ELSEVIER

Behavioural Brain Research 114 (2000) 221–233

BEHAVIOURAL BRAIN RESEARCH

www.elsevier.com/locate/bbr

Circadian rhythms, aging and memory

E.A. Antoniadis *, C.H. Ko, M.R. Ralph, R.J. McDonald

Departments of Psychology and Zoology, University of Toronto, 100 St George Street, Toronto, Ont., Canada M5S 3G3

Received 10 August 1999; received in revised form 7 December 1999; accepted 7 December 1999

Abstract

In human beings and animal models, cognitive performance is often impaired in natural and experimental situations where circadian rhythms are disrupted. This includes a general decline in cognitive ability and fragmentation of behavioural rhythms in the aging population of numerous species. There is some evidence that rhythm disruption may lead directly to cognitive impairment; however, this causal link has not been made for effects due to aging. We have tested this link by examining rhythms and performance on contextual conditioning with the conditioned place preference task, in elderly, age-matched hamsters. Young healthy hamsters developed a preference for a context that is paired with the opportunity to engage in wheel-running (experiment 1). Aged animals with consolidated locomotor rhythms developed similar degrees of preference, whereas the age-matched hamsters with fragmented rhythms did not (experiment 2). The degree of preference was also correlated with activity amplitude. These results support the notion that age-related rhythm fragmentation contributes to the age-related memory decline.

Keywords: Aging; Circadian rhythms; Context conditioning; Hamster; Suprachiasmatic nucleus; Memory

1. Introduction

A decline in cognitive ability accompanying aging has been documented in many species [31]. In addition, numerous physiological, neurochemical and hormonal changes occur in aging organisms, but their direct contributions, if any, to non-pathological cognitive decline are not well known.

One of the characteristics of aging in many species including humans, is the breakdown of circadian rhythms in physiology and behaviour [12,14,15, 17,52,62]. In mammals, the age-dependent disorganization of overt rhythmicity appears to be due directly to a functional decay of the central circadian pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus [17,43,51,67,65]. The pacemaker's decay is not only apparent in the changes of amplitude in circadian rhythms, but also in the altered responsiveness of the clock to environmental stimulation [62]. Reductions in cell number and volume in the nucleus have been reported to be associated with aging [60], and it has been shown recently that age-related rhythm fragmentation can be reversed by transplantation of fetal or culture SCN cells [25,47].

The broad significance of circadian organization is indicated by the relationship between rhythm amplitude and longevity [25]. Experimental fragmentation of circadian rhythms, produced by a phase-shift of the illumination cycle, has been associated with memory decrements in rodents, non-human primates and human beings [1,5,24,40,52,57,61], supporting the notion that circadian rhythms play an important role in the processing and retention of information. This association raises the question of whether the age-related degradation of circadian rhythms contributes directly to the memory decline observed in aged populations.

Studies of circadian rhythms in mammals often use the golden hamster because of the high accuracy and precision of its locomotor rhythm [37]. Expressed in the form of wheel-running in the laboratory setting, this form of locomotion allows the phase of the pacemaker to be estimated to within a few minutes each day. Moreover, for hamsters entrained to an LD 14:10 cycle most of the activity is performed within the first 3–5 h following dark onset. The predictable pattern of daily

* Corresponding author. Tel.: +1-416-9781708; fax: +1-416-9784811.

E-mail address: elena@spych.utoronto.ca (E.A. Antoniadis).

0166-4328/00/$ - see front matter
PII: S0166-4328(00)00145-5

locomotion in the hamster [41] allows the behavioural detection of changes in the physiology of the circadian pacemaker that occur with aging, environmental stress and season. While the majority of studies of learning and memory in rodents use rats and mice, hamsters have been shown to have similar mnemonic capabilities [26,54]. Of particular relevance to our studies are demonstrations of learning associated with exploratory behaviour and reward seeking, since these are likely to be closely regulated by the circadian system [41].

In the present studies we used the conditioned place preference task (CPP) to examine the relationship between rhythm integrity and the ability to form cognitive associations. The CPP is a powerful paradigm for examining the rewarding properties of stimuli and also serves to assess the subject's mnemonic abilities in associating these appetitive aspects of the event and the neutral cues of the surrounding environment [10,66]. This form of learning is referred to as context conditioning. In the CPP task, animals are exposed to a pair of neutral environments, one of which in addition contains a suspected reward stimulus. If the stimulus or event is successful in activating the neural substrates of reward, the animal develops a preference, that is approaches and maintains contact with the environment or context in which the stimulus was experienced, in the absence of the reward [66]. Peripherally administered addictive drugs like amphetamine, cocaine and morphine can produce CPPs [11,23,30]. CPPs have also been produced with natural rewards like food [33], sucrose [9] and sexual partners [50]. The CPP task has been used in the hamster, to demonstrate that amphetamine is rewarding for this species [54]. In addition, female hamsters acquire a conditioned place preference for an environment that has been paired with sexual and aggressive encounters [26].

In the present series of experiments we have used the running wheel as the rewarding feature in a conditioned place preference task. Other conditioning experiments have demonstrated that wheel-running itself is a reinforcer [6,7]. In experiment 1, we determined whether young adult hamsters can acquire and retain a conditioned place preference for a context that has been paired previously with the opportunity to engage in wheel-running. In experiment 2, we asked whether the natural deterioration of circadian rhythms that occurs with aging is correlated with an impairment in context conditioning. In experiment 2, hamsters were age-matched and differed only in the quality of their circadian rhythms. If the breakdown of circadian rhythms predicts mnemonic performance on CPP, it will be indicative of a correlation between the two factors.

2. Experiment 1

The purpose of this experiment was to examine whether hamsters can form associations between neutral contextual stimuli and a rewarding event (wheel-running). The expression of a conditioned place preference would be informative with respect to the learning and memory capacities of hamsters.

2.1. Method

2.1.1. Subjects

Sixteen male golden hamsters (*Mesocricetus auratus* outbred) 45–60 days old, weighing 100–120 g were raised in our breeding colony at the University of Toronto. Throughout the experiment, they were housed individually in propylene cages (22 cm wide × 40 cm long × 20 cm high) and were given free access to water and food. All cages were kept inside a light-tight ventilated box and illumination was provided from an overhead GE 'Vitalite' Cool White fluorescent tube which provided 90–100 lux at the floor of each cage. The hamsters were kept in an LD 14:10 cycle.

2.1.2. Apparatus

The design of the apparatus is depicted in Fig. 1. Two context chambers were used that differed on three dimensions: colour, shape and odour. One context was a black triangle chamber measuring 61 cm × 61 cm base and depth of 30 cm high; and the other context was a white square chamber measuring 41 cm × 41 cm base and height of 20 cm. Both boxes contained a small plastic cylinder (pill bottle) that was mounted on one of the walls of the chamber. On each training day, a drop of each odorant, serving as the olfactory cue, was placed on a cotton ball and inserted within the bottle. Iso-amyl acetate served as the olfactory cue with the black triangle and eucalyptus served as the olfactory cue with the white square. The two chambers were connected during pre-exposure and preference by an alley (16.5 cm long × 11 cm wide × 11 cm high). The entire structure was placed on a Plexiglas table that was 100 cm above the floor. A mirror (91 cm long × 61 cm wide), inclined by 45°, was placed on the floor of the testing room providing the experimenter with a non-intrusive view of the chambers. A video camera was placed two feet in front of the mirror for recording of all phases of the experiment. A stainless steel running wheel, (17 cm diameter) mounted on a plexiglass frame was used during the conditioning phases of the experiment as the rewarding stimulus.

2.2. Experimental procedure

2.2.1. Context conditioning

Animals were removed from the light-tight boxes half an hour before projected activity onset. In this way, all individuals were trained and tested at approximately the same circadian time. Activity onset at circadian time 12 (CT 12) was chosen because these animals are

highly motivated to use a wheel at this time [15], thus we assume that the potential for the wheel to be rewarding is also high. In addition, animals were transferred to the training apparatus in the light, thus avoiding an unexpected dark-light transition. During pre-exposure, animals were placed in the alley and given free access to both chambers for a total duration of 10 min. No running wheel was placed in either of the chambers during this phase. This ensured context neutrality and provided some experience of both contexts.

For conditioning (8 days) animals were placed in one of the two contexts for thirty minutes and access to the other context was blocked. For half the animals (group A) the 'paired' context contained a metal running wheel and the 'unpaired' context contained no running wheel. Previous findings have demonstrated that rodents react to spatial change induced by object removal by renewing exploration of the entire apparatus and/or selectively investigating the location where the object was previously placed [45,58]. In the present experiment, the removal of the wheel from the chamber on the test day, following the conditioning phase, may be sufficient to induce exploration in the paired chamber. To assess the influence of this novelty, a second group of animals (group B), were trained in a situation where the paired chamber contained a rotating wheel, while the 'unpaired' chamber contained a blocked, non-rotating wheel. This treatment, allowed us to determine whether the time spent in the paired chamber for group A was a function of the rewarding properties of wheel-running or a function of the novelty induced by wheel removal from that same chamber.

The order in which the groups experienced each context was counterbalanced so that half the animals were confined in a paired context (black) on days 1, 3, 5, 7 and confined in an unpaired context (white) on days 2, 4, 6, 8. The other half of the group was confined to a paired context (white) on days 1, 3, 5, 7, and confined to an unpaired context (black) on day 2, 4, 6, 8. During the training sessions, wheel-running time was video taped and scored by the experimenter. For this experiment we did not record wheel-running behaviour in the home cages but relied on an extended exposure to the LD schedule to ensure entrainment for all animals.

On test day hamsters were placed individually in the alley and given free access to both chambers. No wheel was present in either of the contexts. Dwell time was accumulated when both forepaws were past the threshold of the doorway into one of the chambers but not when both forepaws were past the threshold of the doorway into the alley. Time spent in the paired context served as a measure of appetitive context learning.

2.3. Results

The mean amount of time spent in the two contexts (white square and black triangle) during the pre-exposure phase was the same for both groups (Fig. 2A). A 2×2 ANOVA (group and location) on the time spent in both chambers revealed a non-significant group effect, [$F\ (1, 14) = 0.20$, $P > 0.05$], a non-significant location effect, [$F\ (1, 14) = 0.21$, $P > 0.05$], and a non-significant group x location interaction, [$F\ (1, 14) = 0.002$, $P > 0.05$]. Planned comparisons revealed that

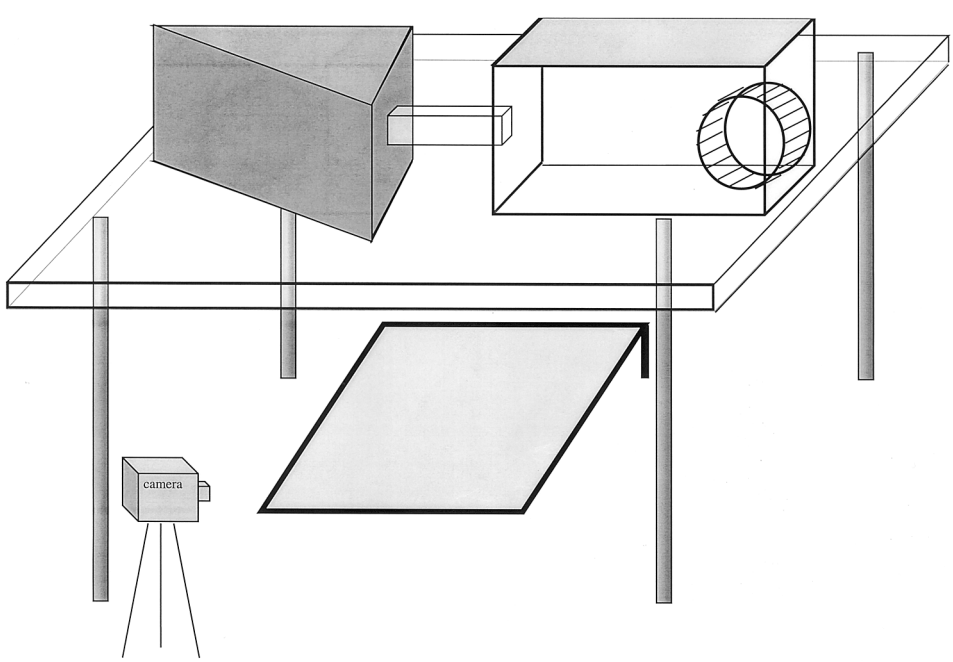

Fig. 1. Design of the apparatus used for experiment 1 and experiment 2.

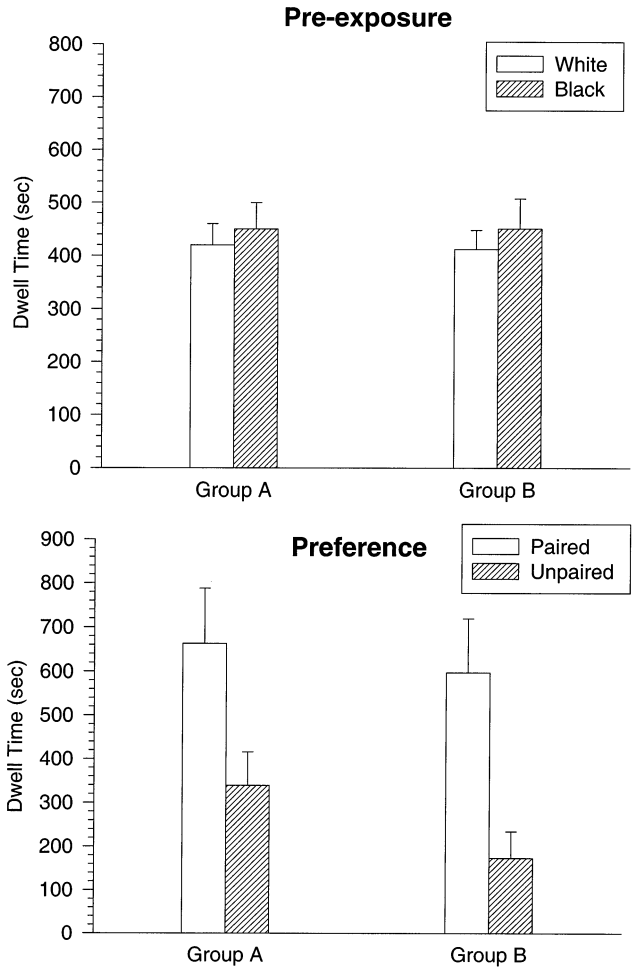

Fig. 2. Top: mean amount of time spent by group A and group B in the black and white chamber during pre-exposure of experiment 1. Bottom: mean amount of time spent in the paired and unpaired context on preference test of experiment 1.

there was no significant difference in the amount of time spent in each chamber, [group A: $F(1, 14) = 0.08$, $P > 0.05$]; [group B: $F(1, 14) = 0.12$, $P > 0.05$]. This lack of preference for any intrinsic features of the chambers strongly demonstrates that this is an unbiased CPP procedure for the hamster [10]. On test day both groups spent a significantly greater time in the paired context. A 2×2 ANOVA (group x location) on time spent in the paired and unpaired chamber revealed a non-significant group effect, [$F(1, 14) = 1.13$, $P > 0.05$], a significant location effect, [$F(1, 14) = 16.76$, $P < 0.05$], and a non-significant group x location interaction, [$F(1, 14) = 0.29$, $P > 0.05$]. Planned comparisons revealed that both groups spent significantly more time in the paired chamber, [group A: $F(1, 14) = 6.29$, $P < 0.05$]; [group B: $F(1, 14) = 10.76$, $P < 0.05$]. These results indicate that both groups spent more time in the paired chamber and that there was no difference between the groups. Taken together, these results suggest that hamsters showed a preference for the chamber paired with the opportunity to wheel-run and that the preference expressed in group A was due to the rewarding properties of wheel-running and not to the novelty induced by wheel removal.

For the conditioning days the amount of time spent running on the wheel for each animal was recorded, and examined in relation to the individual amount of preference exhibited on the test day. There was no significant correlation between the amount of time spent running and the amount of preference shown on the test day ($P > 0.05$).

2.4. Discussion

This experiment clearly showed that both groups of hamsters spent more time in a context paired previously with the opportunity to engage in wheel-running. The initial lack of preference for any of the chambers during pre-exposure in combination with a preference for the paired context during testing, strongly suggests that it is only subsequent to its pairing with a running wheel that the previously neutral context acquired the ability to attract the animal. The potential novelty that might be introduced by the absence of the wheel on test day does not account for the development of the preference. Given that both chambers contained wheels during conditioning in group B and that both contexts were rendered novel during testing, if time spent in the paired chamber was a reaction to novelty, we would expect hamsters to spend as much time in the paired as in the unpaired chamber. This treatment of a blocked wheel for half the subjects allowed us to remove the confound of novelty-induced exploration for group A, enabling us to assert that the rewarding properties of wheel-running attracted the animals and not the novelty induced by wheel removal. Moreover, these results show that the potential for operating the wheel introduced a greater preference than the mere presence of the wheel itself. These results demonstrate that both groups had the mnemonic ability to acquire and retain an association between the rewarding event of wheel-running and the contextual cues that surrounded it. The present experiment was adjusted to be ecologically significant for the hamster by allowing it to engage in locomotor activity at the time when locomotion is at its peak. The development of this paradigm will give us the opportunity to study the effects that circadian rhythms may exert on this type of context conditioning.

3. Experiment 2

The goal of experiment 2 was to examine the relationship between context conditioning and the natural degradation of circadian organization that occurs with

age. Accordingly, we examined and compared the mnemonic abilities of two groups of age-matched aged hamsters whose selection was based solely on the pattern of the individual's circadian locomotor rhythms. Locomotion has a precise daily onset in the natural environment [37], and is measured as wheel-running activity in the artificial environment of the laboratory [2,15,44]. A reduction in the amount of activity and fragmentation of the cyclic pattern is a well-known characteristic of the aging circadian system [62]. However, in previous studies we have found that the rate of decline and chronological age at which it occurs is peculiar to the individual so that in middle aged animals (9–12 months) there is a wide rage of activity patterns from the highly fragmented to the highly consolidated.

3.1. Method

Hamsters of the same chronological age (9–12 months) were trained and tested on the CPP paradigm.

3.1.1. Subjects

Eighteen male golden hamsters (*Mesocricetus auratus* outbred), were raised to 12 months of age at the University of Toronto. Locomotor activity was recorded as wheel-running behaviour. Wheel turns were registered using a microswitch mounted to the outside of the cage that was closed once per turn. Activity (wheel-running) was monitored continuously using Dataquest III (Minimitter CO., Sunriver, OR). Wheel turns were accumulated into 6-min bins. The data were analyzed subsequently by a computer program (Ez-paste) written for this purpose at the NSF Center for Biological Timing, University of Virginia. Following CPP training and testing, animals were rank-ordered on the basis of their prior activity pattern. This procedure was performed by an experimenter who was blind to the learning performance of each animal but who was familiar with the range of activity patterns found in aged hamsters. A continuum of activity patterns was obtained from the highly consolidated to the highly fragmented. For analysis, the eight most consolidated and eight most fragmented were compared for their performance on the CPP task. The middle two animals were not included in the analysis.

3.1.2. Apparatus

The same apparatus was used as in experiment 1.

3.2. Experimental procedure

Same procedure as in experiment 1 but an additional test of motivation and mobility was performed. To test primary motivation and mobility in aged hamsters, we gave each animal access to an estrous female in an open field chamber measuring 24 × 24 in. base and height of 24 in. for a maximum of 10 min. The time taken to mount the female was scored for each hamster and used as a measure of motivation (sexual behavior), sensory processing (somatosensory, visual, olfactory) as well as motor (movement initiation, walking, mounting) abilities.

3.3. Results

The range of circadian locomotor pattern displayed for the 16 aged animals is illustrated in Fig. 3A–F. Panel A shows a healthy locomotor rhythm reflected by a precise and predicable activity onset and consolidated running bouts. Panel F reflects an unhealthy rhythm illustrated by a fragmented pattern of locomotor activity with a variable time of activity onset. Panels B–E show the range of patterns that may be found in animals of this age. Actograms presented in Fig. 4 show the extended recordings for the four most fragmented (left) and most consolidated (right) patterns during the conditioning and testing periods. Both columns depict running activity 9 days prior to the beginning of the experiment and during the 10-day period of the experiment for a total of 19 days. The range of activity patterns and the degree of rhythmic integrity varied within the two groups. There is a continuum in the breakdown of rhythmicity, on the basis of which animals were rank-ordered and separated. Even if the actograms act to illustrate extreme samples from both groups, there is still variability in circadian disruption within these two groups. The numbers on the right of each activity record (actogram) in Figs. 3 and 4 are the total wheel turns per day.

3.3.1. Phase 1

No preference was shown for either of the two chambers during pre-exposure. All animals explored both contexts, and there was no consistent differential dwell time in either chamber (Fig. 5; left). A 2×2 ANOVA (group x location) on the data revealed a non-significant group effect, [$F(1, 14) = 1.94$, $P > 0.05$], a non-significant location effect, [$F(1, 14) = 0.08$, $P > 0.05$], and a non-significant group * location interaction, [$F(1, 14) = 0.60$, $P > 0.05$]. Planned comparisons revealed that there was no significant difference between the time spent in the black triangle and the white square, for the consolidated rhythms group [$F(1, 14) = 0.57$, $P > 0.05$], and for the fragmented rhythms group [$F(1, 14) = 0.12$, $P > 0.05$].

3.3.2. Phase 2

The time spent running during the conditioning phase is expressed as a percentage of the total time that the animal was exposed to the paired context (Fig.

5: middle). Hamsters with fragmented locomotion rhythms spent more time running during the conditioning phase than the hamsters with consolidated rhythms. However, a two-tailed t-test showed that the difference between the two groups was not significant ($T_7 = 1.56,\ P > 0.05$).

3.3.3. Phase 3

On test day, hamsters with consolidated rhythms spent considerably more time in the paired context versus the unpaired context (Fig. 5: right). The 2×2 ANOVA (group x location) revealed a non-significant group effect, [$F\ (1, 14) = 0.03,\ P > 0.05$], a non-sig-

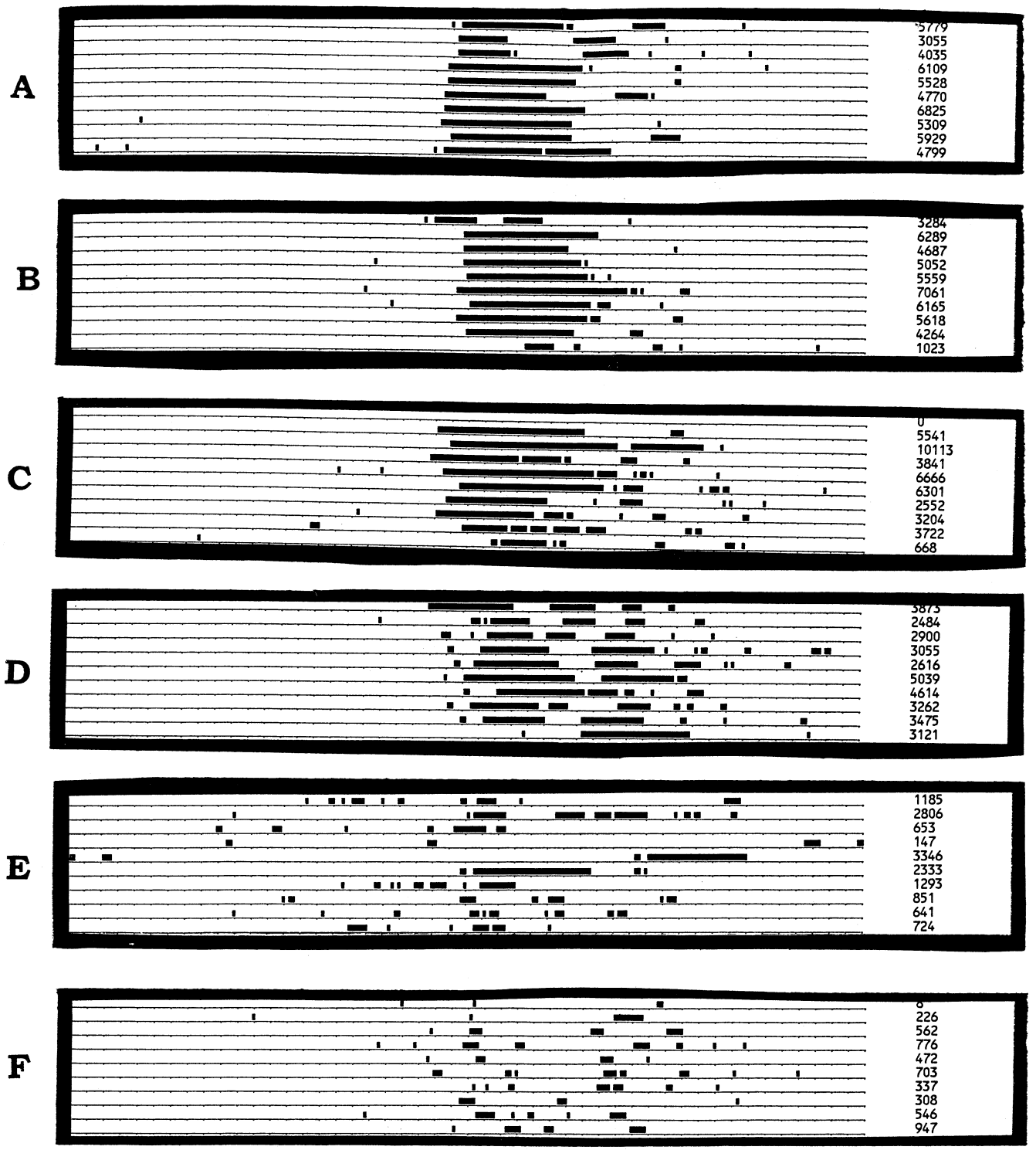

Fig. 3. Continuum of wheel-running records illustrating a gradual, age-induced breakdown of circadian locomotion rhythms.

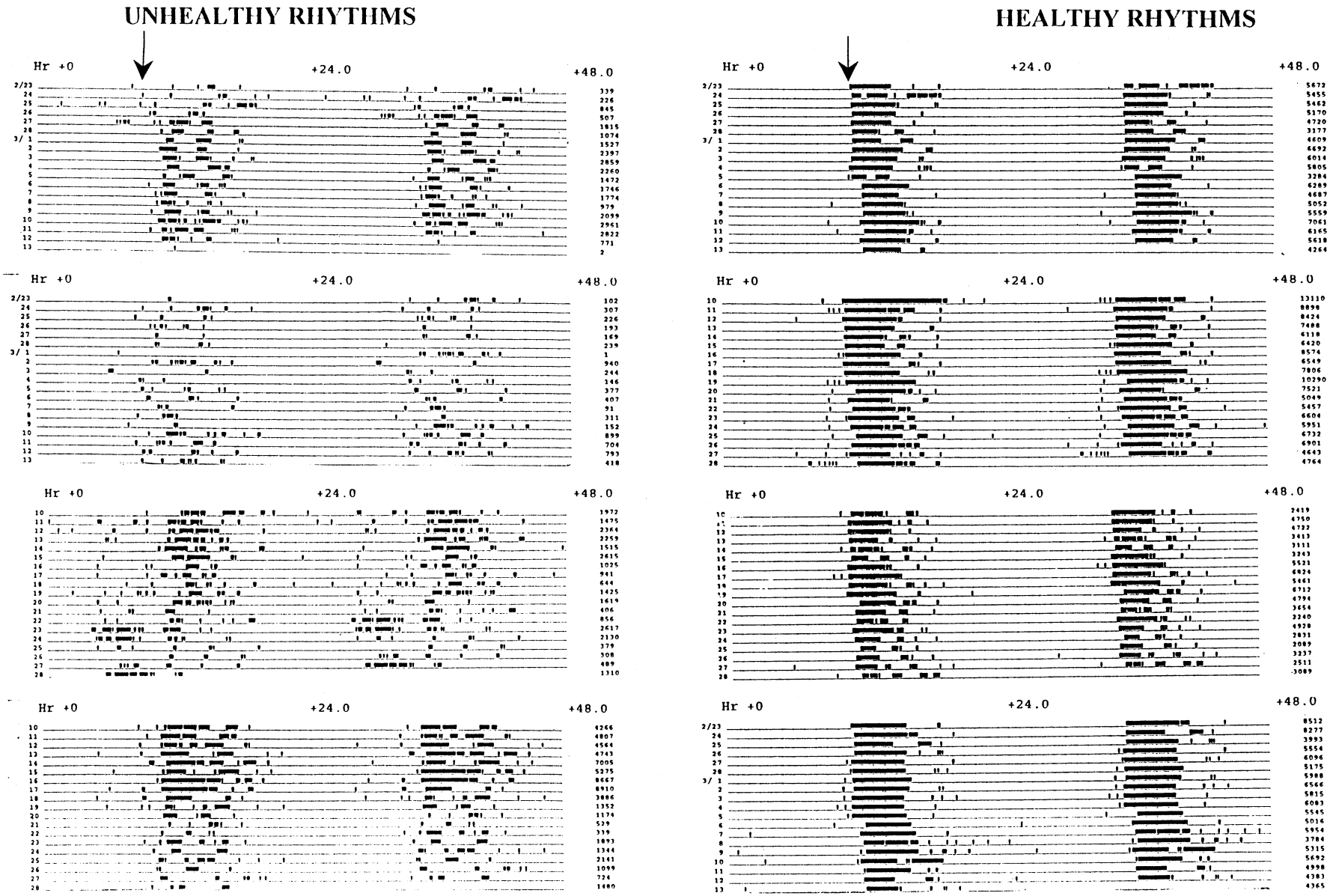

Fig. 4. Left column: running records of hamsters with unhealthy circadian rhythms for a total of 19 days; 9 days before the experiment and the 10-day period of the experiment. Right column: running records of hamsters with healthy circadian rhythms for the same period. Arrows indicate dark onset.

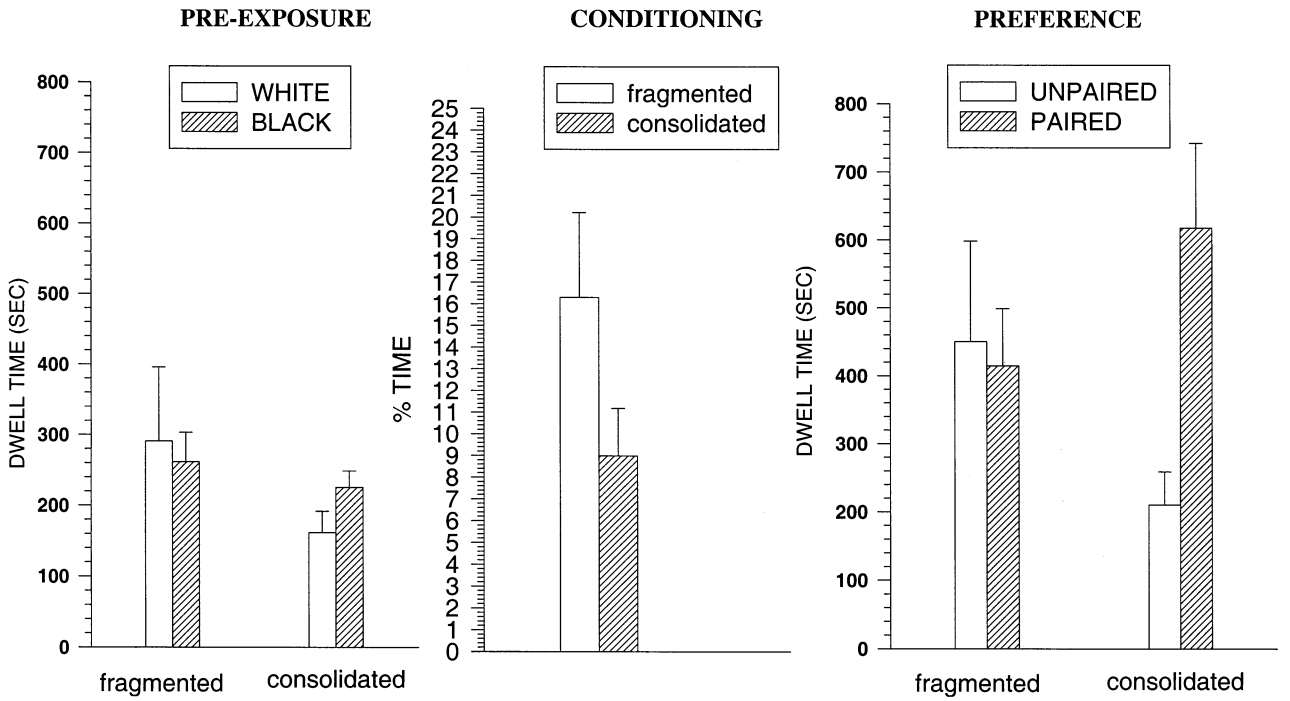

Fig. 5. Pre-exposure (left): mean amount of time spent by both groups in the black and white chamber during the pre-exposure phase of the experiment. Conditioning (middle): mean amount of time spent running during the conditioning phase for the fragmented and the consolidated rhythms group. It is expressed as the percent of total time in the paired chamber. Preference (right): mean amount of time spent by both groups in the paired and unpaired chamber during the preference test.

nificant location effect, [$F(1, 14) = 2.57$, $P > 0.05$], and a close to significant group * location interaction, [$F(1, 14) = 3.69$, $P = 0.07$]. Planned comparisons on each group revealed that the group with consolidated rhythms expressed a preference for the paired context, [$F(1, 14) = 6.22$, $P < 0.05$] but the group with fragmented rhythms did not, [$F(1, 14) = 0.05$, $P > 0.05$].

3.3.4. Test of motivation and mobility

To ensure that the impairment observed in the fragmented rhythms group is related to memory and not to motivational, sensory or motor deficits, we gave hamsters access to a female in her estrous cycle individually and assessed amount of time required to mount the female. As shown in Fig. 6, there was no difference between the two groups in the amount of time required to mount the female, [$F(1, 14) = 0.58$, $P > 0.05$]. This result suggests that the two groups did not differ in their level of motivation, or sensory and motor abilities. To further examine the relation between circadian rhythms and memory performance we developed a 'rhythm score' and a 'preference score'. The rhythm score reflects the amplitude of activity (in home cage) for each group and consists of the mean number of wheel turns during the last five days of the experiment. The preference score reflects the degree of preference for the paired context and is made up of the difference between the time spent in the paired and unpaired context (time paired-time unpaired). Fig. 7 (top) represents the mean activity amplitude for each group and illustrates that the group with consolidated rhythms has a significantly higher level of activity than the age-matched group with fragmented rhythms, [$F(1, 14) = 35.71$, $P < 0.05$]. The scatter diagram (Fig. 7: bottom)

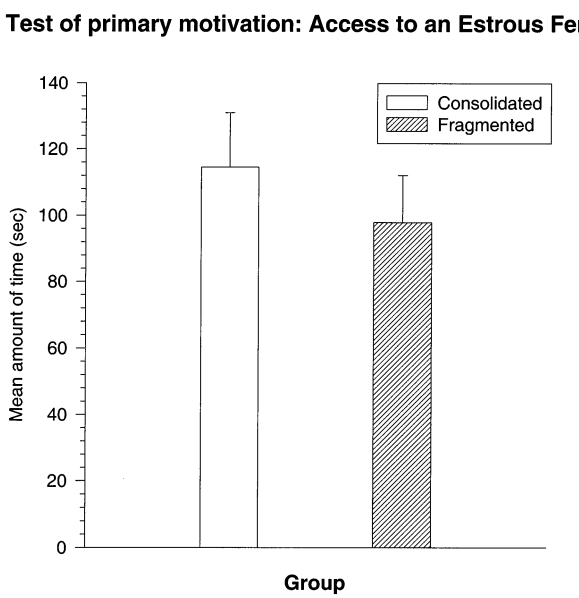

Fig. 6. Mean amount of time (sec) required to mount receptive female in estrous for hamsters with consolidated and fragmented rhythms.

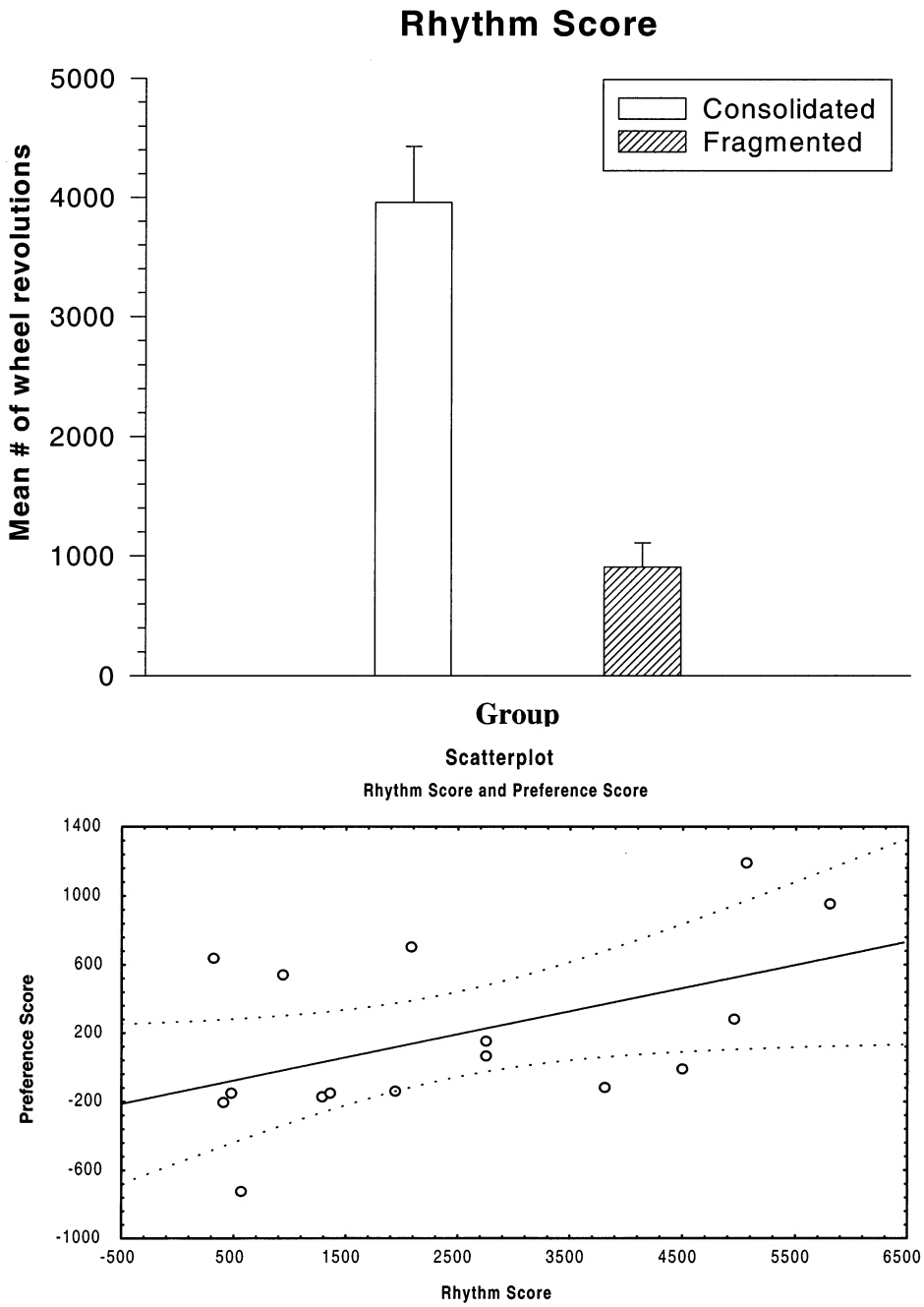

Fig. 7. Top: rhythm score — level of activity expressed in mean number of wheel turns in the home cage for the hamsters with fragmented rhythms and the ones with consolidated rhythms. Bottom: scatterplot illustrating relationship between rhythm score and preference score.

shows the relationship between rhythm amplitude and degree of preference. A regression analysis was performed with rhythm amplitude as the independent variable and degree of preference as the dependent variable. Results indicate that 25% of the variation in degree of preference can be accounted for by rhythm amplitude as a measure of circadian rhythms ($r^2 = 0.25$; Pearson's correlation coefficient: $r = 0.49$). There is a significant relationship between these two variables. [F (1, 14) = 4.66, $P < 0.05$)].

3.4. Discussion

Perhaps the most interesting and significant result from this series of experiments is that in hamsters of the same chronological age, the pattern of their daily locomotor behavior predicts whether or not context conditioning will occur. In experiment 1, young animals with robust locomotor rhythms expressed a preference for a context associated with wheel running. A significant relation between circadian rhythms and memory was

demonstrated in experiment 2, since older animals with consolidated circadian rhythms still developed a preference for a context associated with wheel-running, whereas their age-matched counterparts with fragmented rhythms did not. Running records indicated that hamsters with fragmented rhythms spent similar amounts of time running during conditioning compared to those hamsters of the same chronological age with consolidated rhythms. This unconditioned response data suggests that the lack of preference observed in the fragmented group was probably not due to a lack of motivation to run nor an inability to interact with the rewarding stimulus. This corroborates the observation from experiment 1 that there is no connection between the amount of wheel running and the degree of preference for the paired chamber. The activity records (Fig. 4) served to qualitatively depict the integrity of circadian rhythms for each group. The activity amplitude (mean number of wheel turns), extracted from those same records served to classify quantitatively the integrity of circadian rhythms for each group. Indeed, animals with consolidated circadian rhythms had a significantly higher level of activity than their age-matched counterparts with fragmented rhythms. Not only did the two groups differ significantly on this measure, but activity amplitude turned out to be highly predictive of memory performance, i.e. degree of preference for a context previously paired with reward. All of these results support the idea that the integrity of circadian rhythms predicts mnemonic performance on context conditioning.

The results from experiment 2 indicate that learning and memory deficits are not general consequences of the aging process but are defined by a subset of aged animals whose daily pattern of locomotor behaviour is fragmented and reduced in amplitude. As yet, it is unknown whether there is a causal relationship between rhythm dysfunction and cognitive impairment. However, together with a report of cognitive impairment following experimental rhythm disruption [16], these data suggest such a link. In other words, age-related clock dysfunction contributes to cognitive decline and hastens the demise of the animal [25].

This pattern of data provides a plausible explanation for the demonstration of individual differences in spatial abilities in aged rats [20]. Briefly, these experiments showed that age-matched rats were distinguished on the basis of their spatial abilities and separated into an 'impaired' and 'unimpaired' group. In relation to this finding, although the performance of young rats on a place finding task was bimodal early in training and unimodal with additional training, the performance of aged rats remained bimodal regardless of the amount of training [3,4].

A major question arising from the present experiments is what learning and memory system mediates this form of context conditioning in the hamster. In general, this type of context conditioning has been demonstrated repeatedly to be dependent on some structure or structures in the medial temporal lobe [18,23,33]. Most of these studies have shown that the amygdala is the critical learning and memory system necessary for appetitive context conditioning. Recent evidence suggests that factors specific to the conditioned place preference task play an important role in determining which learning and memory system is activated. In 1991, Hiroi and White [23] found that lesions of the lateral nucleus of the amygdala (LNA) blocked an amphetamine CPP but fornix lesions did not. In 1997, Olmstead and Franklin 39 reported a contrasting dissociation involving the same memory structures, the same learning and memory task but a different rewarding stimulus when morphine served as the reward. They showed that LNA lesions did not have an effect on the morphine CPP but fornix lesions attenuated the preference. In combination, these dissociations indicate that the rewarding stimulus can modulate the activation of memory systems given that the amphetamine CPP required an intact amygdala while the morphine CPP required the hippocampus. These results lead us to suspect that our task is hippocampal-based given that we have found that hamsters with amygdala damage show a CPP when wheel running is used as the reward (manuscript in preparation). This is an important point for understanding the present study because the pattern of learning and memory deficits found in aged humans, monkeys and rats consistently show that senescence affects the types of learning and memory mediated by structures in the medial temporal lobes (hippocampus, amygdala, and perirhinal cortex). Learning and memory tasks in which aged human and non-human animals show impairments include spatial/relational [19,48,64], contextual [42,68] and recognition memory tasks [46,49].

There is an important theory concerning memory formation in the hippocampus that provides insight as to the memory deterioration seen in the aged animals with fragmented rhythms. In 1989, Buzsaki's [8] two-stage theory of hippocampus suggests that any disruption or interference with theta or sharp wave activity (during slow wave sleep) will not only disrupt encoding or consolidation processes in the hippocampus but will also elicit abnormal firing states or patterns in the hippocampus. Initiation of abnormal firing rates in the hippocampus produces epileptic spikes that can produce neurotoxic and behavioral effects associated with the hippocampus [59]. It is tempting to suggest that these disruptive activity patterns in the hippocampus may be a direct cause of learning and memory deficits found in aged animals and also to be a co-factor in the onset of Alzheimer's disease in humans. This hypothesis is supported by evidence showing that low concentrations of beta-amyloid peptide increase neuronal

vulnerability to excitotoxic damage [22,27,32]. Furthermore, there is an increased incidence of epilepsy in aged populations [28] and in Alzheimer's disease patients [29].

Another line of evidence that is suggestive of a link between circadian dysfunction and age-related cognitive decline comes from various studies showing that exposure to high levels of stress in adult rodents leads to elevated glucocorticoid levels. The relationship between these findings and the present study is that it has been shown that increased glucocorticoids levels are also elevated in aged rodents and humans [13,63] who have associated circadian rhythms decline. High levels of glucocorticoids can have two effects on neurons in the hippocampus and related brain structures [34–36,53]. First, continuous exposure to glucocorticoids influence the rate of neuron loss in the aging hippocampus. Second, even under circumstances when glucocorticoids levels are too low to damage neurons, this hormone effects the ability of neurons to survive brain insults like seizures, hypoxia, neurotoxin exposure, or hypoglycemia.

An alternative explanation for the memory decrements observed in our study is the possibility of a general sensory, motor, or motivational decline in aged hamsters. These performance confounds in senescent rats have been tempered by several findings revealing selective memory deficits in some tasks while performance on other tasks remained comparable to that of young rats. For example, young and aged rats performed equally well on a triple platform escape task that required the learning of complex response patterns and on a multiple light discrimination box in which complex sets of cues had to be discriminated [55,56], but showed a deficit in their ability to learn a 14-choice point Stone maze [21]. Recent dissociations on memory tasks in aged animals also exemplify that the deficit resides in the mnemonic process and not the sensory, motor, or motivational aspects of the tasks [19,42,46,48,49,64,68]. In the present experiment, aged hamsters with consolidated and fragmented rhythms did not differ in their latency to mount the female, a result which is taken to indicate that motivation, sensory and motor abilities, are similar in both groups of hamsters.

In conclusion, the above synthesis and the results of the present experiments suggest an intriguing possibility. Circadian dysfunction associated with aging may create a deadly combination of seizures and high glucocorticoid levels in hippocampus that produces cell loss in the medial temporal lobes and associated cognitive impairment. These effects might be accelerated and exacerbated by genetic predisposition and associated beta-amyloid brain pathology like those found in Alzheimer's disease.

Acknowledgements

This work was funded by a Natural Sciences and Engineering Council of Canada (NSERC) grant awarded to R.J. McDonald and M.R. Ralph. We wish to thank Lisa Richardson and Andrew Gristock for excellent animal care.

References

[1] Aggleton JP, Blindt HS, Candy JM. Working memory in aged rats. Behav Neurosci 1989;103(5):975–83.
[2] Albers HE. Response of hamster circadian system to transitions between light and darkness. Am J Physiol 1989;250:R708–11.
[3] Barnes CA. Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. Journal of Comparative and Physiological Psychology 1979;93(1):74–104.
[4] Barnes CA, Suster MS, Shen J, McNaughton BL. Multistability of cognitive maps in the hippocampus of old rats. Lett Nat 1997;388:272–5.
[5] Bonnet MH. The effect of sleep fragmentation on sleep and performance in younger and older subjects. Neurobiol Aging 1989;10(1):21–5.
[6] Borer KT, Kaplan LR. Exercise-induced growth in golden hamsters: effects of age, weight, and activity level. Physiol Behav 1977;18(1):29–34.
[7] Borer KT. The nonhomeostatic motivation to run in the golden hamster. In: Morrison AR, editor. Changing Concepts in the Nervous System. New York: Academic Press, 1982:539–67.
[8] Buzsaki G. Two-stage model of memory trace formation: a role for 'noisy' brain states (review). Neuroscience 1989;31(3):551–70.
[9] Cador M, Robbins TW, Everitt BJ. Involvement of the amygdala in stimulus-reward associations: Interaction with the ventral striatum. Neuroscience 1989;30(1):77–86.
[10] Carr GD, Fibiger HC, Phillips AG. Conditioned place preference as a measure of drug Reward. In: Leibman JM, Cooper SJ, editors. The Neuropharmacological Basis of Reward. Oxford: Oxford University Press, 1989:264–319.
[11] Carr GD, White NM. Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. Life Sci 1983;3(25):2551–7.
[12] Coevorden AV, Mockel J, Laurent E, Kerkhofs M, L'Hermite-Baleriaux M, Decoster C, Neve P, Cauter EV. Neuroendocrine rhythms and sleep in aging men. Am J Physiol 1990;260:E651–61.
[13] Copinschi G, Van Cauter E. Effects of ageing on modulation of hormonal secretions by sleep and circadian rhythmicity. Horm Res 1995;43:20–4.
[14] Czeisler CA, Dumont M, Duffy JF, Steinberg JD, Richardson GS, Brown EN, Sanchez R., David, Rios C, Ronda JM. Association of sleep–wake habits in older people with changes in output of circadian pacemaker, Lancet 1992;340:933–36.
[15] Davis MC, Menaker M. Hamsters through time's window: temporal structure of hamster locomotor rhythmicity. Am J Physiol 1980;239:R149–55.
[16] Devan BD, Goad EH, Petri B, Antoniadis EA, Hong NS, Ko CH, Leblanc L, Lebovic SS, Lo Q, Ralph MR, McDonald RJ. Circadian rhythms and memory consolidation, Neurobiol Learn Mem (in press).
[17] Edgar DL. Sleep–wake circadian rhythms and aging: potential etiologies and relevance to age-related changes in integrated physiological systems. Neurobiol Aging 1994;15(4):499–501.

[18] Everitt BJ, Morris KA, O'Brien A, Robbins TW. The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience 1991;42(1):1–18.
[19] Gage FH, Kelly PA, Bjorklund A. Regional changes in brain glucose metabolism reflect cognitive impairments in aged rats. J Neurosci 1984;4(11):2856–65.
[20] Gallagher M, Burwell RD. Relationship of age-related decline across several behavioral domains. Neurobiol Aging 1989;10:691–708.
[21] Goodrick CL. Learning by mature-young and aged Wistar albino rats as a function of test complexity. J Gerontol 1972;27:353–7.
[22] Gray CW, Patel AJ. Neurodegeneration mediated by glutamate and beta-amyloid peptide: a comparison and possible interaction. Brain Res 1995;691(1–2):169–79.
[23] Hiroi N, White NM. The lateral nucleus of the amygdala mediates expression of the amphetamine conditioned place preference. J Neurosci 1991;11:2107–16.
[24] Holloway FA, Wansley RA. Multiple retention deficits at periodic intervals after active and passive avoidance learning. Behav Biol 1973;9(1):1–14.
[25] Hurd M, Ralph MR. The significance of circadian organization for longevity in the golden hamster. J Biol Rhythms 1998;13(5):430–6.
[26] Joppa M, Rowe RK, Meisel RL. Effects of serotonin 1A or 1B receptor antagonists on social aggression in male and female Syrian hamsters. Pharmacol Biochem Behav 1997;57(2):349–53.
[27] Koh JY, Yang LL, Cotman CW. Beta-amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. Brain Res 1990;533:315–20.
[28] Loiseau J, Singh A, Capps JL. Dissociation of memory and awareness in young and older adults. J Clin Exp Neuropsychol 1986;8:62–74.
[29] Mackenzie IRA, Miller LA. Senile plaques in temporal lobe epilepsy. Acta Neuropathol 1994;87:504–10.
[30] Mackey WB, van der Kooy D. Neuroleptics block the positive reinforcing effects of amphetamine but not morphine as measured by place conditioning. Pharmacol Biochem Behav 1985;22(1):101–5.
[31] Martinez JL Jr, Schulteis G, Janak PH, Weinberger SB. Behavioral Assessment of forgetting in aged rodents and its relationship to peripheral sympathetic function. Neurobiology of Aging 1989;9:697–708.
[32] Mattson MP, Cheng B, Davis D, Bryant K, Lieberburg I, Rydel RE. Beta-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J Neurosci 1992;12:376–89.
[33] McDonald RJ, White NM. A triple dissociation of memory systems: hippocampus, amygdala and dorsal striatum. Behav Neurosci 1993;107(1):3–22.
[34] McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol 1995;5:205–16.
[35] McIntosh LJ, Sapolsky RM. Glucocorticoids may enhance oxygen radical-mediated neurotoxicity. Neurotoxicology 1996;17(3–4):873–82.
[36] Meaney MJ, Aitken DH, Van Berkel C, Bhatnagar S, Sapolsky RM. Effect of neonatal handling on age-related impairments associated with the hippocampus. Science 1988;239:766–8.
[37] Morin LP. Biological rhythms. In: Siegel HI, editor. The Hamster: Reproduction and Behavior. New York: Plenium Press, 1985:323–61.
[38] Oler JA, Markus EJ. Age-related deficits on the radial maze and in fear conditioning: Hippocampal processing and consolidation. Hippocampus 1998;8:402–15.
[39] Olmstead MC, Franklin KBJ. Development of a conditioned place preference to morphine: effects of lesions of various sites. Behav Neurosci 1997;111:1313–23.
[40] Ordy JM, Thomas GJ, Volpe BT, Dunlap WP, Colombo PM. An animal model of human-type memory loss based on aging, lesion, forebrain ischemia and during studies with the rat. Neurobiol Aging 1988;9(5–6):667–83.
[41] Osiel S, Golombek DA, Ralph MR. Conservation of locomotor behavior in the golden hamster: effects of light cycle and a circadian period mutation. Physiol Behav 1998;65(1):123–31.
[42] Park DC, Cherry KE, Smith AD, Lafronza VN. Effects of distinctive context on memory for objects and their locations in young and elderly adults. Psychol Aging 1990;5:250–5.
[43] Pittendrigh CS, Daan S. Circadian oscillations in rodents: a systematic increase of their frequency with age. Science 1974;186:548–50.
[44] Pittendrigh CS, Daan S. A functional analysis of circadian pacemakers in nocturnal rodents. I. The stability and lability of spontaneous frequency. J Comp Physiol 1976;106:223–52.
[45] Poucet B. Object exploration, habituation, and response to a spatial change in rats following septal or medial frontal cortical damage. Behav Neurosci 1989;103(5):1009–16.
[46] Presty SK, Bachevalier J, Walker LC, Struble RG, Price DL, Mishkin M, Cork LC. Age differences in recognition memory of the rhesus monkey (*Macaca mulatta*). Neurobiol Aging 1987;8:435–40.
[47] Ralph MR. Suprachiasmatic nucleus transplant studies using the tau mutation in golden hamsters. In: Klein DC, Moore RY, Reppert SM, editors. Suprachiasmatic Nucleus: The Mind's Clock. Oxford: Oxford University Press, 1991:341–8.
[48] Rapp PR, Kansky MT, Eichenbaum H. Learning and memory for hierarchical relationships in the monkey: effects of aging. Behav Neurosci 1996;110(5):887–97.
[49] Rapp PR, Amaral DG. Evidence for task-dependent memory dysfunction in the aged monkey. J Neurosci 1989;9:3568–76.
[50] Robbins TW, Everitt BJ. Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 1996;6(2):228–36.
[51] Rosenberg R, Zee P, Turek FW. Phase response curve to light in young and old hamsters. Am J Physiol 1991;261:R491–5.
[52] Sandyk R, Anninos PA, Tsagas N. Age-related disruption of circadian rhythms: possible relationship to memory impairment and implications for therapy with magnetic fields. Int J Neurosci 1991;59(4):259–62.
[53] Sapolsky RM. Why stress is bad for your brain. Science 1996;273:749–50.
[54] Schnur P, Morrell J. Morphine conditioned place preference in the hamster. Pharmacol Biochem Behav 1990;37(2):383–5.
[55] Stone CP. The age factor in animal learning. I. Rats in the problem box and the maze. Genet Psychol Monogr 1929;5(1):1–130.
[56] Stone CP. The age factor in animal learning. II. Rats on a multiple light discrimination box and a difficult maze. Genet Psychol Monogr 1929;6:125–202.
[57] Stone WS. Sleep and aging in animals. Relationships with circadian rhythms and memory (review). Clin Geriatr Med 1989;5(2):363–79.
[58] Sutherland RJ. The navigating hippocampus: an individual medley of movement, space and memory. In: Buzaski G, Vanderwolf CH, editors. Electical Activity of the Archicortex. Budapest: Akademiai Kiado, 1985:255–79.
[59] Sutherland RJ, Leung LS, Weisend MP, Schlife J, McDonald RJ. An evaluation of the effect of partial hippocampal kindling on place navigation by rats in the Morris water task. Psychobiology 1997;25:126–32.
[60] Swaab DF, Fliers E, Partiman T. The suprachiasmatic nucleus of the human brain in relation to sex, age, and senile dementia. Brain Res 1985;342:37–44.

[61] Tapp WN, Holloway FA. Phase shifting circadian rhythms produces retrograde amnesia. Science 1981;211(4486):1056–8.
[62] Turek FW, Penev P, Zhang Y, van Reeth O, Zee P. Effects of age on the circadian system. Neurosci Biobehav Rev 1995;19(1):53–8.
[63] Van Coevorden A, Mockel P, Laurent E, Kerkhofs M, L'Hermite-Baleriaux M, Decoster C, Neve P, Van Cauter E. Neuroendocrine rhythms and sleep in aging men. Am J Physiol 1991;260:E651–61.
[64] Weber R, Brown I, Weldon J. Cognitive maps of environmental knowledge and preference in nursing patients. Exp Aging Res 1978;4:157–74.
[65] Weilard NG, Wise PM. Aging progressively decreases and alters the diurnal rhythms of alpha-1 adrenergic receptors in selected hypothalamic regions. Endocrinology 1990;126:2392–7.
[66] White NM. A functional hypothesis concerning the striatal matrix and patches: mediation of S–R memory and reward. Life Sci 1989;45(21):1943–57.
[67] Wise PM, Cohen IR, Weilard NG, London DE. Aging alters the circadian rhythm of glucose utilization in the suprachiasmatic nucleus. Proc Natl Acad Sci USA 1988;85:5305–9.
[68] Zelinski BM, Light LL. Young and older adults' use of context and spatial memory. Psychol Aging 1988;3:99–101.