Impaired Spatial Working Memory Learning and Performance in Normal Aged Rhesus Monkeys

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Abstract

Aged non-human primates may have deficits in a variety of cognitive functions. However, it is possible that at least some age-related performance deficits relate to a deficit in initial task learning. To assess this, aged rhesus monkeys were trained to perform a Self-Ordered Spatial Search task (SOSS) using the same training and testing parameters used previously with normal young animals. Aged animals failed to reach criterion at the easiest task level. In an attempt to improve learning, a group of aged animals were first trained on SOSS using a standard 5 sec ITI, followed by trials with low inter-trial interference (ex., a stimulus used in a trial would not be used again for the next 2 trials) or with trials in which the spatial distance between the stimuli on the screen was maximized. Because performance improved but failed to reach criterion, this was followed by sessions with increasing ITIs (from 5 sec to 10 or 15 sec). Only increasing the ITI improved the performance of the aged animals enough to allow them to learn the task to criterion. Once the criterion was reached, memory was taxed by increasing the delay between stimulus presentations and increasing the number of spatial positions to be remembered. Performance declined for young animals, but even more so for aged animals. The results of the present study suggests that aged primates have difficulty initially learning a complex working memory task, and that the ITI may be an important parameter to manipulate to improve learning. However, once the task is learned, performance of aged animals is inferior to that of young animals, particularly when memory demands are increased. .

Keywords

Aged; Primate; Spatial; Working Memory

1. Introduction

Similar to aged humans, aged non-human primates show a variable decline in a broad array of cognitive functions including memory, executive functioning and attention [1 - 5], some individuals cognitively aging more successfully than others [6 – 8]. Understanding how these processes are affected by normal aging is essential to differentiating between cognitive

decline due to an age-related pathological process such as Parkinson's disease (PD) and normal cognitive decline due to aging. Thus, because of cognitive performance characteristics similar to those in humans, non-human primate models of age-related cognitive decline can play an important role in furthering our understanding of how various cognitive processes are affected by age and in developing and evaluating potential new treatments directed against age-related cognitive decline in humans.

Aged animals have been shown previously to exhibit deficits in performance of complex spatial working memory tasks [2, 9 – 17], which are thought to be dependent on frontal lobe function [4, 18]. Receiving less attention, however, are descriptions of the difficulty with which aged primates acquire new strategies to perform such complex tasks. Several types of tasks have been used to assess spatial working memory learning in primates including the spatial version of the Delayed Recognition Span Test (DRST-S) task conducted in a Wisconsin General Testing Apparatus (WGTA) as well as an automated Self-Ordered Spatial Search (SOSS) task, conducted using a touchscreen system. DRST-S requires animals to displace a new plaque among an increasing set of serially presented identical plaques, while the SOSS task requires animals to touch identical squares located in different spatial locations in a self-ordered sequence without returning to a previously touched square. Both tasks show an age-dependent decline in performance [2, 10, 13-15, 17]. However, at least some findings suggest that aged animals were impaired at initially learning the task and were not able to reach the same performance criterion as young animals [17]. In some instances, aged animals benefited from pre-training on a simpler version of the task [15]. If aged animals have difficulty initially learning a task to the same degree as young animals, then aged animals may continue to underperform relative to young animals especially when attempting more demanding tasks. Thus, comparisons between performance of aged and young animals on a spatial working memory test might be more appropriate if aged animals were trained to perform the task at the same initial criterion level as young animals.

The causes of impaired learning by aged animals are not clear but may be related at least in part to the concept of interference (i.e., memory from a previous trial interfering with performance on the current trial). Aged animals and humans are highly susceptible to interference on working memory tasks in particular [16, 19 – 26]. Other parameters that may affect how well aged non-human primates learn to perform a spatial memory task also include the spatial proximity of the stimuli, the degree of repetition of stimuli between consecutive trials and the inter-trial interval [16]. All of these conditions may contribute to interference between the current trial and the previous trial in the testing session, or introduce interference within a single trial. Thus, it is important to determine if, through decreasing interference, aged animals can initially learn a complex spatial working memory task to the same degree as young animals.

In order to characterize the cognitive profile of aged primates and compare their performance to that of young animals on a spatial working memory task, normal aged male rhesus macaques were trained to perform a SOSS task. Due to difficulties in learning the standard version of this task to the same performance criterion as young animals [27], a subset of aged animals were trained on a version of the SOSS task that manipulated a combination of the inter-trial interval, spatial location of stimuli, and stimulus repetition between trials. Once all aged animals learned the task to the same degree as young animals, their memory was stressed by adding additional stimuli to each trial, and then by increasing the within-trial delay.

2. Methods

2.1 Subjects

Sixteen aged adult male *Macaca mulatta* monkeys (range: 15-22 years of age, mean = 19.7 yrs (one animal was 15 years old; others were 18 years or older); weight 8.0 to 18.1kg at beginning of study) used in this study. In addition, for comparison with normal young adult animals, historical data were used from 11 male *Macaca fascicularis* (range: 5-7 years of age at testing, mean = 5.8 yrs) tested under the same conditions (i.e., all animals were tested using the same testing apparatus, the same testing program, and the same rewards). Animals were maintained on a food-restricted schedule and provided water ad libitum. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Thomas Jefferson University Institutional Animal Care and Use Committee.

2.2 Apparatus and general testing procedures

For training and testing, animals were transferred to a testing cage located in a quiet room away from the main colony room, using a specially designed transfer cage. Affixed to the front of the testing cage was a testing panel that included a touch-sensitive computer monitor, a speaker, and a pellet delivery system for delivery or rewards (Fruit Crunchies - 190mg, Bio-Serv, Inc.). Before testing began, the room lights were turned off and white-noise was provided to reduce outside noise interference.

2.3 Self-ordered spatial search (SOSS) task

The SOSS task was used to assess spatial working memory. We chose this task, instead of a standard spatial delayed response (DR) task because we felt it provided certain advantages over DR for studying spatial working memory. The DR task has significant attention and memory demands: both attention (particularly when cues are presented for a short period of time) and memory (remembering where the cue was presented over increasingly long delay periods) are necessary in order to perform the task correctly. Accurate DR performance requires that the animal pay attention to the cue and possible reward location and remember that information and discriminate the current trial from information presented earlier in the testing. The SOSS task is a spatial working memory task with minimal attentional demands (e.g., SOSS task). The SOSS task requires monitoring of self-generated choices with performance of the task entirely dependent on memory of the visual stimuli, their locations, and previous responses and is not dependent on general attentional functioning [28 - 30]. During training, animals are shown 2 squares simultaneously in different locations on the screen, with 8 possible locations in which the squares can appear (Figure 1A). The monkey must touch each box in sequence without touching the same box twice in order to receive a reward (Figure 1B). First, the animal touches one of the boxes (within 30 seconds of stimulus onset) causing it to turn red for 100 msec, and receives a reward. Both boxes then disappear from the screen for 2 seconds and then reappear in the same locations as before. The animal must touch the previously unrewarded box in order to receive a reward. Once an animal's performance improves, he only receives a reward if the entire trial is correct. If a monkey returns to the box that was already touched, it is recorded as a "commission error" and there is a 10-second time out during which time the screen goes black. If he touches both boxes in sequence without a repetition, the trial is recorded as "correct". If the animal fails to touch the screen during the 30s stimulus presentation, the trial is recorded as an "omission error". The inter-trial interval (ITI) is 5 seconds. A daily session consists of 40 trials with 2 square sequences, and trials were randomized for stimulus location and repetition. Criterion for learning the 2-Square condition of the SOSS task is three consecutive sessions at 85% performance or better.

Once criterion is reached for the 2-Square condition of SOSS, animals advance to the 3-Square condition in which they are presented with 3 squares simultaneously in different locations on the screen and must touch each box in sequence without touching the same box twice in order to receive the reward. A daily session then consists of 10, 2-Square trials followed by 30, 3-Square trials. Once an animal's performance is asymptotic on the 3-Square trials, he advances to the 4-Square condition in which 4 squares are presented simultaneously in different locations on the screen and the animal must again touch each box in sequence without touching the same box twice in order to receive the reward. A daily session then consists of 10, 2-Square trials followed by 10, 3-Square trials, followed by 20 4-Square trials. Once an animal's performance is asymptotic on the 4-Square trials, he receives 30 trials per session with 10 trials each of 2-, 3-and 4-Square conditions pseudorandomly intermixed. All other task parameters for the 3- and 4-Square conditions are the same as in the 2-Square condition.

2.4 Self-ordered spatial search (SOSS) task with delays

After an animal's performance was stable at all 3 levels of the SOSS task, memory demand was increased by lengthening the delays between each presentation within a trial. The delay was initially increased by 1 sec from the starting delay of 2 sec. and continued to increase by 1 sec. Because performance at the 2-Square condition remained high, the increase in delay was stopped only once the animal's performance at the 3- and 4-Square conditions were significantly different from their performance using the standard 2 sec delay. Thus, the final delay for each animal varied from 4 to 8 sec. Once SOSS performance (with delays) was stable, animals were tested on the standard SOSS twice per week and the SOSS with maximum delay once per week. In addition to recording each animal's percent correct responses, commission errors, omission errors and the location of the commission errors in the trial sequence were also recorded. For example, in the 3-Square condition, an animal could make a commission error either at the second or third stimulus; in the 4-Square condition, the animal could make a commission error at the second, third or fourth stimulus presentation within a trial.

2.5 Stimulus manipulation conditions of the SOSS task

Because the SOSS task was particularly difficult for aged animals to learn (compared to young adult animals) when using standard training paradigms, we manipulated three different parameters to assess how each may contribute to learning of this complex spatial working memory task. Therefore, after an average of 23 training sessions (range 20 to 28 sessions) with the 2-Square condition as previously described, six aged animals were randomly chosen to receive additional training with two different stimulus manipulation conditions. In the Near/No Repeat (N/NR) condition, the trial parameters were the same as in the standard 2-Square condition, but 2 squares were chosen such that the spatial distance between the stimuli on the screen was always close (range: 50 – 90mm), and no stimulus appeared in the same location for at least the next 2 trials (Figure 1C). In the Far/Repeat (F/R) condition, the trial parameters were the same as in the standard 2-Square condition, however, 2 squares were chosen such the spatial distance between the stimuli on the screen was maximized (range: 140 – 230mm) so that at least one potential stimulus location was empty between the two stimuli presented. In addition, one of the stimuli locations used was also used in the following trial (Figure 1D). Each animal received 10 sessions of 40 trials for both conditions, but the order of the two conditions was randomized such that three monkeys received the N/NR condition for the first 10 sessions, and three received the F/R condition for the first 10 sessions.

2.6 Inter-trial interval manipulation conditions of the SOSS task

After completing both the N/NR and the F/R conditions, the same six aged animals were given additional training with the standard version of the 2-Square SOSS task, but the ITI was increased. Animals received 10 sessions of 40 trials with a 10s ITI, followed by 10 sessions of 40 trials with a 15s ITI if they did not meet learning criterion with the 10s ITI.

2.7 Data Analysis

All statistical analyses were performed using SPSS 17.0 software. Data are presented as mean ± SEM where appropriate. For SOSS learning, a repeated measures ANOVA was used with the session number as the repeated measure. An independent samples t-test was used to compare the number of total trials required to reach learning criterion. For SOSS 2-, 3- and 4-Square performance, repeated measures ANOVA was used with the number of squares as the repeated measure. For SOSS learning across the training manipulations, a repeated measures ANOVA was conducted with the different manipulations as the repeated measure. When sphericity was not assumed, a Huynh-Feldt correction was used. *Post hoc* Tukey tests were conducted when group differences reached significance.

3. Results

3.1 SOSS Learning

In the 2-Square version of the SOSS task, young mature animals exhibited significant improvements in performance across their first 20 testing sessions [$F(19,152) = 8.648$, $p < 0.001$], whereas aged animals showed no signs of improved learning [$F(19,152) = 0.652$, $p = $ ns] over the same number of sessions (Figure 2). By the third session and beyond, young mature animals performed significantly better than aged animals (all p's < 0.05). Thus, aged animals failed to learn the task at the same rate as the young animals [Session X Age interaction: $F(19,304) = 4.983$, $p < 0.001$].

3.2 Stimulus manipulation and inter-trial interval manipulation conditions of the SOSS task

The performance of aged animals trained in the N/NR condition first did not differ from those trained in the F/R condition first across any of the five manipulation conditions (all p's > 0.05). Therefore, data for these two groups were combined for statistical analyses. Performance on the SOSS task steadily improved across the five stimulus manipulation conditions (Main effect of Condition: $F(4,4) = 19.485$, $p < 0.010$; Figure 3). Further analysis revealed that performance increased between the initial 5s ITI condition and each of the 4 later conditions (all p's < 0.01). In addition, performance improved between the two stimulus manipulation conditions and the 10s and 15s ITI conditions (all p's < 0.05). However, there was only a marginal increase in performance between the first stimulus manipulation condition the animal received and the second, regardless of which condition the animal received first (p = 0.060), and between the 10s and 15s ITI conditions (p = .076).

Using a combination of the different manipulation conditions, all aged animals eventually reached learning criterion on the 2-Square SOSS task. However, the aged animals required significantly more sessions to reach criterion than young mature animals, which all learned the task under the standard 5s ITI condition (66.75 vs. 25.64 sessions respectively; p < 0.001; Figure 4).

3.3 Performance on 2-, 3- and 4-Square SOSS

After aged animals reached learning criterion on the SOSS task, performance baseline measures for percent correct, percent commission errors and percent omission errors were compared with the same measures taken from the young mature animals. The percentage of

correctly performed trials significantly decreased for both groups as the number of squares (i.e., task difficulty) increased (Main effect of Number of Squares, $F_{(2,38)} = 140.052$, $p < 0.001$), and both groups performed significantly above chance on 2-, 3- and 4-Square trials (p's < 0.05; Figure 5A). However, there was a significant Age X Number of Squares interaction ($F_{(2,38)} = 4.57$, $p < 0.05$) such that aged animals performed as well as young animals on the 2- and 3-Square trials, but performed significantly worse than the young group on the 4-Square trials (38.1 vs 57.6 % correct respectively; $p < 0.05$). Similar results were observed for the percentage of commission errors. Both groups made significantly more commission errors as the number of squares increased (Main effect of Number of Squares, $F_{(2,38)} = 140.38$, $p < 0.001$). Again, there was a significant Age X Number of Squares interaction ($F_{(2,38)} = 4.16$, $p < 0.05$) such that aged animals made significantly more commission errors than the young animals only during performance of 4-Square trials (61.0 vs. 42.4% commission errors respectively, $p < 0.05$). There were no significant differences in omission errors either within the aged group or between the aged group and the young group across 2-, 3- and 4-Square trials (all p's = ns).

3.4 Performance on SOSS with maximum delays

The maximum delay for each animal varied based on individual abilities, but there were no differences between the maximum delay achieved for the aged (mean 6.0 ± 1.4 sec; range: 4 to 8) versus the young group (mean 6.8 ± 1.5 sec; range: 5 to 9) ($p = ns$). As with the standard SOSS, the percentage of correct trials significantly decreased for both groups as task difficulty increased (Main effect of Number of Squares, $F_{(2,36)} = 451.21$, $p < 0.001$), and both groups performed significantly above chance on 2-, 3- and 4-Square trials (p's < 0.05). In addition, the significant Age X Number of Squares interaction ($F_{(2,36)} = 3.88$, $p < 0.05$) showed that aged animals performed as well as young animals on the 2- and 3-Square trials, but performed significantly worse than the young group on the 4-Square trials (16.8% vs 31.2% correct respectively; $p < 0.05$; Figure 5B). Both groups also made significantly more commission errors as task difficulty increased (Main effect of Number of Squares, $F_{(2,36)} = 413.65$, $p < 0.001$), with aged animals making significantly more errors than the young animals only during the 4-Square trials (Age X Number of Squares interaction, $F_{(2,36)} = 3.29$, $p < 0.05$). There were no significant differences in the number of omission errors either within the aged group or between the aged group and the young group across 2-, 3- and 4-Square trials (all p's = ns).

When performance for the aged animals on the SOSS with maximum delays was compared to their performance on the standard SOSS task (Figure 5 A, B, and C), the percent of correct responses was significantly reduced (all p's < 0.01) and the number of commission errors was significantly increased (all p's < 0.001) for 2-, 3- and 4-Square trials. For the young animals, there were no differences across delays for the 2-Square trials, but the percent correct responses was significantly reduced (all p's < 0.01) and the number of commission errors significantly increased (all p's < 0.01) for 3- and 4-Square trials during SOSS with delays.

3.5 Performance on SOSS with intermediate delays

Because the maximum delay for each animal varied from 4 to 8 seconds in the aged group, we analyzed SOSS performance using a 4 second delay since that was the maximum delay at which all aged animals could reliably perform the task. The percentage of correct trials significantly decreased as task difficulty increased (Main effect of Number of Squares, $F_{(2,36)} = 302.2$, $p < 0.001$) and both groups performed significantly above chance on 2-, 3- and 4-Square trials (p's < 0.05). A significant Age X Number of Squares interaction ($F_{(2,36)} = 5.53$, $p < 0.01$) showed that aged animals performed as well as young animals on the 2- and 4-Square trials, but performed significantly worse than the young animals on the 3-

Square trials (49.1% vs 69.6% correct respectively; p < 0.01; Figure 5C). Similarly, for commission errors, both groups made significantly more errors as task difficulty increased (Main effect of Number of Squares, $F(2,36) = 255.12$, $p < 0.001$). Again, the aged group made significantly more errors than the young group only during the 3-Square trials (Age X Number of Squares interaction, $F(2,36) = 5.248$, $p < 0.01$). There were no significant differences in omission errors either within the aged group or between the aged group and the young group across 2-, 3- and 4-Square trials (all p's = ns).

When performance of the aged animals on the SOSS with a 4 second delay was compared to their performance on the standard SOSS task and the SOSS task with maximum delays, there was a significant Delay X Number of squares interaction ($F(4,56) = 14.97$, $p < 0.001$). Performance was significantly reduced and commission errors significantly increased during both the 4 second and maximum delay conditions when compared to the standard SOSS for 2-, 3- and 4-Square trials (all p's < 0.01), but there were no differences between performance in either of the delay conditions.

There were no performance differences in the young animals between 2-Square trials, but for 3-Square trials, their percent correct responses were significantly reduced during the maximum delay condition compared to the 4 second and standard SOSS conditions (all p's < 0.05). For the 4-Square trials, their percent correct responses were significantly reduced during the maximum and 4 second delay conditions compared to the standard SOSS condition (all p's < 0.05). During the 3-Square trials, their commission errors were significantly increased during the maximum delay condition compared to the 4 second and standard SOSS conditions (all p's < 0.05), and for the 4-Square trials, their commissions errors were significantly increased during the maximum and 4 second delay conditions compared to the standard SOSS conditions.

3.6 Location of commission errors on SOSS

Examination of the location of the commission errors for the 3- and 4-Square trials of SOSS showed no significant differences between aged animals and young, mature animals during performance of the standard SOSS or either of the two delay conditions, except for the second presentation of 4-Square trials during the standard condition (p < 0.001; Figure 6). During 3-Square trials, both aged and young animals made significantly more errors during the third stimulus presentation of the trial than during the second stimulus presentation, regardless of delay (Main effect of Error Location for Standard SOSS, $F(1,19) = 91.69$, $p < 0.001$; Maximum Delay, $F(1,18) = 76.59$, $p < 0.001$; 4 second Delay, $F(1,18) = 207.92$, $p < 0.001$). In addition, all animals made significantly fewer errors during the second presentation and significantly more errors during the third presentation of the stimulus during the standard SOSS than during SOSS with maximum delays (p's < 0.05). On 4-Square trials, animals made significantly more errors at the fourth presentation of the stimulus compared to the second or third presentation of the stimulus for all delay condition. In addition, they made more errors during the fourth presentation and fewer errors during the third presentation for standard SOSS as compared to both delay conditions (all p's < 0.001).

4. Discussion

The results from the present study show that, while aged rhesus monkeys have difficulty learning a spatial working memory task as quickly as young animals, they can learn to perform the task if trained using the right combination of task conditions. Based on the pattern of change observed in the task acquisition data, the stimulus manipulations employed and not the total time of training, appear to be related to the improvements in task acquisition in the aged animals. While overall, aged monkeys were trained over a longer period of time than were young monkeys, aged animals showed no improvement in task

acquisition until stimulus manipulations were instituted. Additionally, once the aged animals learned the task, their performance was inferior to that of young animals, particularly when memory load was increased.

4.1 Spatial working memory learning

Aged animals were unable to learn the SOSS task under the same initial conditions as young animals, confirming earlier reports of age-related learning problems associated with spatial working memory tasks [15, 17] . However, manipulating 3 different parameters within the SOSS task allowed the aged animals to improve their performance. Increasing the distance between the stimuli (F/R condition) as well as not repeating the stimulus location for at least 3 trials (N/NR) both decreased interference between SOSS trials as evidenced by increased performance over traditional trials for aged animals. While both stimulus manipulation conditions caused an improvement in performance in aged animals, performance leveled off, and did not continue to improve until the ITI was increased to 10sec.

The plateau in performance seen in the two stimulus manipulation conditions may be due to the second parameter in each condition that may have inadvertently increased interference across trials. In the N/NR condition, the lack of stimulus repetition may have initially decreased trial-to-trial interference, but over repeated trials, the close proximity of the stimuli within a trial may have caused some inter-trial interference. In the F/R condition, the far distance between the stimuli may have initially decreased inter-trial interference, but over repeated trials, the repetition of 1 stimulus across each trial may have caused some trial-to-trial interference. Unpublished preliminary data from a single additional animal suggests that if stimuli are both far apart and not repeated for at least 3 trials (Far/No Repeat), then learning continues to improve as with an ITI increase.

Increasing the ITI significantly increased the performance of aged animals on the SOSS task over both traditional 5sec ITI learning as well as both stimulus manipulation conditions (N/NR and F/R), suggesting that increasing the ITI helps to reduce trial-to-trial interference. Thus, increasing the ITI improves learning the most, and may do so at least in part by decreasing inter-trial interference, which is known to affect spatial working memory in older humans [23 – 25]. Despite these manipulations, aged animals still required more trials to reach criterion than young animals. However, it is possible that if aged animals were given the SOSS task with a 15s ITI at the onset of learning, they may have been able to learn the task in as few sessions as the young animals learned the task with a 5s ITI. This needs to be assessed in a future study.

Once aged animals learned the task using the various task manipulations, they were all tested again using the initial standard training conditions that young animals used to learn the task and their performance remained high. Thus, aged animals needed a version of the task with decreased interference in order to learn the task, but once the task was learned, they no longer needed trials with reduced interference to maintain good performance on the task.

4.2 Spatial working memory performance

All aged animals tested were eventually able to learn the simplest 2-Square version of the SOSS task to the same criterion as the young animals (> 90%). This contradicts the one previous study that used the SOSS task in aged primates (3 males and 2 females, age: 24.0 ± 0.8) in which aged animals did not perform as well as the young animals [17]. The animals described by Nagahara et al. [17] were initially trained with 2 squares, then moved on to a 3-square condition, using the same parameters as the current study except with a delay that was of shorter duration (0.25 sec). On the 2-square condition, the aged animals'

performance was stable at 50 – 60% and the young animals' performance was stable at around 80%. With the addition of a third square, both groups' performance declined to 20 – 40%. Thus, both groups of animals in the previous study performed worse than those in the current study on both the 2- and 3-square conditions. It is possible that the aged animals in the Nagahara et al study [17] had not truly learning the initial 2-square version of the task. It is unclear why the young animals failed to learn the 3-square version of the task at all and it was not reported whether the animals omitted any trials or not.

When memory was taxed by increasing the number of square locations to be remembered, aged animals in the current study performed as well as young animals at both 2- and 3-Square trials but had reduced performance on 4-Square trials. Because only a few of the animals omitted a single trial at the 4-Square level, any reduction in performance seen at the 4-Square level in aged animals is likely not a reflection of an overall lack of motivation, but an effect of the increased difficulty of the task itself. This is supported by the finding that aged animals made more errors on the second presentation within a 4-Square trial than young mature animals. However, despite the deficit in performance on 4-Square trials, aged animals still performed well above chance, suggesting they are still capable of performing a spatial working memory task with an increased memory load.

4.3 Spatial working memory performance with delays

Both aged and young groups showed a similar decline in performance with increasing delays during 2- Square trials, yet this was statistically significant only for aged animals. Interestingly, the pattern of decline in performance across delays on the 3-Square trials differed between the two groups. The performance of aged animals significantly declined by the 4s delay and remained low with the maximum delay, whereas the young, mature animals performed well between the minimum and 4s delay and their performance did not decline until the maximum delay was imposed. However, on 4-Square trials, both groups' performance declined by the 4s delay and remained low with the maximum delay. In addition, as the delay increased, both age groups made more errors earlier in the sequence of 3- and 4-square trials than they did during the trials with a minimum delay. This suggests that working memory performance is sensitive to both the delay between stimulus presentations as well as the amount of information to be remembered (i.e., in this case, the number of square locations to be remembered), and that the performance of aged animals is affected by increasing delays sooner (i.e. with a lower delay) than that of young, mature animals. This extends the findings from previous reports that suggested that performance on simple spatial memory tasks was delay dependent in aged animals [9, 31].

It is possible that if aged animals were tested on a version of the task with reduced interference, they may have shown a slower decline in performance, similar to that of young animals. However, under identical testing procedures they are still impaired relative to young animals, suggesting that spatial working memory performance is age dependent.

Working memory is an essential aspect of higher cognitive processes and involves executive and attentional aspects of short-term memory (i.e., the ability to remember information over a brief period of time, manipulate the information and then retrieve it) [32]. The functional integrity of the prefrontal cortex is closely linked to working memory capacity [33, 34] and both noradrenergic [35] and dopaminergic [36] systems in the prefrontal cortex have been suggested to be involved in the control of working memory and these neurochemical systems decline with age [37] as does a variety of cognitive functions associated with the prefrontal cortex [38 – 40]. The current findings showing impaired learning of a spatial working memory task and impaired SOSS performance and working memory abilities in aged primates is consistent with the literature on cellular and molecular dysfunction of the

prefrontal cortex [41] and aging and associated age-related internal monitoring difficulties [42].

4.4 Conclusions

The present study found age-related deficits in learning a spatial working memory task as well as in performance when task difficulty was increased. These findings suggest that aged primates have difficulty in learning, particularly complex tasks, and that stimulus location, stimulus repetition and particularly ITI are important parameters to manipulate in order to improve learning and performance. However, despite these manipulations, aged animals still required more trials to reach criterion than young animals, suggesting an underlying deficit in learning a working memory task. Finally, working memory performance for young animals, and even more so for aged animals, is sensitive to the delay between stimulus presentations and the memory load of the task.

Acknowledgments

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Research Highlights

- Aged primates are impaired in spatial working memory (SWM) learning and performance.

- Difficulty in learning a SWM task can be improved by manipulating task variables.

- Stimuli locations, repetition rate and inter-trial interval influence learning.
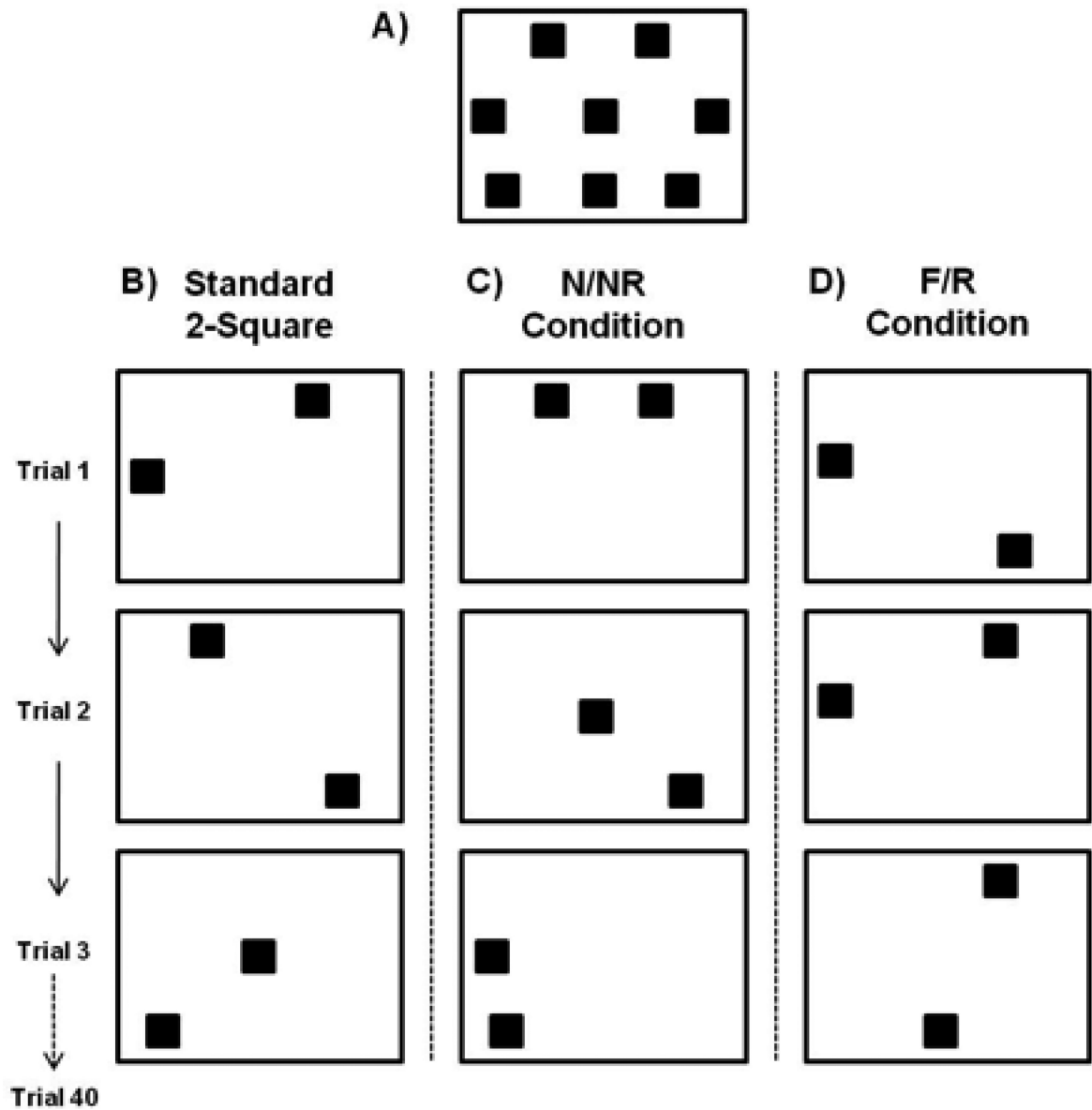
Figure 1.
Examples of SOSS trials for each condition. The eight possible stimulus locations are shown in (A). Examples of the first three trials in the B) standard 2-Square condition, C) Near/No Repeat condition, and D) Far/Repeat condition of the SOSS task.
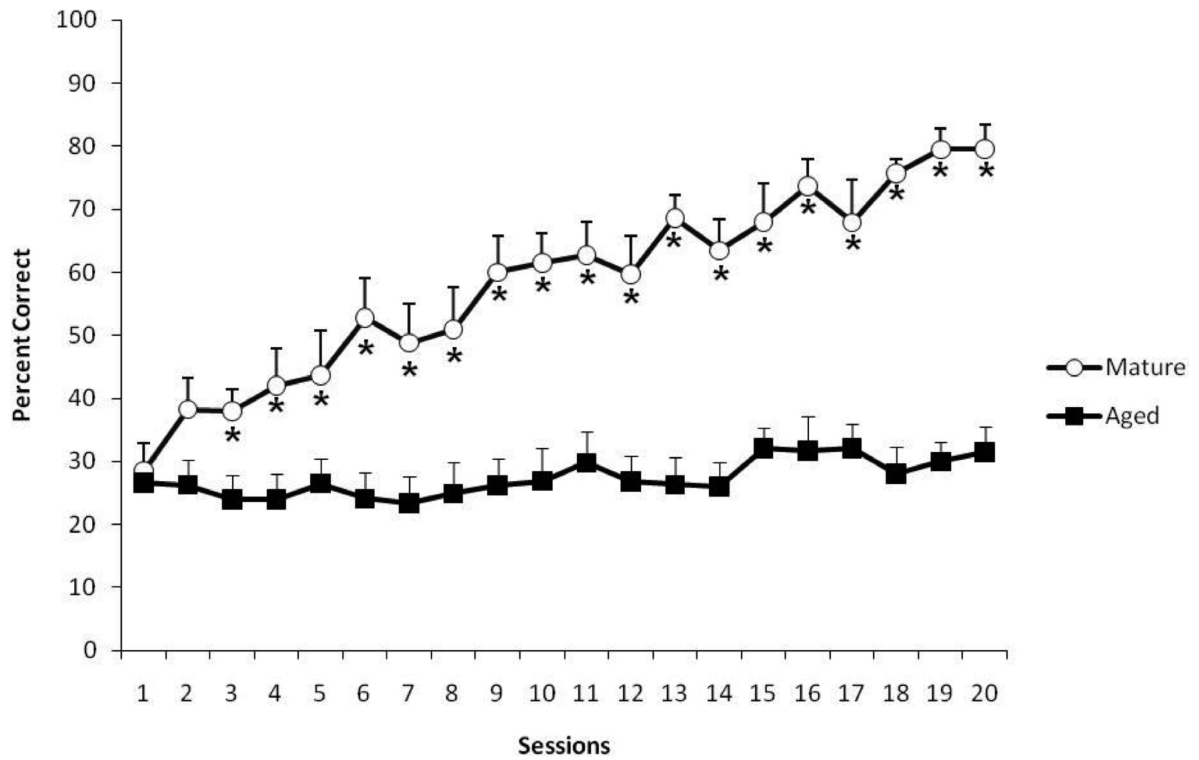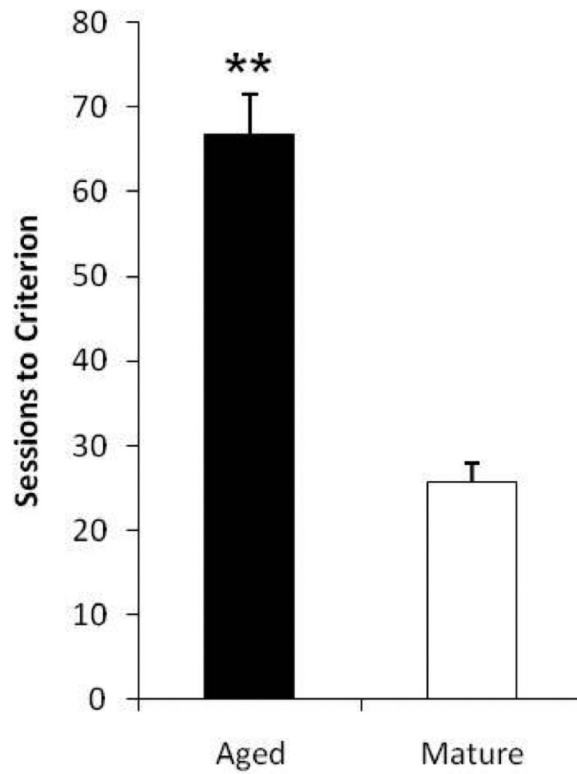
Figure 2.
Acquisition of aged and young adult monkeys on the standard version of the SOSS task with a 5s ITI. Across the first 20 sessions of SOSS learning, young animals showed a steady learning curve, while aged animals' performance remained flat and showed no evidence of learning. By session number 3, young animals consistently performed better than aged animals at every session (*p < 0.05; data points are mean ± SEM).
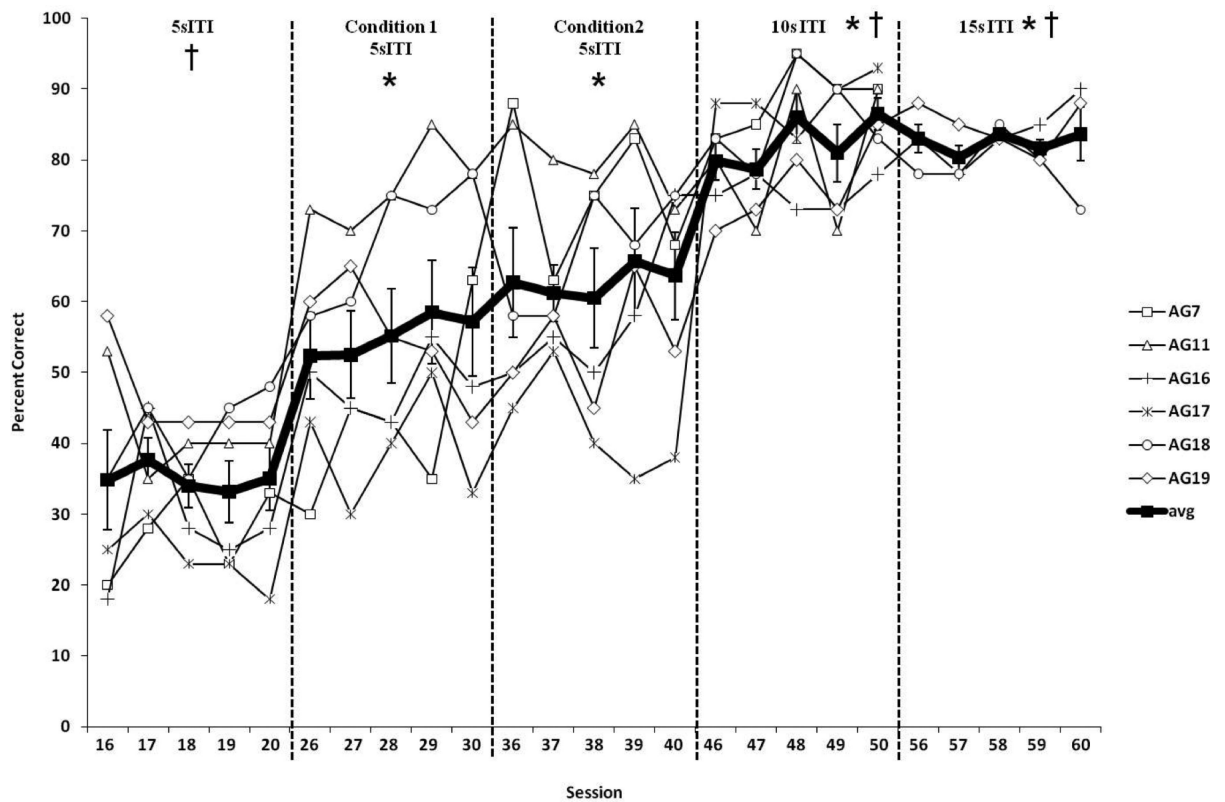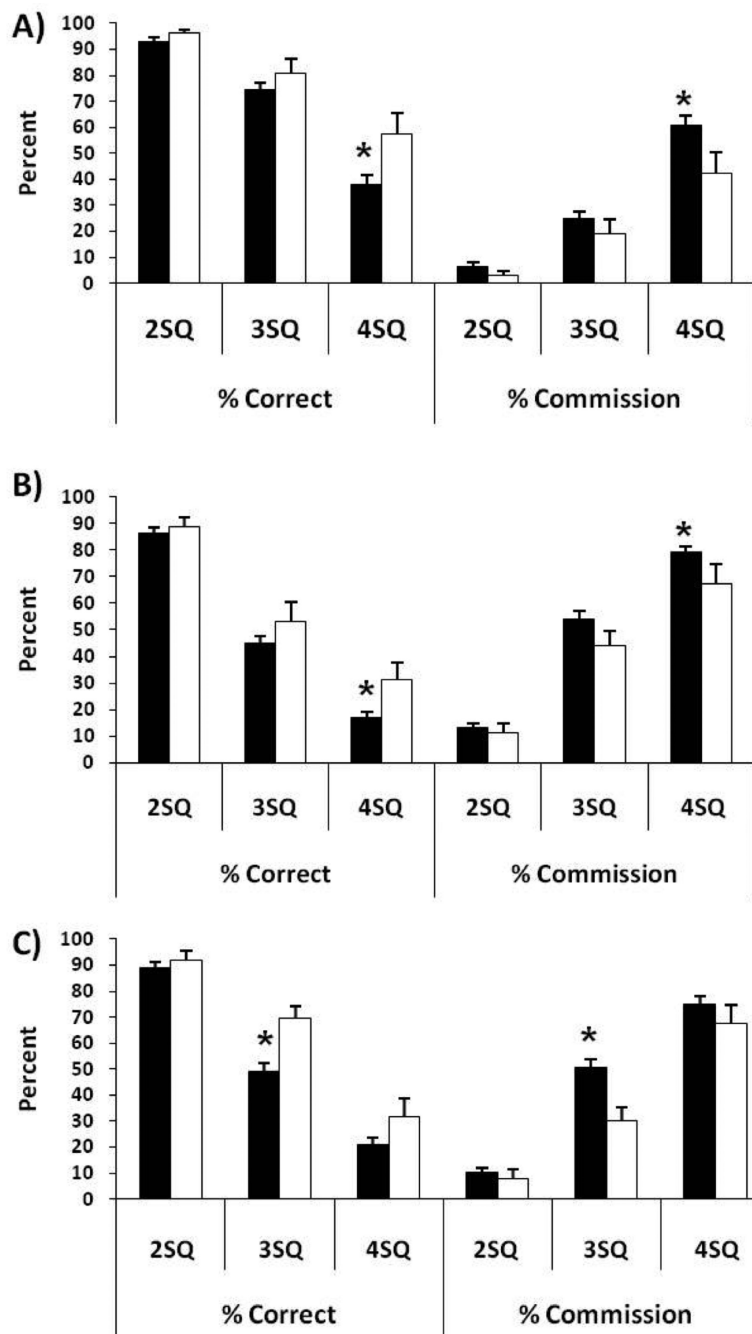
Figure 3.
Learning by aged monkeys on the SOSS 2-Square task across both stimulus manipulation conditions as well as increased ITI conditions. The data represent the last 5 sessions given for each condition. The bold black line is the average performance for the 6 aged animals trained in this manner, represented individually by the narrow black lines. AG7, 11 and 16 received the N/NR condition first and the F/R condition second, whereas AG17, 18 and 19 received the F/R condition first and the N/NR condition second. *, significant difference from the standard 5s ITI condition; †, significant difference from Condition 1 and Condition 2 (all p's < 0.05). Data points are mean ± SEM.
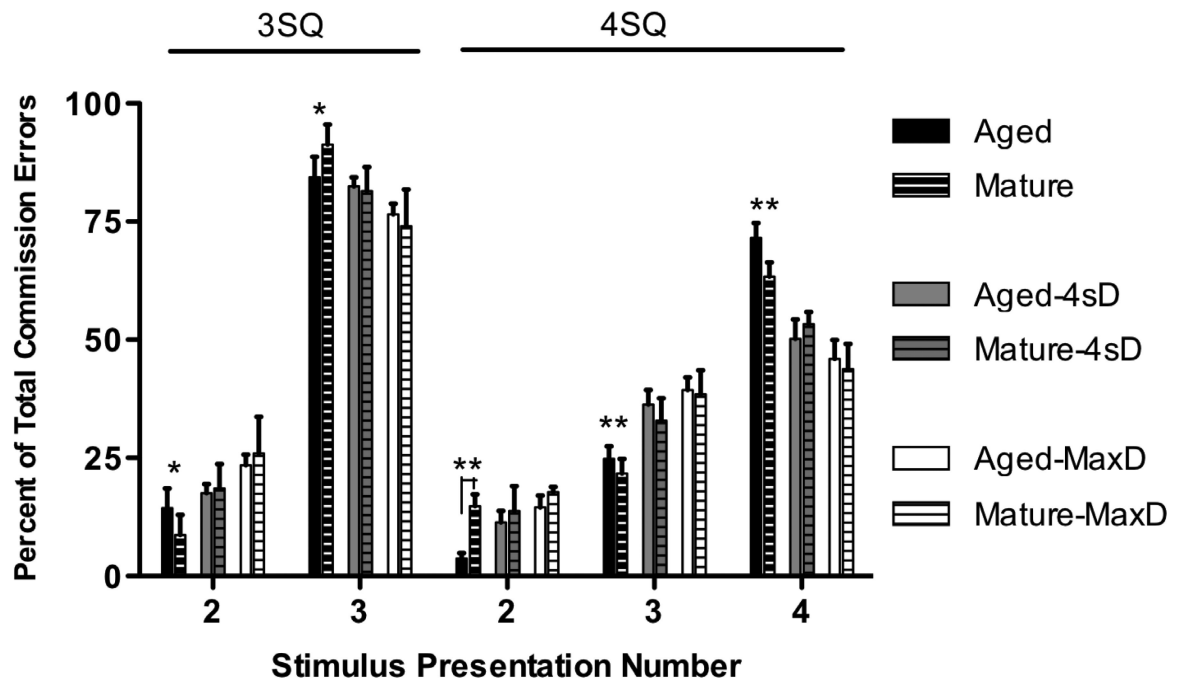
Figure 4.
Number of sessions needed to reach learning criterion in SOSS 2-Square in aged and young, mature monkeys. Aged monkeys required significantly more sessions to reach criterion at the SOSS 2-Square task compared to young, young animals (**p < 0.001). Data are means ± SEM.

Figure 5.
Performance of aged and young, mature monkeys on the SOSS task. In all instances, performance for both groups declined as the number of squares (i.e., task difficulty) increased. Aged animals (black bars) performed as well as the young animals (white bars) on the 2- and 3-Square trials during the standard SOSS task (A) and the Maximum delay condition (B), but they made more errors on the 4-Square trials (*p < 0.05). During SOSS with a 4 second delay (C), aged animals performed as well as young animals on the 2- and 4-Square trials, but made more errors on the 3-Square trials (*p < 0.05). Data shown as means ± SEM.

Figure 6.
Location of commission errors during performance of 3- and 4-Square trials. For 3-Square trials, animals made significantly fewer errors during the second presentation and significantly more errors during the third presentation of the stimulus during the standard SOSS than SOSS with maximum delays. For 4-Square trials, animals made significantly fewer mistakes at the third stimulus presentation, and significantly more at the fourth presentation of the stimulus during the standard SOSS condition compared to the 4 second or maximum delay conditions (*p < 0.05; **p < 0.001). There were no differences between the performance at the 4 second delay and the maximum delay conditions. 4sD: SOSS with 4 second delays, MaxD: SOSS with maximum delays. Data are shown as means ± SEM.