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Circadian Activity Associated With Spatial Learning and Memory in Aging Rhesus Monkeys

G.E. Haley¹, N. Landauer², L. Renner², A. Weiss², K. Hooper², H.F. Urbanski^{1,2,3}, S.G. Kohama², M. Neuringer², and J. Raber^{1,2,4}

¹Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, Oregon 97239, USA

²Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, Oregon 97006, USA

³Department of Physiology and Pharmacology, Oregon Health and Science University, Portland OR 97239, USA

⁴Department of Neurology, Oregon Health and Science University, Portland, OR 97239, USA

Abstract

In rodents, spatial learning and memory tests require navigation, whereas in nonhuman primates these tests generally do not involve a navigational component, thus assessing nonhomologous neural systems. To allow closer parallels between rodent and primate studies, we developed a navigational spatial learning and memory task for nonhuman primates and assessed the performance of elderly (19–25 years) female rhesus monkeys (*Macaca mulatta*). The animals were allowed to navigate in a room containing a series of food ports. After they learned to retrieve food from the ports, a single port was repeatedly baited and the animals were tested until they learned the correct location. The location of the baited port was then changed (shift position). We also determined whether test performance was associated with circadian activity measured with accelerometers. Performance measures included trials to criterion, search strategies, and several indices of circadian activity. Animals learned the task as reflected in their search strategies. Correlations were found between the number of initial or shift trials and circadian activity parameters including day activity, dark:light activity ratio, sleep latency, and wake bouts. Thus, disruptions in circadian rhythms in nonhuman primates are associated with poorer performance on this novel test. These data support the usefulness of this spatial navigational test to assess spatial learning and memory in rhesus monkeys and the importance of circadian activity in performance.

Keywords

Spatial learning and memory; rhesus monkey; circadian activity

Correspondence: Dr. Jacob Raber, 3181 SW Sam Jackson Pkwy, Mail Code L470, Portland, OR 97239, Phone: (503) 494-1524, Fax: (503) 494-6877, E-mail: raberj@ohsu.edu.

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Introduction

In nonhuman primates, spatial memory has been traditionally tested using the Wisconsin General Testing Apparatus (WGTA) (Boothe and Sackett, 1975; Harlow, 1959) or computer-based tasks including the delayed response (DR) task (Ramos et al., 2008; Lacreuse et al., 2002; Rapp et al., 2003). Studies measuring performance in the DR task demonstrate a consistent age-related decline (Rapp and Amaral, 1989). However, these tasks have limitations. They require extensive training and do not assess the animal's ability to navigate through space, as the stimuli are presented within a restricted space and selections can be made without involving whole body movement. All the information is within one field of view, giving the subject an egocentric frame of reference. In contrast, the tasks most often used in rodents to assess impairments in spatial learning and memory with age or following environmental or physiological challenges include the Morris water maze (Morris, 1984; Benice et al., 2006; Rola et al., 2004; Villasana et al., 2006), radial arm maze (Touzani et al., 2003; Olton and Samuelson, 1976), and the Barnes maze (Barnes et al., 1994; Bach et al., 1995; Raber et al., 2004), all of which involve navigation and orientation within a larger relative space. These tasks require the subject's perspective of a world-centered (allocentric) frame of reference, with information found throughout a complex environment in which the participant has to move. In human neuropsychological testing, it is well documented that direct inferences cannot be made about performance on allocentric tests from performance on egocentric tests (Maguire et al., 1998; Maguire and Cipolotti, 1998; Habib and Sirigu, 1987; McCarthy et al., 1996). Therefore, new nonhuman primate tasks requiring navigation are needed to bridge inconsistencies between rodent and nonhuman primate studies, to allow comparison among species, and to facilitate investigation of the neural basis of spatial cognition in nonhuman primates.

Furthermore, the spatial tasks typically used with these two animal models have nonhomologous neural bases. For instance, spatial working memory, as measured in the DR task with monkeys, is particularly sensitive to prefrontal cortex damage (Levy and Goldman-Rakic, 1999). In contrast, spatial learning and memory in rodents, as assessed with navigational tasks, depends heavily on hippocampal function and is impaired by experimental damage to this structure (Raber et al., 2004; Cho et al., 1999). Recent studies in monkeys demonstrate that tasks requiring an allocentric representation are dependent on an intact hippocampus, whereas egocentric tasks are not (Banta Lavenex et al., 2006; Banta Lavenex and Lavenex, 2008). The hippocampus is one of the first brain structures to deteriorate in natural aging (Geinisman et al., 1995; Winocur and Gagnon, 1998), and performance on tasks associated with hippocampal function declines with age in both rodents and humans (Maguire et al., 1996; Rosenbaum et al., 2001).

Object recognition tests such as delayed matching (or non-matching) to sample rely in part on a functional medial temporal lobe (Rapp and Amaral, 1991), as documented in human, nonhuman primate and rodent subjects (Winocur, 1992a; Winocur, 1992b; Zyzak et al., 1995; Shimamura, 1994; Rapp and Heindel, 1994). Elderly rhesus monkeys (≥ 18 years) exhibit a decline in performance on these tasks, although it is more variable and less severe than their impairment in spatial working memory (Rapp and Amaral, 1991). However, object recognition tasks differ from navigational spatial tasks in many essential ways, including the areas within the medial temporal lobe that mediate the tasks (Murray et al., 2007).

A few other investigators have developed spatial tasks requiring navigation for nonhuman primates (Rapp et al., 1997; Hampton et al., 2004; Zhang et al., 2008; Wang et al., 2007), including one for squirrel monkeys using eight food ports, four of them baited with a preferred food reward each trial (Ludvig et al., 2003). In the current study we evaluated the performance of aged rhesus monkeys in a task where only one out of ten ports was baited. Baiting only one

port allows assessment of distinct search strategies, similar to those used in the analysis of Barnes maze data in mice (Raber et al., 2004). In the rodent Barnes maze, sham-irradiated mice with hippocampal damage use more spatial searches to find the escape tunnel than irradiated mice (Raber et al., 2004).

A factor contributing to a decrease in cognitive function in the elderly is age-related disruption of sleep cycles. Elderly people sleep less and the decrease in sleep correlates to a decrease in cognitive function (Haimov et al., 2008; Morin et al., 1998). While similar sleep disturbances have been reported in the rhesus monkey (Perret and Aujard, 2006) together with age-related changes in circadian hormone secretion (Downs et al., 2008; Downs et al., 2007; Urbanski et al., 2004; Sitzmann et al., 2008), the potential relationship between alterations in their circadian rhythms and cognitive performance has not been examined.

In the present study, we tested the hypothesis that aged rhesus macaques can learn the location of a single baited port in a 10-port system. We developed this task and used it to assess performance and search strategies, as a first step toward establishing a spatial memory task for aged nonhuman primates. Furthermore, we monitored circadian activity levels to test the hypothesis that individual performance on the spatial task correlates with activity patterns.

Methods

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Oregon National Primate Research Center (ONPRC) at Oregon Health & Science University. The study involved 16 aged (age range: 19-25 years, mean age = 21.25 years) female rhesus monkeys (*Macaca mulatta*). The animals were housed in pairs in a temperature controlled environment (24°C) under a fixed 12 h light:12 h dark photoperiod (lights on from 7:00 to 19:00 h) with *ad libitum* access to drinking water. They were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC 1996). The animals were acclimated to daily removal from their home cage, transport to the testing room in transfer boxes, and human handlers. Furthermore, all animals had been previously trained in cognitive tasks in an automated testing apparatus. No monkey had chronic neurological disease or surgical intervention that interfered with performance on the task. Subjects tested on the same day were alternated and removed from the room between trials. The animals were not food restricted and received preferred candy or nuts as positive reinforcement in all phases of testing. The animals were fed primate chow (1500h; Purina Mills, St. Louis MO) in amounts appropriate for their weight twice each day, once in the morning (0800h) and once in the late afternoon.

Apparatus

A schematic drawing of the testing room is illustrated in Figure 1. The testing area consisted of a 2.44 × 3.45 m playroom with a custom made 157 × 38 cm bank of ten ports mounted on the far wall from the entrance to the room. Each port was 7.6 × 10.2 × 7.6 cm and had a top-mounted gray, opaque swinging door and infrared photobeam to detect port entry; the photobeam sensors were interfaced with a computer (Med Associates Inc., St. Albans, VT). The distance between the individual ports was 5 cm. The room included a door with a one-way observation window, an adjacent entry port for the animals, and a smaller, one-way observation window in the side wall. The behavioral performance of the animals was tracked live and on videotape using Noldus Ethovision software (Noldus Information Technologies, Leesburg, VA). For data analysis, port doors were numbered 1-10 on the computer screen, starting on the left when facing the ports.

Testing Procedures

There were four phases of testing: *acclimation*, *training*, *initial*, and *shift* phase. Animals were tested five days per week, with at least four trials per day, and each trial was 2 minutes long. Each phase of testing was completed once criteria were achieved and the monkey was immediately moved to the next phase of testing until four trials per day were completed. In the *acclimation* phase of testing, monkeys were placed in the room with all ten ports baited, with the port doors propped open so that the food rewards were prominently visible. The criterion for the *acclimation* phase was retrieval of the treats from more than eight ports per trial in two consecutive trials. During the *training* phase, all ten ports were baited but with the rewards hidden behind opaque doors. The criterion for the *training* phase was retrieval of rewards from at least eight ports in two consecutive trials. For the *initial* phase of testing, only one designated port was baited. Monkeys were pre-assigned randomly to having either the third or seventh port baited. The criterion for the *initial* phase was retrieving the reward from the baited port within the first two ports searched for three consecutive trials. Once the *initial* phase criterion was reached, a different pre-assigned port was baited for the *shift* phase. Monkeys who were initially trained to retrieve the reward from port three were then trained to retrieve the reward from port six (n=5), while monkeys initially trained to retrieve the reward from port seven were trained to retrieve the reward from port four (n=8). As before, the criterion for the *shift* phase was to locate the reward within the first two searches in three consecutive trials. The sequence of ports searched on each trial was recorded as well as the number of trials to reach each criterion.

Activity Recording—Continuous activity and sleep data were recorded for all animals over at least a two-week period using external Actiwatch activity monitors (Mini Mitter, Bend, OR) attached to 3" diameter nylon or 3.5" aluminum collars (Primate Products, Redwood City, CA). These activity sensors measure whole body movements of the animal but not movement of appendages alone or chewing (Papailiou et al., 2008). Monkeys were sedated with 5 mg/kg ketamine to place and remove activity collars, and these days were excluded from the activity data. The activity data were downloaded from the Actiwatch to a computer and analyzed with Rhythmwatch and Sleepwatch software (Mini-Mitter Co., Inc.). Parameters measured were daytime activity (acceleration of movement during the light phase of the day), nighttime activity (acceleration of movement during the dark phase of the day), dark:light activity ratio (night activity/day activity), sleep latency (the time from onset of the dark phase to when the acceleration of movement disappeared) and number of wake bouts (number of periods of acceleration of movement during the dark phase). Examples of activity profiles are illustrated in Figure 2.

Three monkeys were excluded from the activity analysis. Two of these were excluded because their temperaments did not fit with other animals, and therefore were caged individually instead of being pair-caged. Individually caged animals are more active than paired animals, thus their activity data are not comparable. The third monkey was consistently a major outlier, therefore her data were excluded from the analysis. Consequently activity data from only ten animals were used.

Assessment of Search Strategies

The sequence of port selections prior to reaching the baited port were categorized as representing one of three search strategies: *spatial*, *serial*, or *random*. *Spatial* searches were defined as searching the baited port or the one adjacent to it and retrieving the reward within the first four ports searched. *Serial* searches were defined as searching ports in consecutive locations in order to the right or left of the first port choice. *Random* searches were defined as not following either of the proceeding patterns, nor showing another systematic pattern.

Data Analysis

Search strategy and number of trials data were analyzed by Prism (Graphpad Prism, San Diego, CA). Search strategies were analyzed using Friedman's non-parametric test and two-tailed Wilcoxon signed-rank tests. Because the animals were tested to criterion and therefore completed different numbers of initial and shift trials, for each animal the search strategies during the initial and shift phases were assessed separately for the first and second half of all initial and shift trials. Data are presented as means $\pm$ SEM and $P < 0.05$ was considered significant. Pearson product-moment correlations were computed with Prism software.

Activity data was analyzed with Rhythmwatch and Sleepwatch software (Cambridge Neurotechnology Ltd, Cambridge, UK) for activity parameters and sleep quality, respectively. Pearson's product-moment correlations were computed using Prism Software.

Results

Task Learning

During the task, the monkeys navigated from port to port and could not reach all the ports from a stationary position. Thirteen monkeys demonstrated learning in the task. However, three other monkeys failed to pass the training criterion, because they would not search the hidden ports; consequently, they were excluded from the study. Those monkeys completing the study took a mean of 26.8 ± 4.5 trials, to reach criterion for the initial phase and 39.2 ± 8.9 trials, to reach criterion for the shift phase. Once the criterion for a particular phase was achieved, the animal proceeded to the next phase of testing. This created a performance-dependent variable delay that could potentially affect performance during the next phase. However, no difference in performance was observed based on whether the first trial of the next phase started within the same day as the last trial of the previous phase or the following day for either the initial ($P = 0.6$) or shift ($P = 0.9$) phase.

Search Strategies

For the first half of the initial trials, monkeys used spatial, serial, or random search strategies, with serial searches being most frequent. There was a significant difference in the percentage of spatial, serial, and random searches during the first half and the last half of the initial trials ($F_r = 45.10$, $P < 0.001$; Figure 3). In the second half of the initial trials, monkeys used a significantly higher percentage of spatial searches than in the first half of the trials ($P < 0.01$), with significantly higher percentage of spatial searches than serial searches ($P = 0.02$), reflecting their learning of the correct port position. Conversely, the percentage of both serial and random searches decreased in the second half of the trials. Thus, the number of spatial searches for the total initial trials correlated with the total number of trials indicating learning of the task [$R^2 = 0.56$, $P = 0.003$, $n = 13$ (Figure 4A)].

At the beginning of the shift phase, the monkeys returned to the initial port position, before searching for the shift position with a predominately serial search strategy. The same pattern search strategy change was observed in the shift trials, with a significant change during first and the last half of the shift trials ($F_r = 42.21$, $P < 0.0001$). In the first half of the trials, monkeys used a significantly higher percentage of serial searches than spatial searches to find the baited port ($P = 0.02$). In the second half of the shift trials, monkeys used a slightly higher percentage of spatial than serial searches (not significantly different), but they used a significantly higher percentage of spatial searches ($P = 0.01$) and lower percentage of serial searches ($P = 0.03$) when compared to the first half of the trials. The percent of random searches also decreased in the second half of the shift trials and random searches were significantly less than spatial ($P < 0.001$) and serial ($P < 0.001$) searches. The number of spatial searches correlated with the total number of trials to criterion, indicating learning of the task [$R^2 = 0.72$, $P = 0.0002$, $n = 13$].

(Figure 4B)]. There was a strong correlation between the number of repeats of spatial search trials for the initial position and the first spatial search for the shift position [$R^2 = 0.84$, $P = 0.0001$, $n = 13$ (Figure 5)]. Furthermore, the monkeys that had the fewest trials out of the group on the initial phase of testing had the most trials in the shift phase of testing.

Correlation of search strategies and number of trials

Initial position—Across individual animals, as the number of trials increased, the percent of spatial searches decreased in a linear fashion [$R^2 = 0.62$, $R = -0.79$, $n = 13$, $P = 0.001$ (Figure 6A)]. A reverse pattern was observed in the serial searches. As the number of trials to criterion increased, the percent of serial searches increased in a linear fashion [$R^2 = 0.62$, $R = 0.75$, $n = 13$, $P = 0.002$ (Figure 6B)].

Activity

An inverse correlation was observed between day activity and the number of initial trials [$R^2 = 0.41$, $R = 0.64$, $n = 10$, $P = 0.04$]. As day activity recorded from the animals increased, the number of initial trials decreased. A positive correlation was observed between the dark:light activity ratio and the number of initial trials [$R^2 = 0.53$, $R = 0.73$, $P = 0.02$, $n = 10$ (Figure 6C)]. As the dark:light activity ratio increased, the number of initial trials increased.

A direct correlation was observed between sleep latency and the number of shift trials [$R^2 = 0.44$, $R = 0.66$, $n = 10$, $P = 0.03$], between wake bouts and number of shift trials [$R^2 = 0.45$, $R = 0.67$, $n = 10$, $P = 0.03$ (Figure 6D)]. Animals with a longer sleep latency and those with more wake bouts required more shift trials to complete the phase.

Discussion

In this study, we developed a new test to measure spatial learning and memory in the rhesus monkey. While a few other laboratories have developed spatial tests with allocentric representation for nonhuman primates (Rapp et al., 1997; Hampton et al., 2004; Wang et al., 2007; Ludvig et al., 2003), their tests are different from the test used in this study. One major difference is in some of the former tests (Rapp et al., 1997; Hampton et al., 2004) the animals were tethered and this restraint could have interfered with their behavior. The Rapp task is similar to the radial arm maze in rodents, with eight ports on the floor with visual cues to direct the monkeys. Conversely, the Hampton task is a foraging task in an open room with the monkeys on a tether rope. The spatial maze developed by Wang is a three dimensional outdoor maze with four unique compartments. Finally, the Ludvig task used food ports to assess spatial memory in squirrel monkeys (Ludvig et al., 2003). Like the Ludvig test, our test permitted the monkeys to move freely without a tether. This allowed the monkeys full range of the room and exploration of the ports. Baiting the ports with preferred candies or nuts kept the animals motivated to search the ports. The animals in our study were able to learn the location of a single baited port in a series of ten ports, demonstrating that elderly rhesus macaques are able to complete spatial tasks outside of the traditional WGTA. Therefore, this test might provide valuable insight into nonhuman primate spatial memory. While currently our testing room has one series of ten ports, based on the performance of the monkeys, we plan to expand the number of ports and walls containing ports in the testing room to increase the spatial navigational component.

The animals learned the task as evidenced by the use of a spatial search strategy, instead of using serial or random strategy (Figure 3, 4, 6 A & B). A spatial search strategy is an indication of task learning, since this strategy requires knowledge of the approximate correct location (Raber et al., 2004). A serial search strategy leads to success less efficiently, since on average more ports must be searched before the reward is found. Thus, monkeys that were able to more

often employ a spatial strategy also reached criterion on the task more rapidly (Figure 6 A & B).

The same pattern of serial and spatial searches was observed during the shift position trials, but the correlation of spatial searches to number of trials was stronger than the initial position (Figure 4). One reason for the difference in correlation strength is because the monkeys who learned the task well (had fewer initial trials) repeated the initial spatial search during the shift trials, thus requiring more trials to achieve the criterion for the shift phase. The number of repeated initial spatial search trials and the first spatial search for the shift position showed a strong correlation (Figure 5). This correlation indicates that the monkeys remembered the initial position, and once they learned that the position had moved, they quickly began searching spatially for the shift position.

We also examined the relationship between task performance and measures of general activity and sleep quality. Although studies indicate that aged rhesus monkeys have diminished activity levels and disrupted circadian rhythms (Perret and Aujard, 2006; Downs and Urbanski, 2006), little research has correlated sleep quality and cognitive performance. Results from our study indicate that in elderly monkeys, both daytime activity levels and sleep disturbances correlate with performance on this spatial memory task (Figure 6 C, D).

Human studies indicate that sleep disturbances increase daytime sleepiness and decrease cognitive performance (Perret and Aujard, 2006; Stepanski et al., 1984; Stenuit and Kerkhofs, 2008; Roehrs et al., 2000; Kahn et al., 1970; Kahn and Fisher, 1989; Carskadon et al., 1980; Welford, 1962). Sleep latency and the number of wake bouts during the night can be used as measures of sleep quality. Here, these measures directly correlate with the number of shift trials. Animals with longer sleep latencies and more wake bouts took more trials to complete the shift criterion. This result suggests that performance decreases with shorter and more disrupted sleep. In addition, daytime activity was inversely correlated with number of initial trials, indicating that animals that were more active during the day performed better on the initial phase of the task. These results indicate that sleep quality and activity are correlated to performance in this task, which therefore might be valuable for assessing effects of experimentally-induced circadian variation on cognitive performance.

In conclusion, this study demonstrates that elderly monkeys can learn a spatial learning and memory task that requires spatial navigation to retrieve a reward from a single food port out of a series of ten ports. Furthermore, performance on the test was positively correlated with activity levels, acquired through Actiwatch recorders, and a decrease in sleep quality was correlated with a decrease in performance. This new task might be valuable for studying age-related and circadian activity-related cognitive decline in nonhuman primates.

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Figure 1. Schematic representation of the aerial view of the testing area

A represents the door for human handlers to enter the room, equipped with a one-way window for observation. B represents the animal jump door for the monkeys to enter the room. C represents a one-way observation window, smaller than the observation window on the door. D represents the location of the ports. All ten ports were in the same bank of ports, with the port doors facing the human handler door. Two cameras were mounted on the ceiling to view the room, one directed at the port doors and one to view the entire room.

Figure 2. Examples of activity profiles

Representative actograms from two elderly female rhesus macaques who were pair-housed, emphasizing individual differences in activity. The Panels A and B display activity monitored for 35 consecutive days using Actiwatch recorders. Data are double-plotted to aid in the visualization of the activity rhythms. The height of the vertical lines within the actograms is indicative of the intensity of physical activity. The Panels C and D display the corresponding average 24-hour activity during the 35 days of monitoring. The horizontal black and white bars indicate time of night and day on a 12 h light:12 h dark photoperiod. Panels A and C are from an animal with higher overall activity, whereas Panels B and D are from an animal with lower overall activity.

Figure 3. Search strategies change during task, indicating learning of the task

The percentage of each search strategy used in both the first and last half of the initial (I) and shift (S) phases. (* $P < 0.02$, between spatial and serial search strategy, # $P < 0.02$, between first half and last half of trials for one search strategy category)

Figure 4. Correlation between trials to criterion and spatial searches

Scatterplot of data for individual animals demonstrating the correlation of spatial searches and the number of trials to criterion in both the initial phase (Panel A; $R^2 = 0.56$, $R = 0.75$, $n = 13$, $P = 0.003$) and the shift phase (Panel B; $R^2 = 0.72$, $R = 0.85$, $n = 13$, $P = 0.0002$).

Figure 5.

Scatterplot showing correlation between the number of trials in which the monkey repeated a spatial search for the initial position at the beginning of the shift phase and the trial number of the first spatial search for the shift position ($R^2 = 0.84$, $R = 0.92$, $n = 13$, $P = 0.0001$).

Figure 6. Spatial and serial searches and circadian activity correlate with the number of trials
 Panels A and B show individual data plots demonstrating correlations between search strategies and number of trials. Panel A shows the inverse correlation between the percent of spatial searches and the number of initial trials ($R^2 = 0.62$, $R = -0.79$, $n = 13$, $P = 0.001$). Panel B shows the positive correlation between the percent of serial searches and the number of initial trials ($R^2 = 0.57$, $R = 0.76$, $n = 13$, $P = 0.002$). Panels C and D are scatterplots demonstrating correlations between different activity parameters and performance on the test. Panel C demonstrates a positive correlation between the dark:light activity ratio and number of initial trials ($R^2 = 0.53$, $R = 0.73$, $P = 0.02$, $n = 10$), and panel D demonstrates a positive correlation between wake bouts and number of shift trials ($R^2 = 0.45$, $R = 0.67$, $n = 10$, $P = 0.03$).