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MEMORY RETENTION IN WILD-TYPE AND TAU MUTANT SYRIAN HAMSTERS

by

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Summary

Rats are known to display a temporary deficit in memory function 6 h after training on a learning task, a phenomenon known as the 'Kamin effect'. Later studies showed that maximal retrieval recurs in 24 h intervals after a single training and implied the role of the circadian clock in the suppression of memory retrieval at non-24 h intervals. This study aimed to investigate this further by analysing retention deficits following passive avoidance training in the Syrian hamster. The availability of hamsters carrying the *tau* mutation was exploited to address the role of the circadian system in periodic retention deficits. It was expected that *tau* mutant hamsters with an endogenous circadian period of approximately 20 h would have a high retention score at 20 h after training. Surprisingly, deficits in retention were found at 12, 18, 24, and 36 h after training in wild-type hamsters with best performance at 30 h after training. *Tau* mutant hamsters had significant deficits in memory retention at 20, 24, and 30 h, and no clear periodicity in retention could be observed. Step-through latency scores for mutant hamsters were low at all times except training-testing intervals of 0.25 and 6 h. These results demonstrate the absence of clear memory deficit oscillations in both wild-type and mutant hamsters, and may suggest in particular a long-term memory deficit in *tau* mutant hamsters.

Keywords: circadian, memory retention, passive avoidance, Syrian hamster, *tau* mutation.

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Introduction

In 1957, Leon Kamin reported that rats exposed to a single-training passive avoidance task showed a sharp temporary deficit in performance on a trial 1 and 6 h after training, but perfect retention when tested 24 h after training (Kamin, 1957). The temporary suppression became known as the 'Kamin effect' (Denny, 1958) and was usually interpreted as a transient memory dysfunction associated with the transfer from short-term to long-term memory. Later studies by Holloway & Wansley (1973a) demonstrated that both the 6 h deficit and the 24 h maximum in retention recur periodically in 24 h intervals. Such 24 h oscillations were observed in several learning paradigms using passive avoidance (Holloway & Wansley, 1973a), active avoidance (Holloway & Wansley, 1973b) and appetitive learning tasks (Wansley & Holloway, 1975; Hunsicker & Mellgren, 1977).

There are indications that the periodic memory deficits may involve the circadian system. Lesions of the primary circadian oscillator, the suprachiasmatic nucleus (Ralph *et al.*, 1990), eliminated the deficits in memory retention in a passive avoidance test: SCN-lesioned rats tested at 18 and 30 h after training performed indistinguishably from rats tested 24 h after training (Stephan & Kovacevic, 1978). A shift of the light-dark cycle after training impaired rats' performance both in passive and active avoidance tasks (Davies *et al.*, 1974; Tapp & Holloway, 1981). Such shifts in the light schedule did not affect exploratory behaviour or social activities and appeared specific for memory (Fekete *et al.*, 1985).

All these studies were performed in rats, and it is of interest whether the phenomenon of multiple retention deficits also occurs in other rodent species. Establishing such deficits in hamsters would allow us to evaluate the hypothesis that the circadian system is involved by performing tests in mutants with modified circadian period. Thus, the first aim of this study was to determine whether the periodic deficits in memory retention occur in the Syrian hamster. Secondly, *tau* mutant Syrian hamsters were tested to evaluate the possible role of the circadian system in memory retrieval. The *tau* mutation is a single gene, semi-dominant mutation, which shortens the circadian period by 4 h in homozygous mutants (Ralph & Menaker, 1988; Lowrey *et al.*, 2000) compared with the period of circa 24 h in wild-type hamsters. If high retention at 24 h in wild-type hamsters reflects the endogenous circadian periodicity, it might be expected that *tau* mutant hamsters perform better

20 h after training than after 24 h. Evaluation of these predictions should enhance our functional understanding of the circadian system. The exploitation of the circadian system in memory function might contribute to the adaptive significance of endogenous circadian timing (Daan, 1981).

Material and methods

Male Syrian hamsters (*Mesocricetus auratus*) were obtained from breeding stock at the Zoological Laboratory, Haren, The Netherlands. Wild-type (*tau* +/+) and homozygous *tau* mutant (*tau* -/-) were obtained from crossings between heterozygous parents (Oklejewicz *et al.*, 1997). After weaning the circadian phenotype of each offspring was determined by monitoring locomotor activity in a constant dimly illuminated room (red light <0.5 lux).

All experimental hamsters (age 5-6 months) were housed individually in Plexiglas cages (40 × 24 × 15 cm) lined with wood shavings and provided with *ad libitum* laboratory rodent chow (standard Hope farm[®] pellets) and tap water. Wild-type hamsters were entrained to a light-dark cycle of 12 h light and 12 h dark (LD 12:12) and *tau* mutant hamsters were kept in LD 10:10 for at least 14 days prior the experiment (light source: white light tubes, TLD 36W/85, Philips; illumination range 70-170 lux).

A step-through type of passive avoidance apparatus as described by Ader *et al.* (1972) was used. It consisted of a dark compartment (40 × 40 × 40 cm) separated by a sliding door to an elevated, well-lit platform (2 × 25 W light bulbs). The dark compartment was equipped with a floor made from stainless steel bars, which allowed delivering a scrambled footshock.

Hamsters were trained in the light phase starting 2 h after lights on. During two consecutive habituation trials, hamsters were placed on the illuminated platform facing away from the dark compartment. After stepping into the dark compartment the sliding door was closed and 3 minutes of adaptation were allowed. On the third training trial, a pre-shock latency was measured and after the door between compartments was closed a scrambled, unavoidable footshock was delivered (0.6 mA) for 3 s. All three training trials were conducted 24 h apart for *tau* +/+ hamsters and 20 h apart for *tau* -/- hamsters. On the single testing trial, time spent on the illuminated platform (up to 300 s) was measured with a stopwatch and defined as post-shock latency. Hamsters with pre-shock latency higher than 200 s were omitted from the test trial and considered as individuals without preference for the dark compartment ($N = 3$).

In total 86 *tau* +/+ and 72 *tau* -/- hamsters naive to the test were trained and tested. They were randomly distributed among groups tested at different training-testing intervals (TTI) of 0.25, 6, 12, 18, 24, 30, and 36 hours ($N = 11, 16, 11, 14, 14, 11,$ and 9 for *tau* +/+ hamsters and $N = 5, 7, 7, 7, 10, 9,$ and 7 for *tau* -/- hamsters, respectively). *Tau* -/- were additionally tested at 20 h ($N = 7$) and 26 h ($N = 13$) TTI. Each hamster was tested only once after training.

The study was performed under license DEC 2193 from the animal experimentation committee, University of Groningen.

Step-through latency (STL) differences between groups at different TTI were tested for significance with Kruskal-Wallis one-way analysis of variance on ranks and with Dunn's test for multiple comparisons with the 0.25-h group. Paired *t*-test or Wilcoxon test, when appropriate, was applied to test the difference within individuals and Mann-Whitney rank sum test to test for difference between genotypes. A value of $p < 0.05$ (two-tailed) was considered statistically significant.

Results

Both genotypes preferred the dark over the illuminated compartment of the passive shock apparatus. They spent significantly less time on the lit platform during the second habituation trial ($p < 0.001$, paired t -test) than during the first trial, independently of the genotype ($p > 0.05$, t -test). The pre-shock step-through latency (STL) varied from 7 to 138 s for τ u +/+ hamsters and from 7 to 184 s for τ u -/- hamsters with no difference between genotypes ($p = 0.9$, one-way ANOVA). Hamsters from both genotypes associated the dark compartment with the footshock, since latency of the 0.25 h group was significantly higher than the latency for the training and pre-shock groups ($p < 0.01$, repeated measures ANOVA; Fig. 1). Groups tested at different TTI did not differ in average STL during habituation trials ($p > 0.05$, one-way ANOVA).

STLs of τ u +/+ hamsters tested at TTI 6 and 30 h were not significantly different from the group tested at 0.25 h ($p > 0.05$, Kruskal-Wallis ANOVA and Dunn's test). In contrast, the performance was significantly impaired in groups of τ u +/+ hamsters tested at TTIs of 12, 18, 24, and 36 h (Fig. 2A). For τ u -/- hamsters, STL was indistinguishable among groups tested at TTIs of 0.25, 6, 12, 18, 26, and 36 h ($p > 0.05$, Kruskal-Wallis ANOVA and Dunn's test). Groups tested at TTIs of 20, 24, and 30 h had a significantly lower STL than at 0.25 h ($p < 0.05$; Fig. 2B).

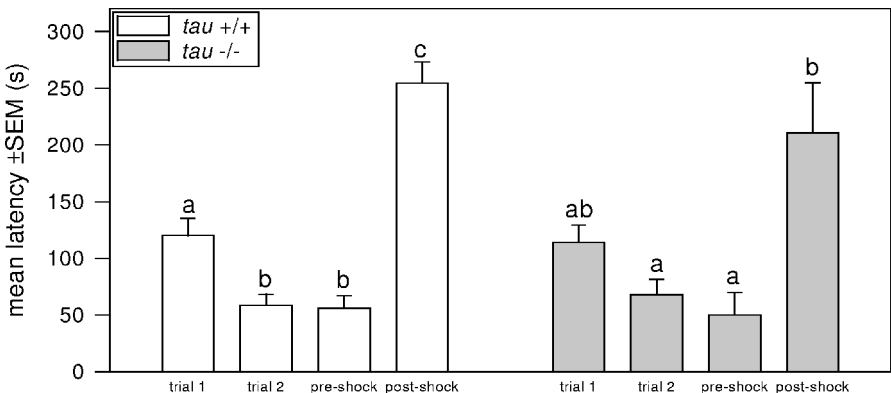


Fig. 1. Mean latency of two habituation trials and the pre- and post-shock trials measured at training-testing interval of 0.25 h in wild-type hamsters (τ u +/+; $N = 11$) and homozygous τ u mutants (τ u -/-; $N = 5$). Significant differences between trials are indicated with different letters. Trials not different from each other have the same letter.

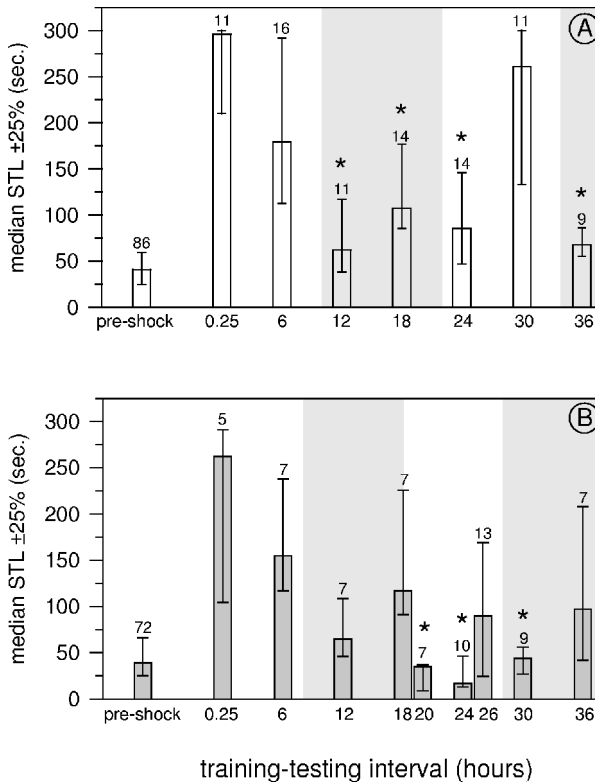


Fig. 2. Median step-through latency (STL) at pre-shock trial and at different training-testing intervals for (A) wild-type hamsters and (B) *tau* mutant hamsters. Number of individuals per group is indicated above bars. Grey area represents the dark period of a light-dark cycle. Statistical significance between TTIs compared with 0.25 h TTI is indicated by a star. Error bars are the first and the third quartiles.

The two genotypes of hamsters did not differ in STL at 0.25, 6, 12, 18, and 36 h TTI intervals ($p > 0.2$, Mann-Whitney test). *Tau* +/+ had a significantly higher latency than *tau* -/- hamsters tested at 24 h and 30 h ($p < 0.001$, Mann-Whitney test).

Discussion

Syrian hamsters like rats and other rodents were capable of learning a passive avoidance paradigm. They had a less pronounced preference for darkness since hamsters stayed approximately three times longer on the illuminated

platform during the pre-shock training trial compared with rats (Holloway & Wansley, 1973a; Fekete *et al.*, 1985).

The findings reported in the literature regarding the best performance at approximately the same time-of-day as training (Holloway & Wansley, 1973a, b) were not fully replicated in the Syrian hamster. In sharp contrast to rats, which have the best memory retention at 0.25, 12, 24, 36 h after training, wild-type hamsters showed this only at the short TTI of 0.25 h and at 6 and 30 h. At 6 and 30 h rats were reported to have impaired memory retention (Holloway & Wansley, 1973a, b).

Since wild-type hamsters showed a different pattern in performance than expected, it is difficult to interpret the results obtained from *tau* mutant hamsters. Mutant hamsters had significantly impaired retention performance at 20, 24, and 30 h after training. However, STL scores appeared reduced at all TTIs. The pattern in figure 2B suggests poor overall retention rather than specific oscillations in memory retention. Both genotypes showed good performance at the short TTI indicating an intact short-term memory. Neither genotype showed the expected high memory performance after 24 h for *tau* +/+ or after 20 h for *tau* -/- hamsters on the basis of the rat data. However, *tau* +/+ hamsters tested at a TTI of 30 h had high average STLs, suggesting that long-term memory performance is better in wild-type than in *tau* -/- hamsters.

The 24 h-oscillation in memory retention in rats depends on the circadian phase of testing relative to the circadian phase of training, and not on circadian phase *per se* (Holloway & Wansley, 1973b). Rats have better memory retention at multiples of a half- and a whole circadian cycle after training, independently of time of day when training took place. Wild-type hamsters on the other hand, seem to have better retention of the shock experience at a certain phase of the circadian cycle, *i.e.* in the late subjective day ('Zeitgeber Time', ZT 8-10), corresponding with TTIs at 6 and 30 h. Similar, mutant hamsters performed better at time-training intervals, which coincide with transition from light to dark (TTI at 6 and 26 h correspond with ZT 11-12; 18 h with ZT 0-1, and 36 h with ZT 23-24). To test whether optimal memory retention is indeed locked to a certain phase of the circadian cycle, it would be necessary to train animals at different times of day and test performance at different zeitgeber times.

It has been demonstrated that *tau* is a point mutation in the gene encoding for casein kinase I ϵ (Lowrey *et al.*, 2000). Casein kinase I has been implicated in the control of cytoplasmic and nuclear phosphorylation processes.

Phosphorylation of cellular and nuclear substrates is a crucial aspect in the formation of memory and a large variety of kinases have been shown to be involved in learning and memory processes in tasks such as passive shock avoidance (Van der Zee *et al.*, 1994; Izquierdo & Medina, 1997; Micheau & Riedel, 1999). It is tempting, therefore, to consider the possibility that long-term memory, which requires a variety of cytoplasmic and notably nuclear processes, is impaired in *tau* $-/-$ hamsters due to the dysfunction in casein kinase I-dependent pathways.

Memory retention of the passive shock avoidance task depends on vasopressin levels in the brain (see for review Laczi *et al.*, 1984; Alescio-Lautier & Soumireu-Mourat, 1998), putatively originating from the suprachiasmatic nucleus. *Tau* $-/-$ hamsters have a high baseline of vasopressin release with low circadian amplitude and fluctuation compared to wild-type hamsters (Van der Zee *et al.*, *subm.*). In addition to the casein kinase I ϵ mutation, these altered vasopressin levels in *tau* $-/-$ hamsters may have contributed to poor memory retention.

In conclusion, we found no evidence for periodic memory retention deficits at 6 h and multiples of 12 h in wild-type Syrian hamsters. *Tau* mutant hamsters did not have the expected high memory retention at times approximately equal to a full circadian cycle after training. These results suggest that the phenomenon of deficits in memory retention as a function of time after training may be expressed differently across rodent species.

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