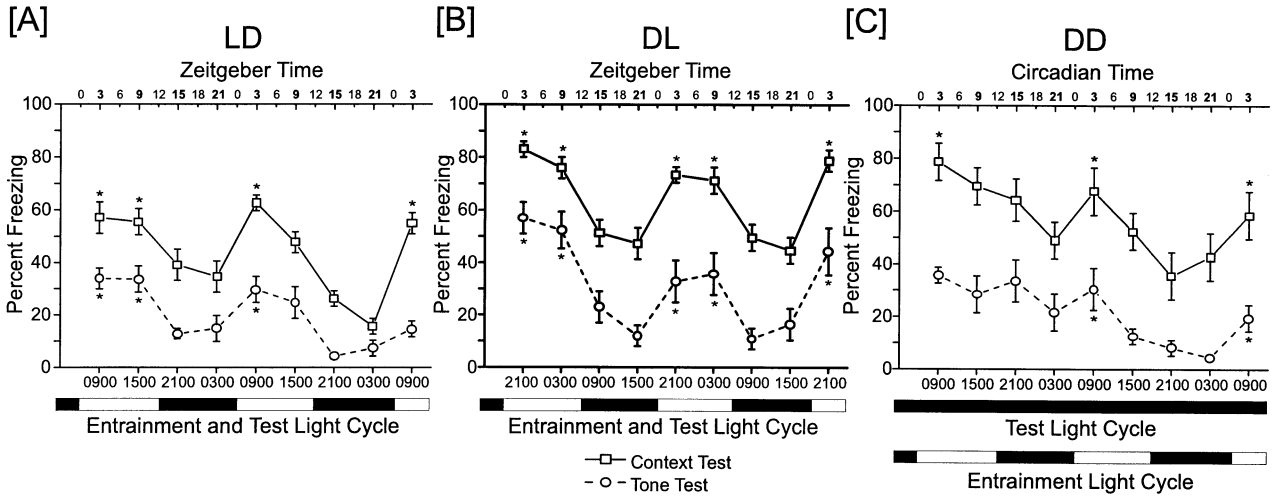

Fig. 6. Rhythms in recall in C-57 mice trained in the day (ZT/CT 3). In all experiments animals were first tested for context 24 h post-training then repeatedly tested every 6 h, for 3 days. On day 4, animals were tested for tone every 6 h for another 3 days. Testing was done at ZT/CT 3, 9, 15 and 21. (A) Mice were maintained on a LD cycle. (B) Mice were maintained on a DL cycle. (C) Mice were maintained in DD. Times of prior LD cycle indicated. Each group contained 8 animals. Within population one way RM ANOVA at the first and second 24-h periods for both the context and tone showed significant differences in recall at different times of test, where * denotes $P < 0.05$.

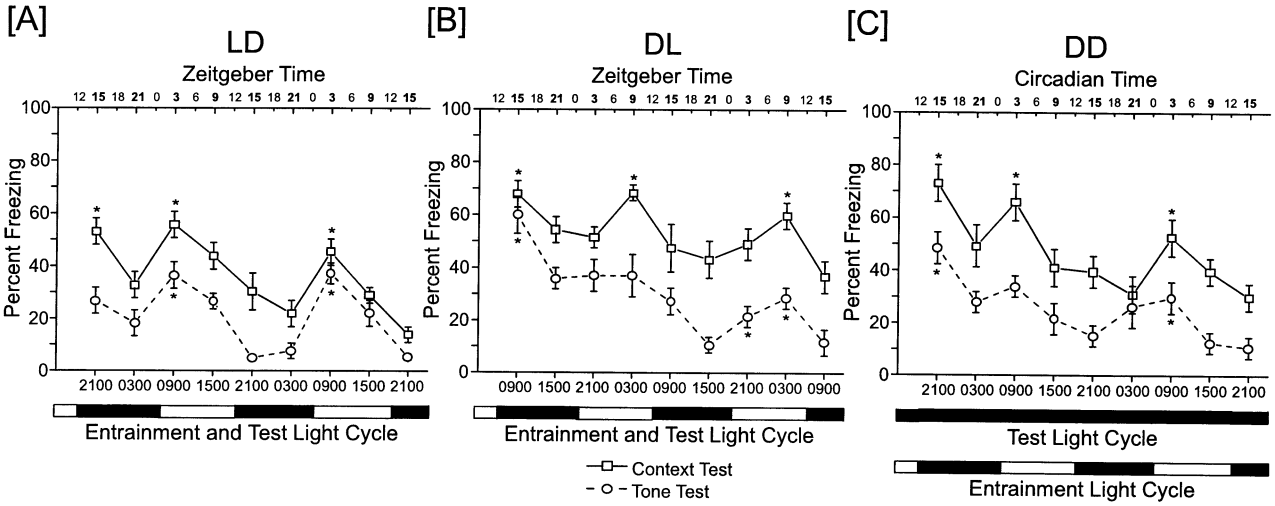

Fig. 7. Rhythms in recall in C-57 mice trained in the night (ZT/CT 15). In all experiments animals were first tested for context 24 h post-training then repeatedly tested every 6 h, for 3 days. On day 4, animals were tested for tone every 6 h for another 3 days. Testing was done at ZT/CT 3, 9, 15 and 21. (A) Mice were maintained on a LD cycle. (B) Mice were maintained on a DL cycle. (C) Mice were maintained in DD. Times of prior LD cycle indicated. Each group contained 8 animals. Within population one way RM ANOVA at the first and second 24-h periods for both the context and tone showed significant differences in recall at different times of test, where * denotes $P < 0.05$.

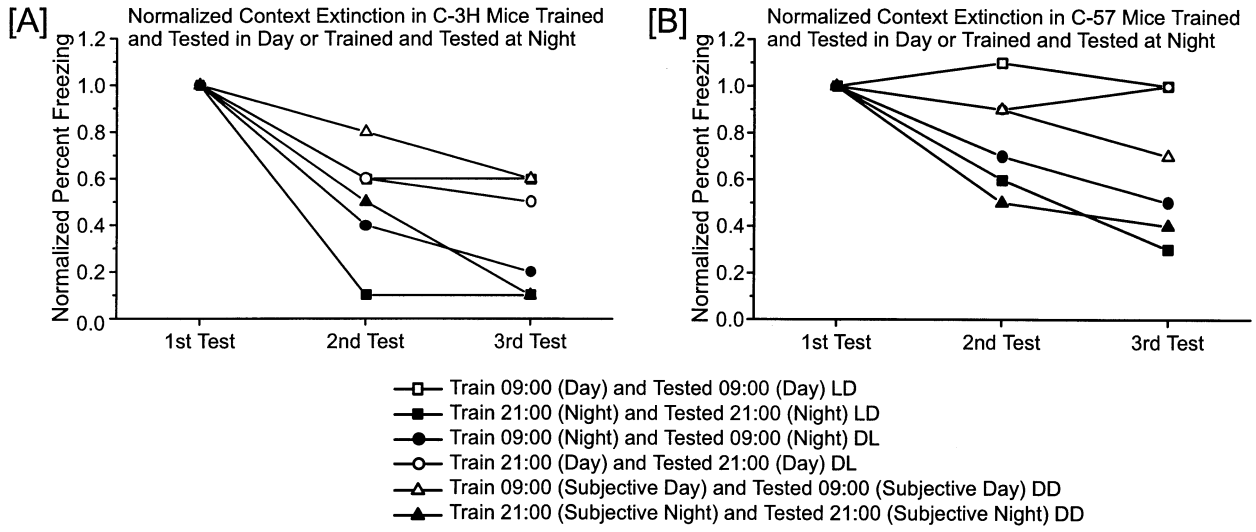

Fig. 8. Rhythms in long-term extinction for context. The degree of extinction in animals repeatedly tested at ZT/CT 3 or ZT/CT 15 was measured. The data was standardized by normalizing percent freezing in animals tested at the second and third test relative to percent freezing in animals in the first test. This enabled better visualization of the graph and a more rigorous way of comparing the gradients between animals repeatedly tested in the day or the night. The gradient of the slopes was taken as the indicator of long-term extinction. For C-3H mice the degree of extinction, in LD and DD experiments, was greater ($P < 0.05$) in animals tested at night. In DL experiments, the extinction was greater during the night however the difference was not significant. For C-57 mice the degree of extinction was significantly greater in animals tested at night during the LD and DL experiments ($P < 0.05$). In DD experiments the degree of extinction was greater in animals tested at night however it was not significant. Each group contained 8 animals.

3.5. Rhythm in long-term extinction of context and tone memories in C-3H mice

With fear conditioning, and other learned behaviors, repeated testing with context or tone without the shock leads to extinction of the learned fear response. In order to examine the possible circadian variation in extinction, we measured the degree of both the short- and long-term extinction. The degree of short-term extinction as measured by the decrease in performance over each 8 min test-trial during the context and tone test did not vary between day and night groups (data not shown). To measure the degree of long-term extinction, we compared the percent freezing of C-3H animals trained and tested at ZT/CT 3 or ZT/CT 15 over the course of 3 days. In contrast to short-term extinction, the degree of long-term extinction measured as the decrease in performance over the 3 days period did show a significant difference between day and night. This diurnal difference persisted in animals maintained on a reverse LD cycle and in DD. The degree of extinction was determined by measuring the gradient of the graph in animals trained and tested in LD, DL and DD conditions (e.g. Fig. 8a). The degree in long-term extinction in C-3H mice following repeated testing for context and tone appears to be circadian. Analysis of the extinction curve in the LD and DD experiments shows that mice trained and tested at night have a greater degree of extinction compared to mice trained and tested in the day (LD Context: ($F(1, 0.0008) = 10$, $P = 0.02$); LD Tone: ($F(1, 0.0009) = 36$, $P < 0.001$; DD Context: ($F(1, 0.0005) = 11$, $P = 0.01$); DD Tone: $F(1, 0.0004) = 8$, $P = 0.03$)). Though there was no significant difference between the day and night groups in the DL experiments, the degree of extinction was generally greater in animals tested at night (DL Context: ($F(1, 0.0001) = 4$, $P = 0.1$); DL Tone: ($F(1, 0.00003) = 3$, $P = 0.2$)). Since the degree of extinction was highest during the night when acquisition was weakest, it is possible that the mice with weaker conditioning also exhibited faster extinction. However, an analysis of our data revealed no significant correlation between the degree of acquisition after training and the degree of extinction (Pearson correlation test). Overall, the data shows that long-term extinction in C-3H mice is modulated by the circadian system.

3.6. Rhythm in long-term extinction of context and tone memories in C-57 mice

Recall in C-57 mice following repeated exposure to context and tone was also analyzed to determine if long-term extinction varied between animals tested in the day or the night (e.g. Fig. 8b). Again, the degree of short-term extinction did not vary from day to night (data not shown). In contrast, analysis of long-term extinction did show that the degree of extinction for context and tone was generally greater in animals tested at night (ZT/CT 15) compared to animals tested in the day (ZT/CT 3). For repeated contextual tests, the de-

gree of extinction was significantly greater in animals tested at night for both the LD and DL experiments (LD: ($F(1, 0.0009) = 96$, $P < 0.001$); DL: ($F(1, 0.0003) = 36$, $P < 0.001$)). For DD experiments the degree of context extinction in animals tested at night was greater, though not significantly different, from animals tested in the day ($F(1, 0.0001) = 5$, $P = 0.07$)). For repeated tone testing, in the LD experiments, the degree of extinction was significantly greater in animals tested at night ($F(1, 0.0001) = 11$, $P = 0.01$). For DL and DD experiments the degree of extinction was still greater, though not significantly different, from animals tested in the day (DL: ($F(1, 0.001) = 4$, $P = 0.08$); DD $F(1, 0.0002) = 2$, $P = 0.2$)). The degree of long-term extinction in C-57 mice, for both context and tone, appears to be circadian.

4. Discussion

In the present study, C-3H and C-57 mice were trained with a context and tone fear conditioning protocol. The mice were then tested over the course of several days for their ability to recall the training. When comparing the performance of animals during the day and night, the mice acquired the conditioning faster in the day than in the night. Furthermore, the recall for context and tone consistently peaked during the day for at least 3 days after training, irrespective of the time of training. Finally, the loss of this training (or extinction) exhibited a rhythm in that mice trained at night exhibited a greater degree of extinction than mice trained during the day. For all of these rhythms in acquisition, recall, and extinction the phase of the rhythm was controlled by the prior LD cycle. When we reversed the phase of the LD cycle, the phase of the rhythm also reversed. Importantly, all three of the rhythms also continued in constant darkness demonstrating the endogenous, and presumably circadian nature, of the rhythms.

Overall, our data suggest that the ability to learn the fear-conditioning task is modulated by the circadian system. There are a couple of aspects of this data that deserve further comment. First, there appears to be a 'ceiling' to the circadian modulation of learning. This was most apparent with our data from the C-57 mice. When these mice were trained on stronger protocols (3 CS–US pairings, US = 1 mA or 2 CS–US pairings, US = 0.3 mA), we did not observe any difference in acquisition between animals trained at ZT 3 or at ZT 15. It is likely that with the stronger protocol the level of learning is above the threshold that would be affected by the circadian system. In addition, our results indicate that the mice learned this task better in the day when they are normally inactive, than at night. In some ways, this was a surprising observation as we were expecting performance to be best during the night when these nocturnal organisms are normally active. This phase dependence may be a special feature of the fear conditioning task or other negative conditioning protocols. Perhaps for a nocturnal animal, day is basically a fearful time and this state-dependence leads to improved performance. A survey of previously published reports on day/night differences on acquisition in animals during training is not consistent. It seems that different learning processes are differentially affected by time of day. For example, some reports have shown that acquisition of a shuttle avoidance task and 8-arm radial mazes are better in animals during the dark period [33,52]. In contrast there is also some evidence that acquisition, for an active avoidance task, is quicker in animals during specific times of the day compared to other times of the day or night [7,34]. Other groups have reported no difference in the acquisition of the shuttle avoidance task in control, untreated, animals during the day or the night [13,17,29]. One possible explanation for the conflicting reports on the effects of time of day on acquisition may be due to differences between species and variations in training protocols and behavioral paradigms that make use of different neural substrates. For example, it is unclear whether these groups trained their animals in complete darkness during the acquisition procedures at night since it is possible that training or even testing of animals in a lit room during their night-phase may influence the animal's performance. An example different neural substrates being differently influenced by time of day was recently reported by Rudy and Pugh [56] whose study showed that time of conditioning influenced contextual, but not auditory, learning by affecting some aspect of the acquisition or consolidation process of context memory. It should be noted, however, this finding is in some ways inconsistent with our data since we found rhythms for both contextual and tone learning. There is already abundant evidence from lesion and pharmacological studies in a variety of species that indicate neural substrates of learning and memory are organized into multiple distinct neural systems [4,11,28,43,45,51]. Thus, it would also be logical to assume that different types of memory systems can be differently affected by the circadian system.

To explore circadian modulation of recall, for context and tone, following training in the day or night, mice were tested every 6 h for several days. It was clear that the animals exhibited a daily rhythm in their performance for both context and tone tests whether the animals were placed either on a LD, DL, or in DD. This type of modulo-24-h rhythm in recall was similar to that described in rats tested on an active avoidance task. These rats exhibited better recall when tested 24 h post-training than did rats tested at other times post-training [34]. This type of observation suggests that

learned information may be stored in a 'time-stamped' manner to be retrieved easier at some time of day than at others. As Daan [19,20] has previously argued, it makes adaptive sense for an animal to use previous temporal experience to guide future behavior. Experimental support for this concept of time-stamping has come from a variety of studies demonstrating that a range of species have the ability to learn to associate a specific time and place with a food reward [8,40,49]. However, while our study provides support for circadian modulation of acquisition and recall of learned information, the data does not support any simple concept of time-stamping being important in our fear conditioning protocol. This model would clearly predict that animals trained during the night would perform best on subsequent nights while animals trained during the day would perform best during the day. Instead, our study clearly shows that animals had better recall during the day, irrespective of the time of training. This observation would suggest that 'time-stamping' is not necessarily the only mechanism by which the circadian system can affect recall. There are certain methodological differences in our study and others that may explain our findings. For example, we repeatedly tested individual animals over the course of several days whereas the other groups had used separate population of animals to be tested at different times following training on an aversive task (e.g. [34]). There are, however, reports that also describe 'time-stamping' in the same animal tested over a period of days (e.g. [8]). The difference between our study and those experiments that tested the same animal over the course of several days is that we used a fear-conditioning task in which mice learned to fear a stimulus while these groups employed a appetitive task where animals had learnt to come to a feeding station at a particular time and place.

Extinction of a conditioning response occurs when an animal is re-exposed to the CS (context or tone) in the absence of the US (footshock). This is measured as a progressive decrement in the expression of the conditioned response (CR). Our data show that when C-3H and C-57 mice are repeatedly tested in the day (ZT/CT 3) or the night (ZT/CT 15) the degree in long-term extinction is greater in animals tested at night than in those tested during the day. Interpretation of the long-term extinction data is complicated, since extinction can be indicative of the animal having relearned that the conditioned cage is not aversive (i.e. learned to ignore stimuli that are of little or no biological significance). Alternatively, the animal may have simply forgotten about the aversive experience. Previous studies have shown that when animals are repeatedly exposed to a conditioning chamber the response of the animal shows a general decline (e.g. [16]). Our data indicate that the degree of this decline is not necessarily constant and can vary with the time of training within the 24-h cycle. In some ways, this could be viewed as a temporal variation of a phenomenon called the 'renewal effect'. This effect describes the renewed response in animals when placed in the conditioning environment following extinction as a result of repeated testing in a different context [10,27]. Thus it is possible that the peaks in freezing observed in animals tested during the day is some form of renewal which occurs on a daily basis. Recent evidence has shown that extinction of some behavioral tasks is sensitive to some of the same pharmacological agents as long-term memory. This finding has lead Berman and Dudai [6] to propose that extinction may be the result of molecular cascades leading to the development of new memory. It is therefore possible that the peaks in freezing in our experiments, during the day but not at night, indicate better recall at such times of old information and a corresponding decrease in ability to form new memory or association of the context or tone. In support of our hypothesis that the circadian system has the ability to influence extinction of a learned response, Ternes [61] reported diurnal differences in extinction when rats were repeatedly tested on a taste aversion task. While, Fekete et al. [25] presented some evidence showing that extinction in rats trained on a passive avoidance task was greater in those animals that had been exposed to a phase shifted light-cycle compared to non-phase-shifted controls.

It may be possible, though unlikely, that our observed differences between the day and night groups may be due to differences in sensory, motor or motivational differences and not memory per se. A discriminative procedure where animals were trained in two different contexts (context A and B) and two different cues (CS A and B) which were either associated with shock or no shock would have overcome this confound. In general, there are three types of evidence that learning and memory function can be regulated by the circadian system. First, there is a long history of work demonstrating diurnal differences in the ability of animals to acquire or recall a memory task (e.g. [33,49,52]). This literature includes studies demonstrating that peak performance of learned tasks occur at 24-h intervals after training [34]. The current study adds to this literature, in part, by demonstrating that the phase of the rhythms is controlled by the prior LD cycle and continues in constant darkness. This later observation is critical, as it is part of the requirement for a circadian rhythm that it is generated endogenously in the absence of a rhythm in the environment. Second, there have been at least two reports that lesioning the SCN, the core of the mammalian circadian timing system, interferes with the expression of the rhythm in performance on learned tasks [9,58]. Finally, a recent study demonstrated that desynchronizing the circadian system by exposing animals to rapidly shifting LD cycles, interferes with recall (but not acquisition) of

spatial learning task [21]. Thus, it seems likely that learning and memory function, like most physiological processes, is subject to modulatory regulation by the circadian system.

4.1. Underlying mechanisms

The mechanisms that underlie the circadian modulation of learning and memory function are unknown. Many physiological and neurochemical processes in the body such as hormone secretion, cellular communication, and even gene transcription show daily variation. Therefore, there is a large list of possible processes that could underlie the circadian modulation of learning. It is worth noting that some of the hormones whose secretion is modulated by the circadian system have already been implicated in learning and memory. For example, secretion of corticosteroids rises to a maximum at or just before the time of waking and remains elevated during an animal's active period. Thus, in rodents the level of circulating corticosterone is low during the day and high at night and also during periods of stress. Diurnal variations in stress may explain differences in acquisition and recall in mice trained and tested at different times. Many studies have shown that corticosteroids can influence cognitive processes in both humans and animals in such a way that certain doses augment memory while very high concentrations can impair memory [57]. For example, injections of type II glucocorticoid antagonist was shown to impair the rat's ability to learn the Morris water maze when administered prior to or immediately after training. Furthermore, post-training infusion of glucocorticoid agonist into the dorsal hippocampus was found to enhance memory (for a review see Ref. [44]). Pugh et al. [53] reported that adrenolectomized (ADX) rats showed impairment of long-term, but not short-term, memory for context learning following fear-conditioning training. This effect was ameliorated if ADX rats were treated with glucocorticoids. Rudy and Pugh [56] reported that immediate post-training injection of the corticosterone into rats trained and tested on the fear-conditioning paradigm at certain times evoked stronger recall, as measured by greater freezing, compared to control animals. Additionally, there is a positive correlation between the footshock intensity during fear-conditioning training, fear-related freezing and plasma corticosterone levels [17,18,57]. Recently, Kelliher et al. [35] reported that nocturnal rodents expressed greater stressful behavior and had correspondingly larger increases in serum corticosterone compared to basal levels when tested during the day. Thus, one possible explanation for differences in the degree of acquisition and recall observed in our animals could be indicative of variations in the level of stress hormones during training at different times of the day.

Besides corticosterone, other hormones such as melatonin are secreted rhythmically and have been suggested to regulate learning functions. In the present study, we intentionally used the C-3H strain that secrete melatonin rhythmically and the C-57 strain that does not [22,30]. The strains performed very similarly on the measurements of recall and extinction. The data from the C-57 animals clearly indicate that rhythmic secretion of melatonin is not required for rhythms in learned behaviors. The only observable difference between the two strains of mice appears to be during acquisition. Even though both strains of animals trained in the day learn the fear-conditioning task faster than their counterparts trained at night, the profiles of the learning curves for C-3H and C-57 mice are quite different. In C-3H mice, freezing after the last training stimulus was significantly greater in animals trained in the day compared to those animals trained at night. However, in the C-57 mice significant differences in freezing between the day and night trained groups was only seen after the first training stimulus. To put it another way, high melatonin levels during the night could serve to inhibit learning the fear-conditioning task and thus be responsible for part of the rhythm in acquisition. This possibility will need to be specifically investigated in future studies.

Another possible explanation for the observed differences in the rate of acquisition could be circadian modulation of the cellular pathways in the amygdala and hippocampus that may be independent of hormonal regulation. Many biological processes exhibit daily rhythms including NMDA receptor activated currents [15], levels of Ca^{2+} [14], cyclic nucleotides [26] and gene transcription including those of transcription factors such as immediate early genes like c-fos [60]. Many of these same processes have been implicated in the acquisition and expression of fear conditioning. For example, pharmacological studies have shown that NMDA receptor activation in the amygdala is necessary for the acquisition and expression of the fear-conditioning task [42]. More recent studies have shown that both pre-training and pre-testing injections of the NMDA antagonist 2-amino-5-phosphonovaleric acid (APV) inhibits acquisition and recall, respectively, of context or tone [38]. Thus, another possible explanation for the variation in acquisition may be due to circadian fluctuations of NMDA receptor number or receptor sensitivity in the hippocampus or amygdala. Consistent with the idea of circadian variation in cellular processes, diurnal variations have also been observed in the strength of synaptic plasticity. Harris and Teyler [31] observed that post-tetanus LTP was more robust in area CA1 of the hippocampal formation in slices prepared during the day, while in slices prepared at night post-tetanus LTP was more robust in the dentate gyrus. Similarly, a larger magnitude LTP was recorded in the

pyramidal cells of the CA1 in slices prepared during the day in comparison to slices prepared at night [54]. Although the proximate mechanisms by which the circadian system regulates fear conditioning are not known, there are a number of candidate mechanisms that can be explored in future studies.

5. Conclusion

The data presented here show that different aspects of memory, namely acquisition, recall and long-term extinction for simple associative memory in mice is modulated by the circadian system. Since learning and memory function is based on biological processes and most biological processes are rhythmic, it should not be surprising that circadian rhythms were seen in acquisition and recall of learned behaviors. It may be that these daily rhythms represent a bi-product or epi-phenomena of a temporal organization imposed on biological systems. We feel that it is more likely that these rhythms are adaptive and serve specific functions. Since time is a critical parameter of the environment, it would seem adaptive for organisms to use time as a variable in learning. For example, it would be beneficial for animals to associate certain times of the day or night with either a rewarding stimulus such as availability of food sources or aversive stimulus to warn of potential dangers such as the likely presence of predators. Regardless of the ultimate causes, understanding the mechanisms for this modulation may be important for the study of both circadian systems and learning and memory.

Acknowledgements

Supported by Whitehall Foundation grant # F98P15, NIH # HL64582 and MH59186. We are also grateful to Dr M.S. Fanselow (University of California, Los Angeles, USA) whose laboratory generously aided in the collection of the preliminary data upon which this study was based.

References

[1] Anagnostaras SG, Gale GD, Fanselow MS. Hippocampus and contextual fear conditioning: recent controversies and advances. Hippocampus 2001;11:8–17.
[2] Aschoff J. Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. Z Tierpsychol 1979;49:225–49.
[3] Barnes CA, McNaughton BL, Goddard GV, Douglas RM, Adamec K. Circadian rhythm of synaptic excitability in rat and monkeys CNS. Science 1977;197:91–2.
[4] Bechara A, Tranel D, Damasio H, Adolphs R, Rockland C, Damasio AR. Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. Science 1995;269:1115–8.
[5] Beling I. Uber das Zeitgedachtnis der Bienen. Zeitschrift fur vergleichende Physiologie 1929;9:259–338.
[6] Berman DE, Dudai Y. Memory extinction, learning anew, and learning the new: dissociations in the molecular machinery of learning in cortex. Science 2001;291:2417–9.
[7] Bialik RJ, Pappas BA, Roberts DC. Deficits in conditioned avoidance responding following adrenalectomy and central norepinephrine depletion are dependent on postsurgical recovery period and phase of the diurnal cycle. Behav Neurosci 1984;98:847–57.
[8] Biebach H, Falk H, Krebs JR. The effect of constant light and phase shifts on a learned time-place association in garden warblers (Sylvia borin): hourglass or circadian clock? J Biol Rhythms 1991;6:353–65.
[9] Biemans BAM, Van der Zee EA, Gerkema MP, Daan S. Circadian oscillations in memory function: correspondence to changes in muscarinic chloninergic receptor density in the rat suprachiasmatic nucleus? SRBR 2000.
[10] Bouton ME. Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. Psychol Bull 1993;114:80–99.
[11] Carobi C, Garinei G. Effect of capsaicin on learning, retention and extinction of spatial and active avoidance tasks in adult rats neonatally treated. Brain Res Cogn Brain Res 1995;2:221–7.
[12] Carr JA, Wilkie DM. Rats use an ordinal timer in a daily time-place learning task. J Exp Psychol: Anim Behav Processes 1997;23:232–47.
[13] Coll-Andreu M, Marti-Nicolovius M, Morgado-Bernal I. Facilitation of shuttle-box avoidance by the platform method: temporal effects. Physiol Behav 1991;49:1211–5.
[14] Colwell CS. Circadian modulation of calcium levels in cells in the suprachiasmatic nucleus. Eur J Neurosci 2000;12:571–6.
[15] Colwell CS. NMDA-evoked calcium transients and currents in the suprachiasmatic nucleus: gating by the circadian system. Eur J Neurosci 2001;13:1420–8.
[16] Corcoran KA, Maren S. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. J Neurosci 2001;21:1720–6.
[17] Cordero MI, Merino JJ, Sandi C. Correlational relationship between shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear conditioning. Behav Neurosci 1998;112:885–91.
[18] Cordero MI, Sandi C. A role for brain glucocorticoid receptors in contextual fear conditioning: dependence upon training intensity. Brain Res 1998;786:11–7.
[19] Daan S. Adaptive strategies in behavior, book adaptive strategies in behavior. New York and London: Plenum Press, 1984:275–98.
[20] Daan S. Learning and circadian behavior. J Biol Rhythms 2000;15:296–9.
[21] Devan BD, Goad EH, Petri HL, Antoniadis EA, Hong NS, Ko CH, et al. Circadian phase-shifted rats show normal acquisition but impaired long-term retention of place information in the water task. Neurobiol Learn Mem 2001;75:51–62.
[22] Ebihara S, Marks T, Hudson DJ, Menaker M. Genetic control of melatonin synthesis in the pineal gland of the mouse. Science 1986;231:491–3.
[23] Ebihara S, Tsuji K, Kondo K. Strain differences of the mouse's free-running circadian rhythm in continuous darkness. Physiol Behav 1978;20:795–9.
[24] Fanselow MS, Bolles RC. Naloxone and shock-elicited freezing in the rat. J Comp Physiol Psychol 1979;93:736–44.
[25] Fekete M, van Ree JM, Niesink RJ, de Wied D. Disrupting circadian rhythms in rats induces retrograde amnesia. Physiol Behav 1985;34:883–7.
[26] Ferreyra GA, Golombek DA. Rhythmicity of the cGMP-related signal transduction pathway in the mammalian circadian system. Am J Physiol Regul Integr Comp Physiol 2001;280:R1348–55.

[27] Frohardt RJ, Guarraci FA, Bouton ME. The effects of neurotoxic hippocampal lesions on two effects of context after fear extinction. Behav Neurosci 2000;114:227–40.
[28] Gaffan D. Dissociated effects of perirhinal cortex ablation, fornix transection and amygdalectomy: evidence for multiple memory systems in the primate temporal lobe. Exp Brain Res 1994;99:411–22.
[29] Ghiselli WB, Patton RA. Diurnal variation in performance of free-operant avoidance behavior of rats. Psychol Rep 1976;38:83–90.
[30] Goto M, Oshima I, Tomita T, Ebihara S. Melatonin content of the pineal gland in different mouse strains. J Pineal Res 1989;7:195–204.
[31] Harris KM, Teyler TJ. Age differences in a circadian influence on hippocampal LTP. Brain Res 1983;261:69–73.
[32] Hasher L, Zacks RT, Rahhal TA. Timing, instructions, and inhibitory control: some missing factors in the age and memory debate. Gerontology 1999;45:355–7.
[33] Hauber W, Bareiss A. Facilitative effects of an adenosine A1/A2 receptor blockade on spatial memory performance of rats: selective enhancement of reference memory retention during the light period. Behav Brain Res 2001;118:43–52.
[34] Holloway FA, Wansley RA. Multiple retention deficits at periodic intervals after active and passive avoidance learning. Behav Biol 1973;9:1–14.
[35] Kelliher P, Connor TJ, Harkin A, Sanchez C, Kelly JP, Leonard BE. Varying responses to the rat forced-swim test under diurnal and nocturnal conditions. Physiol Behav 2000;69:531–9.
[36] Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. Science 1992;256:675–7.
[37] LeDoux JE. Emotion circuits in the brain. Ann Rev Neurosci 2000;23:155–84.
[38] Lee HJ, Choi JS, Brown TH, Kim JJ. Amygdalar nmda receptors are critical for the expression of multiple conditioned fear responses. J Neurosci 2001;21:4116–24.
[39] Loudon AS, Semikhodskii AG, Crosthwaite SK. A brief history of circadian time. Trends in Genetics 2000;16:477–81.
[40] Marchant EG, Mistlberger RE. Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6j mice. Brain Res 1997;765:273–82.
[41] Maren S, Aharonov G, Fanselow MS. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav Brain Res 1997;88:261–74.
[42] Maren S, Aharonov G, Stote DL, Fanselow MS. N-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. Behav Neurosci 1996;110:1365–74.
[43] McDonald RJ, White NM. A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behav Neurosci 1993;107:3–22.
[44] McGaugh JL. Memory—a century of consolidation. Science 2000;287:248–51.
[45] McNay EC, Gold PE. Memory modulation across neural systems: intra-amygdala glucose reverses deficits caused by intraseptal morphine on a spatial task but not on an aversive task. J Neurosci 1998;18:3853–8.
[46] Mistlberger RE, de Groot MH, Bossert JM, Marchant EG. Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. Brain Res 1996;739:12–8.
[47] Monk TH, Buysse DJ, Reynolds CF III, Berga SL, Jarrett DB, Begley AE, et al. Circadian rhythms in human performance and mood under constant conditions. J Sleep Res 1997;6:9–18.
[48] Monk TH, Weitzman ED, Fookson JE, Moline ML. Circadian rhythms in human performance efficiency under free-running conditions. Chronobiologia 1984;11:343–54.
[49] Moore D, Siegfried D, Wilson R, Rankin MA. The influence of time of day on the foraging behavior of the honeybee, Apis mellifera. J Biol Rhythms 1989;4:305–25.
[50] Moore RY, Eichler VB. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 1972;42:201–6.
[51] Murray EA, Gaffan D. Removal of the amygdala plus subjacent cortex disrupts the retention of both intramodal and crossmodal associative memories in monkeys. Behav Neurosci 1994;108:494–500.
[52] Pagano RR, Lovely RH. Diurnal cycle and ACTH facilitation of shuttlebox avoidance. Physiol Behav 1972;8:721–3.
[53] Pugh CR, Tremblay D, Fleshner M, Rudy JW. A selective role for corticosterone in contextual-fear conditioning. Behav Neurosci 1997;111:503–11.
[54] Raghavan AV, Horowitz JM, Fuller CA. Diurnal modulation of long-term potentiation in the hamster hippocampal slice. Brain Res 1999;833:311–4.
[55] Reebs S. Time place learning in golden shiners (Pisces: Cyprinidae). Behav Processes 1996;253:253–62.
[56] Rudy JW, Pugh CR. Time of conditioning selectively influences contextual fear conditioning: further support for a multiple-memory systems view of fear conditioning. J Exp Psychol Anim Behav Process 1998;24:316–24.
[57] Sandi C. The role and mechanisms of action of glucocorticoid involvement in memory storage. Neural Plast 1998;6:41–52.
[58] Stephan FK, Kovacevic NS. Multiple retention deficit in passive avoidance in rats is eliminated by suprachiasmatic lesions. Behav Biol 1978;22:456–62.
[59] Stephan FK, Zucker I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci USA 1972;69:1583–6.
[60] Sumova A, Travnickova Z, Mikkelsen JD, Illnerova H. Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. Brain Res 1998;801:254–8.
[61] Ternes J. Resistance to extinction of a learned taste aversion varies with time of conditioning. Anim Learn Behav 1976;4:317–21.
[62] Wakamatsu H, Yoshinobu Y, Aida R, Moriya T, Akiyama M, Shibata S. Restricted-feeding-induced anticipatory activity rhythm is associated with a phase-shift of the expression of mPer1 and mPer2 mRNA in the cerebral cortex and hippocampus but not in the suprachiasmatic nucleus of mice. Eur J Neurosci 2001;13:1190–6.
[63] Wallenstein GV, Vago DR. Intrahippocampal scopolamine impairs both acquisition and consolidation of contextual fear conditioning. Neurobiol Learn Mem 2001;75:245–52.
[64] Weitzman ED, Moline ML, Czeisler CA, Zimmerman JC. Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. Neurobiol Aging 1982;3:299–309.
[65] Young MW. Life's 24-hour clock: molecular control of circadian rhythms in animal cells. Trends Biochem Sci 2000;25:601–6.