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# Age and time-of-day effects on learning and memory in a non-matching-to-sample test

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**Abstract**

This study provides further evidence that the time-of-day (TOD) when testing is conducted affects cognitive performance in old rats and, for the first time in an animal model, in young adult rats as well. Groups of young and old rats were entrained to a 12-h light–dark schedule and administered tests of learning and memory in a non-matching-to-sample (NMTS) task in a water maze. Testing was conducted at the beginning of the rats' activity cycle (AM) or at the end of the cycle (PM). In addition to age differences in performing the task, there were major findings with respect to time of testing: (1) young rats tested in the PM were better than young rats tested in the AM at learning the NMTS rule and in the delayed-NMTS (DNMTS) task; (2) old rats tested in the AM were better than PM-tested old rats on the DNMTS task, with the former attaining performance levels that approximated those of young rats; (3) the TOD effect in old rats extended to a DNMTS reversal (DNMTS-R) condition in which rats, originally tested in the AM, subsequently were administered the test in the PM, and vice versa; (4) the TOD effects in young and old rats in the DNMTS and DNMTS-R tests were strongest at relatively long delays, suggesting that hippocampal function may be particularly vulnerable to such effects; (5) there was evidence in the old rats of a relationship between diurnal drinking patterns and performance at the longest delay in the DNMTS test. These results, which parallel similar findings with human subjects, emphasize a linkage between circadian rhythmicity and cognitive performance throughout adulthood, and indicate the importance of circadian disruption in old age as a contributing factor to age differences in learning and memory performance.


*Keywords:* Aging; Cognition; Time-of-day effect; Circadian rhythms; Animal model

## 1. Introduction

During the course of normal aging, alterations in circadian rhythmicity produce changes in a wide range of behavioural (e.g., sleep–wake cycles [9,17], eating and drinking patterns [5,14]), and physiological (e.g., brain glucose uptake [6] neurotransmitter production [3]) processes. These changes are related to a shift in optimal arousal and activity from later in the day in young adults to early morning in older adults. Recent studies indicate that circadian-related changes extend to cognitive functions. In a series of experiments, involving a variety of cognitive tests, young adults consistently performed better in the afternoon than in the morning, while older adults showed the reverse pattern. Interestingly, when tests were administered to both age groups at peak times for older adults, i.e., morning, age differences were minimized and, in some cases, even eliminated [7]. Also of interest, the cognitive performance of old and young people typically correlated with scores on the Morningness–Eveningness Questionnaire, a reliable and valid psychometric index of circadian rhythmicity [8].

The impact of circadian rhythm disruption on age differences in cognitive performance has also been demonstrated in an animal model. In a recent study [24], old and young rats were entrained to a 12-h light–dark schedule and subsequently administered tests of delayed-alternation and inhibitory avoidance conditioning at the beginning or end of the dark cycle. Age-related disruption of circadian rhythmicity was confirmed by water-intake measures that revealed altered diurnal drinking patterns in the old rats. Young rats were not affected by the time of testing but, on both behavioural tasks, old rats performed better when tested early in the dark cycle, when activity/arousal levels were highest. Other studies have also linked age-related learning and

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memory loss in animals to circadian disruption [2,15,16]. In the Antoniadis et al. [2] study, conducted with middle-age hamsters, performance decline on a test of conditioned place preference was reported only in animals that exhibited degraded circadian rhythms as reflected in fragmented activity patterns.

The time-of-day (TOD) effects seen in humans and animals also provide important insights from a neuropsychological perspective. For example, in humans, the effect has been reported most frequently on cognitive tests that challenged attentional processes or executive functions linked to the frontal lobes [26]. By comparison, Winocur and Hasher [24], working with rats, observed the TOD effect primarily on measures of long-term memory known to be sensitive to hippocampal impairment. These results do not preclude involvement of other brain regions but they highlight the vulnerability of hippocampal and frontal-lobe functions to circadian disruption in old age.

The present research extends our neuropsychological investigation of the TOD effect to an age-sensitive non-matching-to-sample (NMTS) test conducted in a water maze. This task consists of a series of paired sample and test trials. For the sample trials, a distinctive stimulus cued the location of an invisible platform where the rat could escape the water. In the subsequent test trial, the same stimulus was presented along with a different stimulus and the rat must swim to the new stimulus to find the platform. NMTS rule-learning, which incorporates conditional and working memory components, is highly sensitive to frontal-lobe dysfunction [13], but is not typically affected by hippocampal lesions [1,27]. However, by increasing the interval between sample and test trials, the task puts increased demands on hippocampus-controlled memory function [23]. The NMTS task, designed this way, yields dissociable learning and memory functions related respectively to the frontal lobes and hippocampus, and possibly modulated by synchronized rhythmicity of the circadian system [25]. A primary question addressed here is whether TOD effects, if demonstrated on the NMTS task, are selective to frontal lobe or hippocampal measures, or impact learning and memory performance in a more general way.

Another objective relates to the view that a reduction in inhibitory control is a central feature of cognitive aging and that, in humans, the TOD effect has been observed on tasks that require suppression of irrelevant information and other forms of inhibitory control [7]. On the other hand, in our previous work with aged rats, both the delayed alternation and inhibitory avoidance tasks included substantial inhibitory components but there was only slight evidence that this factor contributed to age and TOD effects on performance. Accordingly, it was thought desirable to ask whether such effects occur on a test in which successful performance does not depend on the suppression of inappropriate response tendencies.

Finally, in our previous research, rats received behavioural testing in the early or late phase of their activity cycle. Since cognitive performance in aged rats appears to be linked directly to the time at which testing occurs, the question arises as to whether the TOD effect would continue to be expressed if time of testing were reversed. For example, would old rats tested early in the activity cycle, after previous testing later in the cycle, now perform better than their counterparts tested in the reverse sequence? To investigate this possibility and test the robustness of the TOD effect, following delayed NMTS testing, all groups were subjected to a reversal condition in which rats originally tested in at the beginning of the dark cycle were administered the delayed task late in the dark cycle, and vice versa.

## 2. Method

### 2.1. Subjects

Twenty-four old and 20 young, male Long–Evans rats, born and reared in the Trent University Breeding Centre, participated in this research. At the time of behavioural testing, old rats were approximately 23 months old and young rats were 6 months old.

All rats were naïve to the NMTS task but, as young adults, had received some unrelated behavioural testing. Throughout the experiment, which was approved by the Trent University Animal Care Committee, rats were regularly examined by a veterinarian.

### 2.2. Apparatus

All testing was conducted in a circular pool (122 cm in diameter), located in the centre of a room (360 cm × 360 cm). The room, which was different than the one in which rats were housed, was illuminated by overhead fluorescent lights. The pool was filled with water, rendered opaque by diluted, non-toxic white tempera paint and maintained at room temperature (21 °C). Standard lab furniture (e.g., a rack of cages, testing equipment, a stool, and a cabinet) was distributed around the room and several pictures were mounted on the walls. Throughout testing, the water was cleaned daily and changed every 5 days.

An inverted flower pot (24 cm high) with a white surface that served as a platform (10 cm in diameter) was situated a few centimetres below the surface of the water. The stimuli for the sample and test trials were black and white cylinders (30 cm long × 3 cm in diameter), suspended 20 cm above the surface of the water. The position of the cylinders was controlled manually by the experimenter through a system of pulleys, weights, and wires that ran inconspicuously outside the perimeter of the pool and along the ceiling. The water maze was divided into four equal quadrants. The dividing lines between the quadrants were invisible during testing, but the experimenter became expert at identifying the borders through extensive prior practice.

### 2.3. Procedure

Six weeks before behavioural testing, all rats were transferred from group cages to individual cages with food and water available at all times. They were maintained on a 12-h light–dark schedule with lights on only between 8.00 PM and 8.00 AM. Entrainment to this schedule was confirmed by measuring water intake at the end of the dark and light cycles.

Before the beginning of testing, old and young rats were assigned, in approximately equal numbers, to early (AM) or late (PM) subgroups for testing purposes. Thus, four subgroups were created: Old-AM (old rats tested early in the dark cycle; $n = 12$); Old-PM (old rats tested late in the dark cycle, $n = 12$); Young-AM (young rats tested early in the dark cycle, $n = 10$); Young-PM (young rats tested late in the dark cycle, $n = 10$). Two old rats became ill during testing in the final DNMTS-R condition and, on the advice of the veterinarian were removed from the experiment and the colony. As a result, their data for the DNMTS-R condition are not available.

Testing for the AM subgroups commenced within 1 h of the beginning of the dark cycle and, for the PM subgroups, within 1 h of the end of the dark cycle. Within the subgroups the order in which each rat was tested varied from day to day. All rats were administered the following conditions.

#### 2.3.1. NMTS learning

The NMTS task consisted of a series of paired sample and test trials. At the beginning of each sample trial, the black or white cylinder was suspended directly above the submerged platform. Both cylinders were present during the subsequent test trial but the cylinder that was not present during the preceding sample trial was suspended over the platform and cued its location. Thus, if on a given sample trial, the black cylinder cued the platform, then on the succeeding test trial, the white cylinder cued the platform. The black and white cylinders were selected as sample stimuli for each pair of trials according to a semi-random schedule that ensured that each cylinder was the sample stimulus on 50% of the trials over this phase of the experiment. For each test trial, the platform was moved to another quadrant with the non-sample cylinder located directly above it. The sample stimulus was also moved to a different quadrant. The position of the submerged platform was changed after each sample and test trial, according to a random schedule, in order to eliminate the use of spatial cues. All quadrants were used equally for locating cues in the sample and test trials and, within the quadrants, the platform was positioned randomly.

At the beginning of each sample trial, the rat was placed in the pool at the same location (in the centre of the south-east quadrant), facing the wall of the pool, and allowed to swim to the submerged platform under the sample cylinder. The rat remained on the platform for 10 s. On rare occasions, when a rat would fail to find the platform within 60 s, it was picked up and placed on the platform for 10 s. The rat was then removed and placed under a heat lamp while the platform was moved and the cylinders put in position for the test trial. The organization of the cylinders and platform took about 5 s. The rat was then placed in the pool at the usual location and allowed to swim to the submerged platform or until 60 s had elapsed. In either case, the rat was allowed 10 s on the platform before being returned to a holding cage under a heat lamp, to await the next pair of trials. In the NMTS learning, and subsequent conditions, rats were tested in squads of five rats. During NMTS learning, approximately 2 min separated each pair of trials. Ten daily sessions, each consisting of four pairs of sample and test trials, were administered.

#### 2.3.2. Delayed-NMTS (DNMTS)

The day after the completion of NMTS training, rats were administered five additional daily sessions. Each session consisted of four paired trials, with intervals of 0, 20, 40, or 80 s between the sample and test trials. (Note: The intervals do not include the 5 s required for repositioning the cylinders and platform.) The order of the delays varied each day according to a random schedule. During DNMTS testing, the interval between pairs of trials varied, ranging from approximately 2 min, when the sample-trial interval was 0 s, to approximately 9 min, when the sample-trial interval was 80 s. In all other respects, the procedure for delayed testing was identical to that of NMTS learning.

#### 2.3.3. DNMTS-reversal (DNMTS-R)

The day following DNMTS testing, all rats received an additional five sessions of delayed testing but this time the test-times were reversed. Thus, rats that were previously tested early in the dark cycle (AM) were now tested at the end of the cycle (PM), and vice versa. For this condition, old and young subgroups, initially tested in the AM and switched to the PM for DNMTS-R testing are designated Old-AM → PM and Young-AM → PM, respectively. Old and young subgroups, initially tested in the PM and switched to the AM, are designated Old-PM → AM and Young-PM → AM, respectively. In all other respects, the procedures were identical to those of DNMTS testing.

## 3. Results

### 3.1. Light–dark entrainment

The average daily water consumption by old and young rats at the end of 4 weeks of adaptation to the light–dark cycle is summarized in Table 1. The data presented in Table 1 are water intake amounts averaged over the last 3 days of the entrainment period. Overall, young rats drank more water than old rats, $F(1, 42) = 9.25$, $P < 0.004$ over the 24 h period. Of particular interest was the finding of a highly significant age × cycle interaction, $F(1, 42) = 48.66$, $P < 0.00001$, that was due to old rats drinking substantially less

Table 1
Amount of water (in ml) consumed by Old and Young Groups at the end of their dark and light cycles

| | Dark cycle | | Light cycle | |
|---|---|---|---|---|
| | *M* | S.D. | *M* | S.D. |
| Old | 37.70 | 3.55 | 25.32 | 4.93 |
| Young | 46.57 | 5.04 | 22.01 | 2.56 |

Scores are averaged over the last 3 days of entrainment.

than young rats during the dark cycles but slightly more than the young rats during the light cycle.

To assess alterations in drinking patterns, an index of entrainment was calculated for each rat by subtracting the average amount of water consumed during the light cycle over the last 3 days before behavioural testing, from the amount consumed during the corresponding dark cycle. A comparison of this index between Old and Young Groups revealed reduced diurnal differences in the old rats, $t(42) = 7.11$, $P < 0.001$. The pattern of these results indicate an age-related disruption of rhythmic water-intake patterns that is consistent with previous reports [4,5,24].

### 3.2. Behavioural results

Latency and error scores for each sample and test trial were recorded by the experimenter. Latencies represent the time taken to swim to, and climb on to the platform after the rat was placed in the water. Errors represent the number of entries made to incorrect quadrants. An error was designated only if the rat's entire body (excluding the tail) was in an incorrect quadrant. In all conditions, both measures were submitted to analysis of variance (ANOVA) with post hoc analysis performed with the Neuman–Keuls test. All tests of significance were performed at an alpha level of 5%.

Because there were no performance differences associated with age or time of testing on the sample trials, data are reported only for test trials.

#### 3.2.1. NMTS learning

The performance of Old and Young Groups in the NMTS learning condition is presented in terms of mean latency to find the platform (Fig. 1A) and in terms of mean errors (Fig. 1B), across the 10 training sessions.

*3.2.1.1. Latency.* Fig. 1A shows that all groups reduced the amount of time required to find the platform over the 10 session training period and that, by Session 7, performance on this measure stabilized in all groups. ANOVA applied to these data yielded a highly significant age × TOD interaction, $F(9, 360) = 2.95$, $P < 0.002$, that was due completely to the relatively slow latencies of the Young-AM Subgroup on Sessions 1 and 2. Post hoc comparisons with the Neuman–Keuls test, indicated that this subgroup was significantly slower in both sessions (all $P$'s $< 0.01$). There

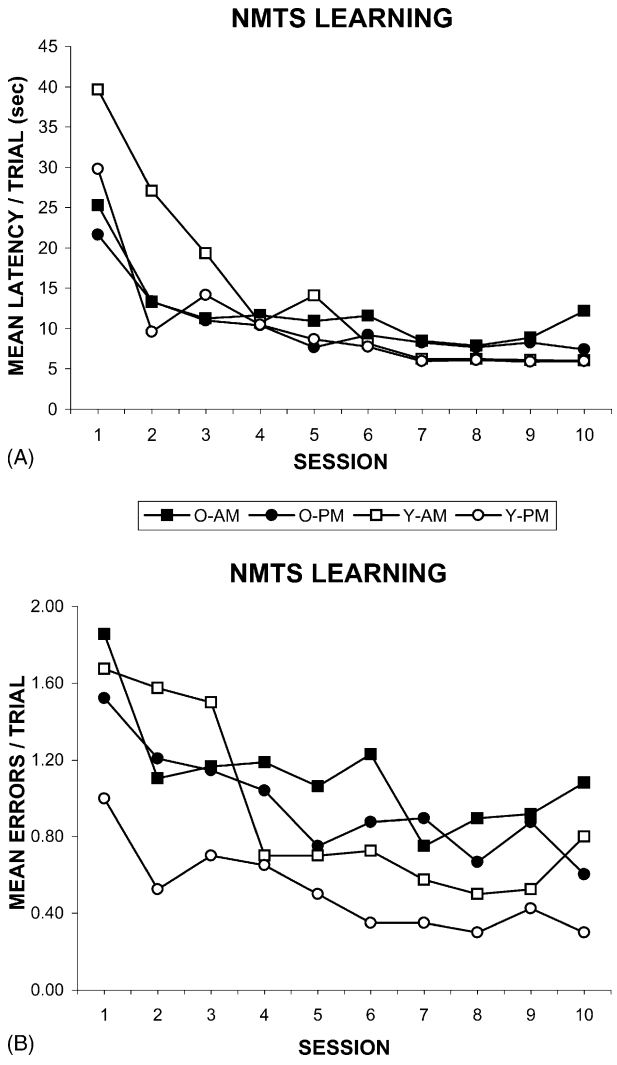


Fig. 1. Response latencies (A) and error scores (B) for Old and Young Groups, tested early (AM) or late (PM) in the activity cycle, on the NMTS learning task.

were no differences on Sessions 3–10. The only main effect that approached statistical significance was due to TOD, $F(1, 40) = 3.72$, $P = 0.06$, an effect that is qualified by the interaction discussed above.

*3.2.1.2. Errors.* ANOVA applied to the error scores (Fig. 1B) revealed a significant age × TOD × session interaction, $F(9, 360) = 1.90$, $P = 0.05$. There was also a significant age × TOD interaction, $F(1, 40) = 4.92$, $P < 0.03$. These differences are attributable to the large differences in errors between the Young-AM and Young-PM Subgroups, relative to the Old-AM and Old-PM Subgroups on Days 1–3. Post hoc analysis revealed significant differences between the Young-AM and Young-PM Subgroups on Sessions 1–3 (all $P$'s $< 0.03$) but no other significant differences.

In addition to these effects, old rats generally made more errors than young rats, a finding that was confirmed by a

significant main effect of age, $F(1, 40) = P < 0.001$. As well the progressive reduction by all groups in terms of number of errors contributed to a significant sessions effect, $F(1, 40) = 14.27$, $P < 0.001$.

*3.2.1.3. Summary.* Overall, the results indicate that old rats made more errors than young rats in learning the NMTS rule, but that this difference was not reflected in the latency measure. There was a TOD effect in that young rats tested in the AM made more errors and took more time to reach the platform. This effect was apparent in the early stages of training on both measures, and throughout training on the error scores. Old rats did not exhibit a TOD effect in NMTS learning.

*3.2.2. DNMTS*

The mean latency to find the hidden platform on the test trials and the mean number of errors at each ITI were averaged for each of the 5 test days and are presented in Fig. 2A and B, respectively.

*3.2.2.1. Latency.* There were no differences on the latency measure between Young-AM, Young-PM, and Old-AM Subgroups at any ITI. However, the mean latencies for the Old-PM Group increased beyond ITI-20 and were markedly slower than those of the other groups at ITIs-40 and 80. This was confirmed by a significant age × TOD × ITI interaction, $F(3, 120) = 10.06$, $P < 0.001$. Separate ANOVAs conducted on the latency measures of the Old and Young Groups confirmed that the triple interaction was due to increased latencies in the Old-PM Subgroup relative to the Old-AM Subgroup (TOD × ITI interaction—$F(3, 66) = 12.64$, $P < 0.001$). Post hoc comparisons revealed that this interaction was due to significant differences at ITIs-40 and 80 (both $P$'s $< 0.01$). ANOVA performed on the latency data of the Young Group yielded no significant interaction or main effect.

*3.2.2.2. Errors.* ANOVA conducted on the error scores revealed an age × TOD × ITI interaction, $F(3, 120) = 11.63$, $P < 0.001$. In this case, the effect of age qualified the effects of both ITI and TOD. With respect to the age × ITI interaction, $F(3, 120) = 10.40$, $P < 0.01$, old rats consistently made more errors than young rats but, as can be seen in Fig. 2B, the difference was much greater at ITIs-40 and 80 than at ITIs-0 and 20. Age differences were further analyzed with the Neuman–Keuls test and found to be significant at ITIs-40 and 80 (both $P$'s $< 0.001$).

The age × TOD interaction was also significant, $F(3, 120) = 26.20$, $P < 0.0001$, but here there were two TOD effects. Separate ANOVAs revealed significant TOD × ITI interactions for the old, $F(3, 66) = 8.51$, $P < 0.001$ and young, $F(3, 54) = 4.37$, $P < 0.008$, groups. In the Old Group, this interaction was due to the Old-PM Subgroup having made more errors than the Old-AM Subgroup at ITI-80 ($P < 0.001$). In the Young Group, the reverse was true. Here, the Young-AM Subgroup generally made more errors than Young-PM Subgroup, although the difference was statistically significant only at ITI-80 ($P < 0.007$).

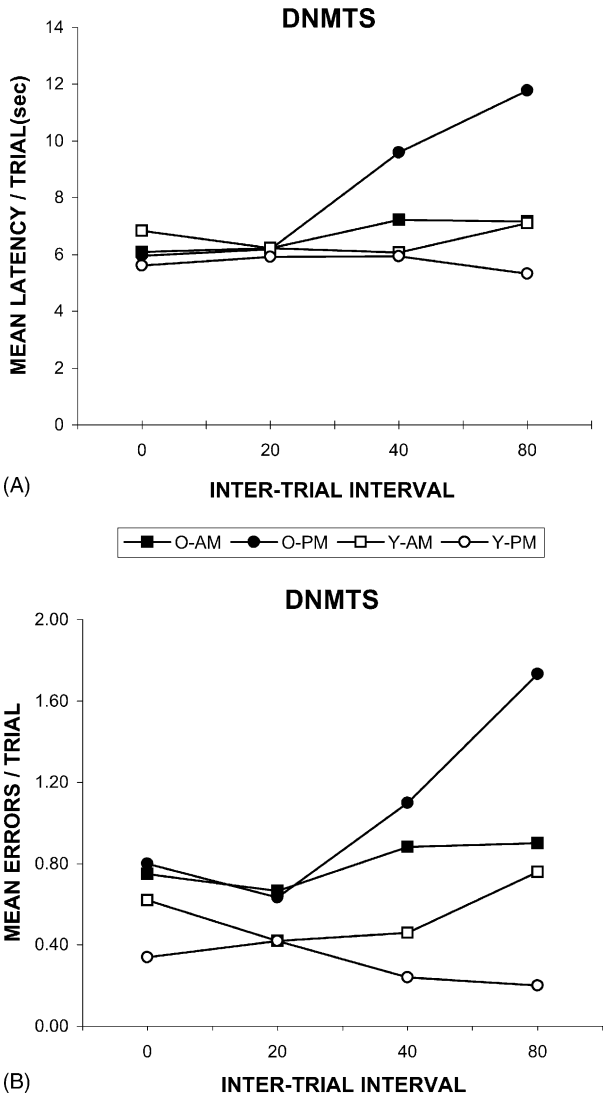


Fig. 2. Response latencies (A) and error scores (B) for Old and Young Groups, tested early (AM) or late (PM) in the activity cycle, on the DNMTS task.

*3.2.2.3. Summary.* The results of the DNMTS condition revealed that, in general, old rats performed worse than the young rats on the error measures with the age effect most marked at long ITIs. The latency measure only showed differences for older rats tested in the PM at long ITIs. The ability of old and young rats to perform this task was influenced by time of testing. Old rats tested in the PM were especially disadvantaged, relative to old rats tested in the AM. In contrast, young rats tested in the AM performed worse than young rats tested in the PM at ITI-80, but this effect was observed only on the error scores.

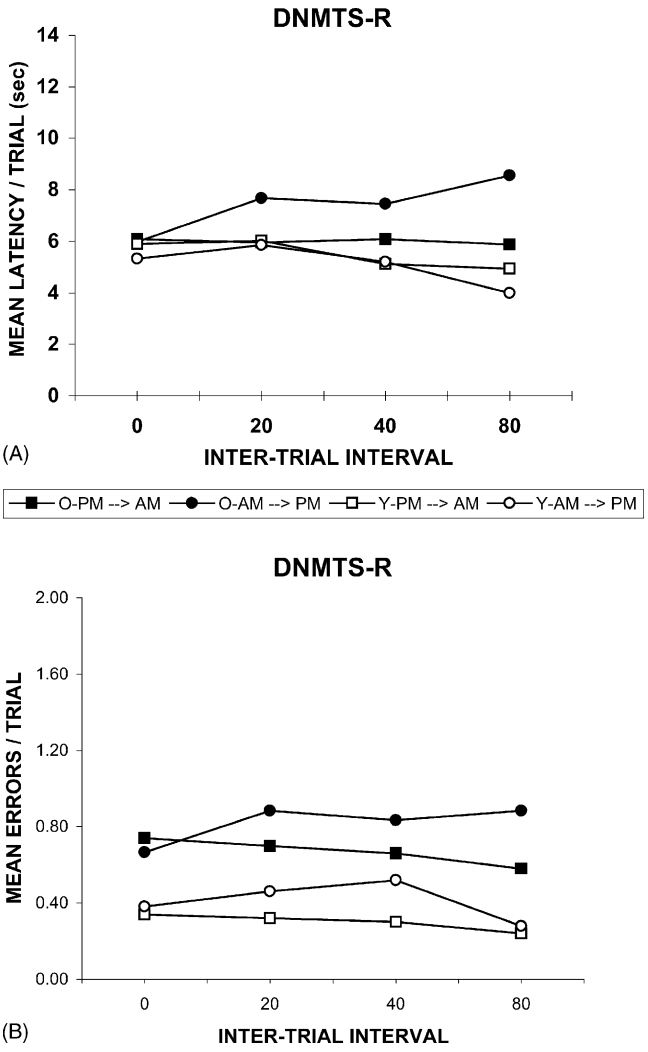


Fig. 3. Response latencies (A) and error scores (B) for Old and Young Groups, tested early (AM) or late (PM) in the activity cycle, on the DNMTS-R task.

### 3.2.3. DNMTS-R

The mean latency to find the hidden platform on the test trials of the reversal condition and the mean number of errors at each ITI were averaged for each of the 5 test days and are presented in Fig. 3A and B, respectively.

*3.2.3.1. Latency.* ANOVA applied to the latency data revealed a significant age × ITI interaction, $F(3, 114) = 3.51$, $P < 0.02$. Old rats generally performed worse than young rats, with the differences reaching statistical significance at ITIs-40 and 80 (both $P$'s $< 0.02$). There was also a significant age × TOD interaction, $F(1, 38) = 5.29$, $P < 0.03$, that was due entirely to differences between the Old-AM → PM and Old-PM → AM Subgroups. Separate ANOVAs conducted on the Old and Young Groups yielded a significant TOD × ITI interaction only for the Old Group, $F(3, 60) = 2.77$, $P < 0.05$. Subgroup differences approached significance at ITI-20 but were reliable at ITI-40 ($P < 0.05$) and ITI-80 ($P < 0.006$).

*3.2.3.2. Errors.* Overall, there were no significant interactions involving the error measure, but there was a strong main effect of age, $F(1, 38) = 45.94$, $P < 0.0001$ that was due to the greater number of errors made by the old rats. There was a general tendency for the Old-AM → PM Subgroup to make more errors than the Old-PM → AM Subgroup at ITIs longer than zero. The age × ITI interaction was not statistically significant, $F(3, 60) = 1.56$, $P = 0.21$. However, a Neuman–Keuls test provided evidence of a TOD effect in revealing a significant difference between the Old Subgroups at ITI-80, $P < 0.02$, but not ITI-0. The same analyses performed on the young rats did not show a significant interaction but there was a non-significant tendency for the Young-PM → AM Subgroup to make more errors than the Young-AM → PM Subgroup at ITI-40.

*3.2.3.3. Summary.* In the DNMTS-R condition, the old rats generally performed worse than the young rats on both measures. On the latency measure, this effect was apparent only at the longer ITIs. The performance of old rats was qualified by the time of testing, with old rats tested in the PM performing worse than old rats tested in the AM, especially at longer ITIs. The general tendency of the young rats tested in the PM to perform worse than the young rats tested in the AM provided weak evidence for a TOD effect in this group.

### 3.3. Relationship of water-intake patterns to behaviour

To determine if there was a relationship between altered circadian rhythms, as measured by diurnal differences in water intake, each rat's entrainment index was correlated with performance in those conditions where TOD effects were observed. These conditions were, for the old rats, ITI-80 in the DNMTS and DNMTS-R tests, and, for the young rats, during NMTS learning and ITI-80 of the DNMTS test. Pearson product–moment correlations yielded significant correlations between the entrainment indices and error, $r = -0.80$, $P < 0.01$, and latency, $r = -0.76$, $P < 0.01$, scores of the Old-PM Subgroup at ITI-80 of DNMTS testing. No other correlations reached statistical significance.

## 4. Discussion

The present results clearly demonstrate that the time of day at which testing is conducted affects cognitive performance in old rats. As well, they showed, for the first time, that young adult rats are also vulnerable to this effect. Interestingly, the TOD effect was expressed differently in the two populations. Old rats, tested in the AM out-performed their counterparts tested in the PM, whereas the reverse was true for young rats. Another important finding is that

performance differences between Old-AM and Old-PM rats were consistently larger than the differences between Young-AM and Young-PM Subgroups. The results parallel similar findings of studies with human populations [5,8,9] and provide further evidence that age-related disruption of biological rhythms is an important factor in assessing cognitive aging. Because of the shift towards greater activity in the AM that is part of normal aging, older subjects are disadvantaged when tests are administered at off-peak hours. When this factor is taken into account, age differences can be significantly reduced, a finding reported here and elsewhere in the animal [24,25], and human [11,12,26] literatures.

Before analyzing the TOD effects, the age differences on learning and memory aspects of the NMTS task warrant discussion. Over the 10-day training period, the old rats made significantly more errors than the young rats in learning the NMTS rule. The relatively slow learning by the aged group is consistent with several reports of age-related deficits in learning matching- and non-matching-to-sample tasks, as well as other tasks (e.g., response alternation, conditional associative learning) that incorporate conditional and working memory components [13]. Curiously, despite making more errors during NMTS learning, old rats were not slower than young rats in reaching the platform. There is no obvious explanation for this discrepancy, but the use of a water maze may have been a factor. Water mazes are highly stressful for rats and the combined effects of age and stress may have interacted in unknown ways with the consequences of making more errors. If, for example, old rats experienced heightened stress and, as a result, swam faster, they may have been more error prone. Interestingly, old rats did not show this pattern in the DNMTS and DNMTS-R conditions. However, it was observed in the young rats, as part of the TOD effect they exhibited in the DNMTS condition. This appears to be the first study in which a complex learning task was administered in a water environment, and the results point to a need to examine the comparability of water-based and land-based tests.

It is worth noting that the nature of non-spatial NMTS tasks allows for the possibility of relatively efficient performance through different response strategies. For example, having learned to associate the platform with the presence of a stimulus cue, on test trials, rats could simply approach one of the two cues. If that turned out to be the correct one, the rat would find the platform; if not, it would redirect to the other cue. This approach would result, by chance, in only two errors over a four-trial session (0.5 error per trial). However, this was clearly not the case. Examination of error scores in both delayed conditions (Figs. 2B and 3B) shows that old rats tested in the PM consistently made more errors at long ITIs than short ITIs, while the Young-AM Subgroup showed the same pattern in the DNMTS condition. The error rates in these conditions were well above that expected by chance if selection of cues on test trials was randomly determined. If rats adopted a random cue selection approach, there would be no reason to expect an increase in errors when ITIs increased. Rather, since they would not be remembering the cues of the preceding sample trial, they would continue to randomly choose one of the cues at the beginning of the test trial, and make errors at a constant rate, regardless of the ITI.

The NMTS task was chosen for this research because it is sensitive to the effects of aging, and because it incorporates dissociable measures of learning and memory that have been linked to different brain regions. The ability to learn matching- and non-matching-to-sample rules under standard training conditions has been identified with frontal-lobe function [13,23]. When the interval between sample and test trials is substantially lengthened, thereby placing increased demands on episodic memory processes, successful performance requires support of the hippocampus [22,23]. The frontal lobes and hippocampus are known to be vulnerable, in physiological terms, to the aging process [13,18,21] and the present results confirm that this vulnerability is reflected in a decline in functions that depend on the integrity of these regions.

Whereas old rats generally performed worse than young rats in learning and memory components of the task, TOD effects in the old rats were observed only at long ITIs in the delayed tests. In these conditions, the performance of old rats tested early in the activity cycle approached that of young rats. This pattern is identical to that reported by Winocur and Hasher [24], using a variable-interval delayed alternation task, and support the conclusion that long-term memory processes under hippocampal control are sensitive to TOD effects and related alterations in circadian rhythmicity.

The TOD effect in old rats parallels that seen in aged humans, where age differences in cognitive performance were also maximal later in the day and substantially reduced in the morning. Although similar in their essential features, there may be some important differences between TOD effects observed in aged animals and humans. For example, whereas old rats, tested at off-peak times, were most disadvantaged on tests of hippocampus-mediated memory function, in aged humans, TOD effects have been observed primarily on tasks that place heavy demands on attentional processes and executive functions. As well, many of these tasks require the active suppression of predisposed responses. Together, the results of the human studies tend to implicate frontal lobe dysfunction in the TOD effect. Given the limited research in this area, it would be premature to conclude that the animal and human-based data are in conflict. What is needed is systematic investigation of TOD effects in both populations, using neuropsychological tests that are sensitive to a wide range of functions, including those that are affected when various brain regions are compromised by old age.

Another unresolved issue relates to the link between age-induced changes in cognitive performance and alterations in circadian rhythmicity. Studies with human subjects have yielded reliable correlations between cognitive test scores and scores on the Horne–Ostberg Morningness–Eveningness Questionnaire, a survey that provides an index

of arousal that reflects circadian activity. In line with this finding, the present results yielded a significant relationship between diurnal drinking patterns in old rats and their performance at the longest ITI of the DNMTS test. Specifically, the old rats that showed least entrainment to the dark–light cycle also performed worse in this condition. However, this was the only condition in which this relationship was observed and it must be noted that no such relationship was seen in our previous research [24]. While the present results are encouraging in this regard, the reliability of the finding needs to be established. Rhythmically-controlled drinking patterns may well be an indicator of age-related changes in cognition, although it may be possible to identify stronger links between neurocognitive function and other circadian rhythms. One strategy for addressing this important question is to try to relate circadian changes that directly impact brain function (e.g., glucose metabolism, availability of central neurotransmitters) to differences in cognitive performance across peak and off-peak arousal times.

An interesting feature of the present results is the finding that young rats, tested in the AM, generally performed better than young rats tested in the PM. These differences were substantial in the NMTS learning and DNMTS conditions and persisted to a lesser degree in the DNMTS-R condition. As noted above, the TOD effects in young and old rats differed in that they were in opposite directions and, in contrast to the aged group, young rats showed the effect in the NMTS learning condition. However, in one important respect, they were similar—in both groups, the TOD effects in the DMTS condition were greatest at the longer ITIs. While TOD effects have been reported in young human adults, in demonstrating this effect in an animal model, the present results offer convergent evidence that biologically-controlled diurnal rhythms influence cognitive performance across adulthood, and possibly even across the entire life span [10].

The results of DNMTS-R testing underscore the linkage between circadian rhythmicity and cognitive performance, at least in the old rats. As reported above, the DNMTS condition yielded a strong TOD effect in which old rats tested early in the activity cycle performed better at the longer ITIs than old rats tested late in the cycle. When time of testing was reversed in the DNMTS-R condition, the same TOD effect was observed, with old rats transferred from late to early testing performing better on both measures than old rats transferred from early to late testing. The dramatic improvement of the Old-PM → AM Subgroup is particularly noteworthy. In demonstrating the effects of early and late testing in the same animals, the combined results of the two delayed conditions speak to the robustness of the TOD effects in old rats.

Interestingly, although young rats that were switched from AM to PM testing in the DNMTS-R condition generally made fewer errors than the young rats that were switched from PM to AM testing, differences did not reach statistical significance. This outcome may be taken as an indication that the TOD effect in the DNMTS condition may have been weaker in the young rats. In line with this possibility, and as can be seen in Fig. 2, the young rats exhibited the TOD effect only on the error measure whereas in the old rats the effect was seen on both latency and error scores. A contributing factor to a reduced TOD effect in the young rats' may have been their superior resiliency and increased capacity to benefit from their experience in the DNMTS condition, regardless of time of testing.

It has been suggested that TOD effects, particularly in elderly subjects, are linked to a loss of inhibitory control [26]. Indeed, the TOD effect has been observed reliably, in animals and humans, on tasks that require subjects to withhold a dominant response and frequently to suppress irrelevant information. The water-based NMTS task used in this study contrasts from other tasks in that it does not require the inhibition of a specific response. It might be argued that, during the test trials, attention to the potentially distracting sample stimulus must be suppressed, and that the old rats were deficient in that regard. As well, it is conceivable that different strategies came into play throughout training and testing, and that, for optimal performance to occur, inappropriate strategies would have to be suppressed. However, if such factors contributed significantly to the TOD effect, the effect would have been manifested more generally than it was in both young and old rats. A reasonable conclusion is that disinhibitory tendencies can exacerbate the TOD effect under certain conditions, but that the effect can also be expressed on cognitive tasks that demand little or no inhibitory control.

A related possibility is that the various subgroups were differentially affected by the build-up of non-specific interference over the course of training and testing. The effects of proactive interference could not be measured in this study but, to the extent that they were operative, they likely interacted with the effects of ITI and contributed to the maximal disruption at long intervals in the delayed conditions. Such an outcome is in line with evidence that memory loss in young adult rats with hippocampal damage and old rats with presumed hippocampal dysfunction, is greatest under conditions that combine high interference and long delays [19,20].

Finally, it might be argued that the relatively poor performance of the Old-PM and Young-AM Subgroups is due to fatigue, reduced motivation, or other performance-related factors. A number of points counter this argument. All subgroups were highly motivated and physically capable of performing the task, as reflected in comparable latencies and error scores on sample trials in all conditions of the experiment, and on the test trials during NMTS learning, where all subgroups displayed rapid improvement over the 10-session training period. As well, the selectivity of the TOD effects argues strongly against a performance-related argument. Although time-of-testing affected young and old rats somewhat differently, in both groups, the effect was specific to certain conditions. As indicated above, there was no TOD effect in either population at short ITIs in DNMTS and DNMTS-R

testing and, in the aged group, there was no indication of a TOD effect during NMTS learning. The most likely interpretation of these results is that CNS function is influenced by circadian rhythmicity and that, with advancing age, changes in diurnal rhythms can exacerbate age differences in aspects of cognitive performance. Our research with a rat model clearly points to the vulnerability of memory processes under hippocampal control, but other brain regions (e.g., frontal lobes) are probably also affected. The challenge of identifying the learning and memory processes that are susceptible to TOD effects, and the particular circadian rhythms that directly affect underlying brain function in young and old adults, is emerging as a priority in our research program.

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