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Attention, executive functioning and memory in normal aged rhesus monkeys

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Abstract

Understanding how cognition declines in normal aging is vital in order to distinguish between normal cognitive decline due to aging and cognitive decline due to an age-related pathological process such as Parkinson's disease (PD). Several cognitive domains including memory, executive functioning and attention are all adversely affected with age in humans, as well as by PD, yet less is known about how these processes are affected by aging in non-human primates. Thus, in order to characterize baseline performance in aged primates prior to inducing Parkinson-like pathology, male rhesus macaques aged 15–22 years were tested on several tasks analogous to those used in cognitive aging studies in humans. The tasks included simple visual discrimination to assess learning and reference memory, discrimination reversal to assess cognitive flexibility and response inhibition, continuous performance to assess sustained visual attention, and attention set-shifting to assess cognitive flexibility and set-shifting ability. Deficits were detected in some aspects of learning, cognitive flexibility, response inhibition and sustained visual attention, whereas reference memory and set-shifting did not appear to be affected. Additionally, there was a greater amount of variability in cognitive abilities across the aged animals than observed in young adult animals. These findings will form an important baseline for comparison with cognitive performance after PD-like pathology is superimposed on the normal aging process.

Keywords

Aged; Primate; Memory; Attention; Executive function

1. Introduction

Cognitive functions decline with normal aging in humans (for review see [1]). This decline has been shown across several cognitive domains including memory, executive functioning and attention. Understanding how these functions change with age is important because it will help to distinguish between normal cognitive declines due to aging versus cognitive decline due to a pathological process. Because monkeys exhibit changes in learning and memory as they age [2,3], and, as with humans, some individuals appear to age more

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successfully than others [3–5], they are a good model in which to study age-related alterations in a variety of cognitive processes.

While the influence of normal aging on cognition, and primarily memory, has been well studied in non-human primates, there have been comparatively few studies on the influence of aging on other cognitive domains known to be affected by age-related diseases like Parkinson's disease (PD), such as attention and executive functioning. Executive functions include such processes as planning, response inhibition, task management and set shifting, and are thought to depend on the integrity of the frontal lobes, which may be particularly vulnerable to the aging process [6–8]. For example, the Wisconsin Card Sorting Task (WCST), a test of cognitive flexibility and set-shifting [9], is a commonly used test of executive functioning in humans, and has recently been adapted for use in non-human primates [10]. Using a task based on WCST principles, middle aged (12–19 yrs) and aged (20–30 yrs) monkeys had deficits in their ability to set-shift and made perseverative errors after each shift [11,12].

Another common test of executive function in primates is visual discrimination reversal learning (VDRL). There have only been a few studies that have examined VDRL in aged primates and these studies have had mixed results despite all studies using comparably aged animals. Some studies found that aged animals required more trials to reach criterion during learning than their young counterparts [13–16], while others found no such differences [14,17,18]. Several studies also found that aged animals had impaired performance of a reversal task, but after several reversals, performed as well as young animals [14,17,18]. Yet, other studies reported no reversal deficits at all in aged animals [15]. Voytko [13,14] reported reversal learning deficits in aged animals using objects and patterns but found no spatial deficit per se, while Lai et al. [18] reported deficits in spatial reversal learning but not object reversal learning.

Very few studies have examined attention in aged primates and as discussed above for VDRL, results have been mixed. For example, in one study, aged animals (21–31 years) that had to attend to a white bar in the center of a touchscreen and then make a response to the same bar either appearing on the right or left of the original stimulus had longer reaction times than young, mature animals (3–7 years) [2]. However, in another study, aged animals (28–31 years) that were given a valid, invalid or no cue for which side of the screen the target would appear showed no differences in reaction time, compared to young mature animals (10–13 years), under any of the three conditions [19].

Rhesus macaques have been a valuable resource for studying normal cognition and the changes associated with aging as well as for modeling cognitive deficits associated with Parkinsonism, although this latter research has up to this point utilized exclusively young adult animals [20–22]. The animals assessed in this study are part of a larger project aimed at examining the interaction of aging and PD-associated neural damage on cognition. Prior to inducing a Parkinson-like state in these animals, it is first important to evaluate their baseline cognitive capabilities. Because humans with age-related diseases, such as PD, can exhibit a range of deficits across a number of different cognitive domains, particularly those related to the functioning of the frontal lobes, it is necessary to first examine the extent to which these potentially at-risk cognitive processes are influenced by the aging process before a PD-like pathological process is introduced. Thus, the present study was conducted to characterize these cognitive domains in a group of normal, aged male rhesus macaques trained to perform the following tasks (analogous to those used in cognitive aging studies in humans): simple visual discrimination (assesses learning and reference memory), discrimination reversal (assesses cognitive flexibility and response inhibition), continuous

performance (assesses sustained visual attention and ability to ward off distraction), and attention set-shifting (assesses cognitive flexibility and set-shifting ability).

2. Methods

2.1. Subjects

Seventeen aged adult male *Macaca mulatta* monkeys (range: 15–22 years of age, mean = 19.7 yrs; weight 8.0 to 18.1kg at beginning of study) were used in this study. In addition, for comparative purposes, historical data obtained from normal young mature adult animals were examined (two male *Macaca mulatta* and 15 male *Macaca fascicularis* (range: 5–9 years of age at testing, mean = 6.6 yrs). The normal young animals were trained and tested in the same lab as the aged animals, using the same equipment, procedures and protocols as were used for the training and testing of the aged animals. There were no significant differences in the performance of these latter 2 groups of animals and thus their data have been combined for analysis. Animals were trained to perform a variety of cognitive tasks during which time they 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 and a pellet delivery system for delivery of rewards (Bio-Serv, Fruit Crunchies - 190mg) for each correct response. Before testing began, the room lights were turned off and white-noise was provided to reduce outside noise interference. Animals were trained to perform several tasks, according to their individual learning and performance abilities.

2.3. Simple visual discrimination (SD)

This task is a measure of simple discrimination learning and reference memory. A blue square and a blue triangle are presented at the same time on the right and left of the screen. The left/right position of the 2 stimuli varies pseudo-randomly from trial to trial. The blue square is always the positive (rewarded) stimulus and touching it results in a positive tone and reward. A maximum of 200 trials are presented to the animal daily in 20 trial blocks, until the animal reaches a criterion of 90% correct over 3 consecutive blocks. The total number of blocks, or sessions, it takes an animal to reach criterion is considered their SD learning. Once criterion is reached, the animal is presented with only 1 block (session) of 20 trials per day. Once the animal maintains an average performance of at least 90% correct over 5 consecutive blocks, it is presented with 1 block of 20 trials once per week for the duration of the study.

2.4. Visual discrimination reversal learning (VDRL)

Animals received VDRL only after 3 consecutive weeks of performing at or above criterion on the SD task. The VDRL uses the same stimuli as the SD task, but for this task, the blue triangle becomes the positive stimulus. An animal is given a maximum of 10 – 20 blocks of trials (i.e., up to 200 trials) during one testing session within a single day. Animals are tested until a criterion of at least 90% correct responses is reached within a single block. The number of trials it takes to reach criterion is the measure of the ability to learn the reversal. After successfully performing the reversal, the animal is given one session, or 20 trials of the original SD task once a day until they return to a performance level of at least 90% correct

responses within a single testing session. The number of sessions it takes each animal to return to criterion on SD is considered their SD-Recovery. The animals then go back to the standard SD schedule of performing one session per week. They receive a second VDRL only after three consecutive weeks of SD performance above criterion.

2.5. Attention set shifting ability (ATSS)

This task measures cognitive flexibility and set-shifting ability and is based on the Wisconsin Card Sorting Test. The task is modeled after the intradimensional-extradimensional set-shift task from the Cambridge Neuropsychological Test Automated Battery (CANTAB; [10]) which requires the animal to shift attention across several dimensions of a complex set of stimuli composed of shapes and lines. This task consists of 7 subtests. The animal must meet a criterion of 6 correct responses in a row, within a maximum of 200 trials, in order to proceed to the next subtest. A full session of ATSS is only given once every 3 months.

1. Simple visual discrimination (SD): This task is as described above, with the exception that a different set of blue shapes are used and the criterion is 6 correct responses in a row.
2. Simple visual discrimination reversal (SDR): This task employs the same two shapes just seen in SD. However, the previously unreinforced shape is now reinforced. For example, if S1 (of pair S1–S2) was reinforced during SD, then S2 is reinforced during SDR.
3. Compound visual discrimination (CD): An additional dimension of a line (L) is added to the original stimulus of a shape (S). If the animal previously saw S1 and S2, a grouping of white lines is now superimposed on the shapes. Each shape can be randomly paired with each line on a given trial (i.e. pairs of S1L1 and S2L2, or S1L2 and S2L1). The reinforcement contingencies remain the same as in SDR, such that a response made to the compound stimulus containing S2 is reinforced.
4. Compound visual discrimination reversal (CDR): The task employs the same shapes and lines (S1 and S2, L1 and L2) just seen in CD. However, the previously unreinforced shape is now reinforced, such that all responses made to the compound stimulus containing S1 are reinforced.
5. Intradimensional shift (IDS): The animal is given a novel set of compound stimuli comprised of the same two dimensions, shapes and lines (randomized pairs of S3L3 and S4L4, or S3L4 and S4L3). The intradimensional shift is within the shapes category. For example, all responses made to the compound stimulus containing S3 are reinforced.
6. Extradimensional shift (EDS): The animal is presented with a novel set of compound stimuli comprised of the same two dimensions, shapes and lines (ex. randomized pairs of S5L5 and S6L6, or S5L6 and S6L5). To make an extradimensional shift, the animal must now attend to the line instead of the shape. For example, all responses made to the compound stimulus containing L5 are reinforced.
7. Extradimensional shift reversal (EDSR): The task employs the same shapes and lines as in EDS (ex., randomized pairs of S5L5 and S6L6, or S5L6 and S6L5). However, the previously unreinforced line is now reinforced, such that all responses made to the compound stimulus containing L6 are reinforced.

The number of trials to criterion is measured for each subtest of the ATSS task. Reversal subtests measure cognitive flexibility and the ability to inhibit a response to a previously

rewarded stimulus. For IDS, the number of trials to criterion measures the animals' ability to shift response to a novel stimulus within the dimension (i.e. shape). Finally, the number of trials to criterion in EDS measures the animals' ability to shift their response across dimensions (i.e. shape to line) and is measures of set shifting ability and cognitive flexibility.

2.6. Continuous performance task (CPT)

This task is a measure of sustained visual attention. Colored rectangles (yellow, black, or white) appear in the center of the screen and the animal is rewarded for touching the target rectangle (yellow) and for withholding responses to non-target rectangles (black and white). Animals are trained until they reach an asymptotic performance level when 30% of the trials over a 200 trial session are the target. The animal is then given two CPT sessions per week, 1 each of CPT 2,2,1 and the more difficult CPT 1,1,1. For CPT 2,2,1, the stimulus remains on the screen for a maximum of 2 seconds (or until the animal touches it), the inter-stimulus interval is 2 sec, and if the animal touches a non-target stimulus, there is a 1 second penalty (time out) period. For CPT 1,1,1, the stimulus remains on the screen for a maximum 1 second (or until the animal touches it), the inter-stimulus interval is 1 sec, and if the animal touches a non-target stimulus, there is a 1 second penalty period. The measures recorded for each testing session are, 1) hits - the number of correct touches to the target, 2) commission errors - the number of touches to a non-target stimulus, and 3) omission errors - the number of times the target appears but is not touched.

2.7. Data Analysis

All statistical analyses were performed using SPSS 17.0 software. Data are presented as mean $\pm$ SEM where appropriate. For SD and CPT, an independent sample t-test was conducted to compare group differences. For VDRL and ATSS, a repeated measures ANOVA was conducted with session number 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. Additionally, when interactions between factors were not significant, planned comparisons were performed between the aged group and the mature group, using one-sided planned comparisons [23].

Specific sub-analyses were performed on VDRL data. It has previously been shown that aged animals spend significantly more time than young animals choosing the object rewarded prior to the reversal when performing their first VDRL [14,18]. In order to examine this, data analysis was subdivided into 3 stages: Stage 1 is 0–3 correct responses in a 10 trial block (inability to inhibit previous response), Stage 2 is 4–6 correct trials in a 10 trials block (chance), and Stage 3 is 7–10 trials correct in a 10 trial block (reversed response). A repeated measures ANOVA was conducted across Stages.

3. Results

The overall performance characteristics of the aged animals and their individual variability in task performance are shown in Table 1. In addition, because all of the behavioral data for the two mature *M. mulatta* animals fell within the range of the 15 mature *M. fascicularis* animals, the data were combined into one Mature group for comparison with the Aged group.

3.1. Simple visual discrimination (SD)

Aged animals took significantly longer to reach criterion during the learning phase of SD than did young adult animals (33.7 ± 4.8 versus 14.6 ± 2.9 sessions respectively; $p < 0.01$; Figure 1A). However, once the aged animals learned the task, they performed as well as

young adult animals ($95.7\% \pm 0.8$ correct for aged monkeys versus $97.6\% \pm 0.8$ correct for young adult monkeys; Figure 1B).

3.2. Visual discrimination reversal learning (VDRL)

Aged animals required significantly more sessions to reach criterion on the VDRL task (4.8 ± 0.5 compared to the young adult group (2.8 ± 0.5 ; $p < 0.05$). Additionally, aged animals required significantly more sessions before recovering from the SDR than did the younger animals (3.3 ± 0.3 vs. 2.1 ± 0.2 respectively; $p < 0.01$; Figure 2A and 2B). Further analysis of VDRL data revealed that both aged and young adult groups needed significantly fewer sessions to reach criterion during later testing sessions [Session effect: $F(1,15)=5.67$, $p < 0.05$] than on the first testing session, yet there was no significant Group $\times$ Session interaction [$F(1,15) = 2.25$, $p = \text{ns}$]. A planned comparison showed no group differences during the first testing session, but the aged group took significantly longer to reach criterion compared to the young adult group when all VDRL sessions except the first were compared ($p < 0.01$; Figure 2A).

When VDRL performance on the first testing session was examined by Stage, there was an overall effect of Stage [$F(2,34) = 5.72$, $p < 0.01$], such that both aged and young adult groups spent significantly more time in Stage 3 than Stages 1 or 2 (Figure 2C). However there was no Group $\times$ Stage interaction [$F(2,34) = 0.68$, $p = \text{ns}$]. Similar analyses performed on results from the second and later sessions similarly showed an overall effect of Stage [$F(2,30) = 150.76$, $p < 0.001$]. That is, both groups spent most of their time in Stage 3, and there was no Group $\times$ Stage interaction [$F(2,30) = 2.01$, $p = \text{ns}$; Figure 2C]. Planned comparisons revealed that young adult animals spent more time in Stage 3 than aged animals ($p < 0.05$) and that aged animals spent less time in Stage 3 during their first VDRL session than during subsequent VDRL sessions [$F(1,11)=4.58$, $p < 0.05$].

For SD-Recovery, despite the overall Group difference, the repeated measures ANOVA revealed no differences between first and later sessions [$F(1,15)=1.24$, $p = \text{ns}$] and no Group $\times$ Session interaction [$F(1,15) = 0.39$, $p = \text{ns}$]. When performance on the first testing session was examined by stages, there was an overall effect of Stage [$F(2,34) = 69.24$, $p < 0.001$], such that both groups spent more time in Stage 3 than Stages 1 or 2 (Figure 2D). There was a marginal Group $\times$ Stage interaction [$F(2,34) = 3.12$, $p = 0.06$], and further analysis revealed that aged animals spent more time in Stage 1 ($p = 0.08$) and less time in Stage 3 ($p < 0.05$) than young adult animals. Interestingly, during their first testing session, 8 of the aged animals spent 1 or more sessions in Stage 1 (62%), whereas only 1 mature animal spent a single session in Stage 1 (20%). The two groups did not differ in time spent in Stage 2.

When performance on subsequent SD-Recovery sessions were examined, there was an effect of Stage [$F(2,30) = 94.39$, $p < 0.001$], such that animals in both groups spent more time in Stage 3 than Stages 1 or 2, but there was no Group $\times$ Stage interaction [$F(2,30) = 0.39$, $p = \text{ns}$]. A planned comparison showed that aged animals spent significantly more time in Stage 1 than did young adult animals ($p < 0.01$; Figure 2D). Fifty-eight percent of the aged animals spent 1 or more sessions in Stage 1, whereas none of the young adult animals spent a single session in Stage 1. In addition, aged animals spent less time in Stage 3 during their first SD-Recovery than during subsequent SD-Recoveries [$F(1,11) = 5.20$, $p < 0.05$]. The aged and young adult groups did not differ in time spent in Stage 2 or 3.

3.3. Attention set shifting ability (ATSS)

No differences between first and subsequent sessions for any of the 8 subtasks were found for either the aged [Subtask $\times$ Session: $F(7,28) = 1.722$, $p = \text{ns}$] or young adult group [Subtask $\times$ Session: $F(7,21) = 1.266$, $p = \text{ns}$]. Therefore, all testing sessions were combined

for analysis within each group (Figure 3). No overall Group [$F(1,29) = 0.43$, $p = ns$] or Group $\times$ Subtask effect [$F(7,203) = 1.61$, $p = ns$] was found, but there was an overall Subtask effect [$F(7,203) = 10.35$, $p < 0.001$]. Both groups took longer to reach criterion in the EDSR subtask than all other subtasks ($p < 0.05$ for all comparisons). Additionally, both groups took longer to reach criterion in the EDS subtask compared to SD, SDR, CD, IDS ($p < 0.05$ for all comparisons), and both groups took longer to reach criterion in the CDR subtask compared to SD, SDR, CD and IDS ($p < 0.01$ for all comparisons).

3.4. Continuous performance task (CPT)

There were no differences between aged and young adult animals in the number of omission errors for either of the two conditions (i.e., CPT 2,2,1 or CPT 1,1,1) or in the number of commission errors for CPT 1,1,1 (Figure 4A). However, aged animals made significantly more commission errors than did the young adult animals in the easier CPT 2,2,1 condition ($p < 0.05$; Figure 4B). In addition, the aged animals showed a positive correlation between SD learning and the number of CPT 2,2,1 commission errors ($r = 0.66$, $p < 0.01$; Figure 4C) and between SD learning and the number of CPT 1,1,1 commission errors ($r = 0.73$, $p < 0.001$; Figure 4D).

4. Discussion

The results of the present study show that aspects of learning, executive function and attention are impaired in aged rhesus monkeys, and that these deficits are task specific. That is, aged animals were impaired in one test of executive function, VDRL, but not in another, ATSS, and only one version of CPT reliably differentiated the attentional abilities of aged versus young adult animals. However, these data need to be viewed in the context of significant individual variability in task performance in the current aged population, which may account for lack of more significant effects in many of the behavioral tasks currently assessed.

4.1. Learning and memory

Aged animals were significantly impaired relative to young adult animals in learning a simple visual discrimination problem. This supports previous work that suggested that aged monkeys (female, 23–27 years; female, 28–34 years) require more trials to learn to discriminate between two visual stimuli the first time they experience the stimuli [14,15], but not others that found no learning deficit in aged animals (males and females, 18–22; males and females, 20–28 years) [17,18]. The reasons for the differences in outcomes from these studies are not clear but may relate to the physical demands of the tasks used in the various studies (i.e., computer automated vs. Wisconsin General Test Apparatus (WGTA); nature of the objects/discriminations). The clearest impairment in SD learning (similar to the current finding) was reported in a study using a touchscreen system [14], similar to that used in the current study. Lai et al. [18] presented animals with a spatial version of a discrimination task before testing them on an object oriented version. Animals were impaired on the former but not the later discrimination, relative to young animals (6–11 years). There may have been an order of task effect such that if the object version had been presented first, the animals would have shown a learning deficit. Rapp [15] used a non-automated WGTA, the stimuli were patterns taped onto placards and were visually distinctive, but not texturally different. In this study, the effect of aging on discrimination learning was not straight forward. There was not a significant difference in performance between young and aged animals, but 3 of the 5 aged monkeys performed well outside the range of the young animals. Thus aged animals may not show a discrimination learning deficit if they can physically manipulate the stimuli they are learning to discriminate.

Once the current set of aged animals learned the visual discrimination, their reference memory abilities in all subsequent tests did not differ from young adult animals. Previous studies have suggested that aged primates have reference memory problems, particularly in the context of performing a spatial reference memory task [24]. However, the spatial reference memory task used required a spatial span sequence to be learned and remembered, was a more difficult task than our assessment of reference memory and may have had a greater executive component than the task we used. In addition, it is not clear that all animals actually learned the spatial span task by the end of the study period and possibly a learning deficit rather than a memory deficit was detected in some animals [24].

4.2. Executive function

Aged animals were significantly impaired relative to young adult animals on one test of executive function, VDRL, but not another, ATSS. For VDRL, aged animals required more trials to reach criterion than young adult animals. The performance of aged and young adult animals did not differ during their first VDRL session, but aged animals took longer to reach criterion during their second and later VDRL sessions. This suggests that initially, both groups had difficulty learning to switch their responses from the previously rewarded stimulus to the new rewarded stimulus. However, after repeated sessions, the young adult animals showed greater flexibility in their ability to switch between stimuli, compared to the aged animals. When performance was broken down further into stages, aged animals spent more time in Stage 3 during second and later testing sessions than during the first session, though still not as much as did young adult animals. This suggests that aged animals learned to switch to the target stimulus more quickly with repeated tests, but still required more trials to finally reach criterion.

The VDRL deficit currently detected is generally consistent with previous findings in aged animals [13,14,17,18] but differs from previous studies in 2 ways. First, our aged animals were not impaired on the first VDRL session as previously reported [13,14], but were impaired in performing subsequent VDRL sessions. Second, our aged animals were impaired in Stage 3 and not in Stage 1 as previously reported [14,18]. One reason for this discrepancy could be that the animals in the current study are somewhat younger than the animals used in the previous studies (average age of the current group = 19.7 yrs versus 24.0 yrs [18] and 29.9 yrs [14]). Additionally, the current study only used males, while the two previous studies used both males and females. However, neither study specifically examined sex differences so it is not possible to know if aged males perform differently than aged females on this task.

This is the first study to our knowledge to also examine SD-Recovery in aged animals. As with VDRL, aged animals required more trials to reach criterion in SD-Recovery than did young adult animals. In addition, for all SD-Recovery sessions, aged animals spent more time in Stage 1, and for their first SD-Recovery session, they spent less time in Stage 3 than young adult animals. Thus, aged animals perseverated more than the young adult animals (Stage 1), but once they got past chance performance and showed re-learning of the original discrimination, they reached criterion more quickly than the young adult animals (Stage 3).

While aged animals showed a distinct deficit in VDRL, they were not impaired on another test of executive function, the ATSS task. The aged animals in the current study performed as well as young adult animals on initial discrimination learning and reversals as well as on intra- and extra-dimensional shifts. It is not surprising that the aged animals showed no deficit on the visual discrimination subtask of the ATSS even though they were impaired on learning an SD task (discussed above). Previous studies showed that aged animals are only impaired in acquiring SD the first time they see it [14,15,18] and perform as well as young animals on any additional discriminations. One study did describe deficits in aged animals

when learning several discrimination pairs, however, animals were presented with two new discrimination pairs each day and the pairs consisted of objects with overlapping lines that had to be ignored [16], making this a much more difficult task than that used in the ATSS task. The added lines may have caused interference, (i.e., the memory of a previous trial interferes with performance on the current trial), and aged animals and humans have been shown to be highly susceptible to interference [7,17,25]. However, even with the more difficult trials, by day 4 aged animals learned to discriminate between 2 novel objects as well as young animals.

In a non-human primate version of the WCST, the Conceptual Set-Shifting Task (CSST), middle-aged (12–19 years) [12] and aged (24–30 years) [11] primates were impaired in their ability to learn the initial discrimination, as well as shift between sets (i.e. from shape to color). They required more trials to reach criterion and made more perseverative errors after each shift. However, it may be difficult to directly compare the present results to those previously reported [12]. Moore et al. required animals to reach a criterion of 10 correct trials in a row, whereas our protocol required animals to reach a criterion of 6 trials in a row. It is possible that with the additional number of trials needed to reach criterion in the prior study the animals had an opportunity to make more set breaks before reaching criterion. Additionally, the prior study presented 3 different stimuli to the animal per trial while our task used only 2 stimuli per trial. Adding a third stimulus to each trial adds an additional distracter, which may cause a level of interference that the aged animals may not be able to overcome as easily as young adult animals. Also, in the prior study, the 3 stimuli could appear in any of 8 positions on the screen, whereas the stimuli in our task always appeared in the same two spatial locations on the left and right of the screen. Although the spatial component is irrelevant to solving the task, it may introduce interference between trials. Thus, aged animals may require a more difficult version of the task in order to detect an impairment in their set-shifting abilities if such an impairment exists.

4.3. Attention

Aged animals made significantly more commission errors than did young adult animals in the CPT 2,2,1 condition, but not the CPT 1,1,1 condition. While the CPT 1,1,1 data had the same trend as the CPT 2,2,1 data, these results did not reach statistical significance, most likely because of the large variability in performance within the aged group. The number of commission errors for the young adult group ranged from 0 to 5.8 while the aged group ranged from 0 to 52.8, with 24% of the animals performing above the range, or worse than, the young adult group.

The CPT task is a measure of sustained attention over time. While there is little known about attentional processes in aged primates, the evidence from the human literature suggests that sustained attention may not be significantly altered by normal aging [1,26,27]. However, the ability to inhibit responding, a measure of impulsivity, is an aspect of selective attention that is known to be affected by aging [1] and may be reflected in the large number of commission errors made by many of the aged animals. Interestingly, the CPT 2,2,1 and CPT 1,1,1 data from the aged animals correlated positively with their SD-Learning scores. That is, animals with worse performance on CPT also took longer to learn the SD task. Animals that had more difficulty inhibiting their responses to the incorrect target while learning SD showed the same pattern of behavior in CPT. This suggests that in some of the aged animals, there may be a fundamental deficit in the ability to inhibit responding to an incorrect target (i.e., impulsive responding) that affects performance across other cognitive domains.

4.4. Summary

The present study detected age-related cognitive deficits across several domains, with animals showing task-specific impairments. Deficits were detected in some aspects of learning, cognitive flexibility, response inhibition and sustained visual attention, whereas reference memory and set-shifting abilities were not different from younger animals. These findings provide an important baseline for continuation of this work in which Parkinson-like pathology will be superimposed on the normal aging process in order to study the interaction of aging and PD-associated neural damage on cognition. Considering the difficulty in treating cognitive dysfunction in PD, such information will be important for the ultimate development of pharmacotherapies to enhance cognition in an age-related disease such as PD. Additionally, it will be important to know if treatment strategies previously found to be effective in younger cognitively impaired Parkinsonian animals remain so in older Parkinsonian animals.

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Figure 1. Trials to criterion (A) and average performance (B) for Simple Visual Discrimination (SD)

Aged animals required more trials to reach criterion on SD than the mature group (A), but once they learned the task, the two groups performed at equal levels (B). Error bars represent SEM. (** $p < 0.01$).

Figure 2. Performance on Visual Discrimination Reversal Learning (VDRL) and Recovery (SD-Recovery)

When compared across all session, aged animals took longer to reach criterion on VDRL (A) and SD-Recovery (B). Both groups required more testing sessions to reach criterion the first time they were given the task than on subsequent sessions of the task, but the aged group still required more sessions to meet criterion than the mature group on these later sessions (A). When the VDRL data were broken down by stages, aged animals spent less time in Stage 3 than mature animals on second and later sessions (C). When SD-Recovery data were broken down by stages, aged animals spent less time in Stage 3 than mature animals for the first testing session, and more time in Stage 1 than the mature animals for all testing sessions (D). In addition, for both VDRL and SD-Recovery, aged animals spent less time in Stage 3 on their first testing session than on subsequent testing sessions. Error bars represent SEM. (†, $p = 0.08$; *, $p < 0.05$; ** $p < 0.01$)

Figure 3. Performance on Attention Set Shifting (ATSS)

There were no group differences for any of the subtests of ATSS. However, both groups took longer to reach criterion for EDSR than all other subtests, and to reach criterion for EDS and CDR than all subtests but EDSR. Error bars represent SEM.

Figure 4. Performance on the Continuous Performance Task (CPT) and correlations of performance on CPT with SD Learning

Aged animals had more commission errors than mature animals on CPT 2,2,1 (A), but the two groups did not differ on omission errors for CPT 2,2,1 or either type of error for CPT 1,1,1 (B). In aged animals, the more commission errors they made on CPT 2,2,1 (C) and CPT 1,1,1 (D), the more trials they needed to reach criterion for SD Learning. Error bars represent SEM. (* $p < 0.05$).

Table 1

Individual variability for each aged animal.

Aged	Test Age	SD-Learn	SD	VDRL			SD-Recovery			CPT221			CPT111			ATSS				
				1st - Total Sessions	2 or more - Total Sessions	1st - Total Sessions	2 or more - Total Sessions	Com	Om	Com	Om	Com	Om	SD	SDR	CD	CDR	IDS	EDS	EDSR
AG1	22	++	++	++	++	+	++	++	+	++	+	++	++	+	+	+	+	+	+	-
AG2	15	++	++	+	++	+	++	++	++	+	++	++	++	+	++	+	+	-	+	++
AG3	20	-	+	++	+	++	+	+	-	+	+	+	+	+	+	+	+	-	++	++
AG5	20	+	+	++	+	++	++	+	+	+	+	+	+	+	++	+	+	+	+	+
AG6	20	++	-	+	-	-	+	++	+	++	+	+	+	-	+	Fail				
AG7	20	-	++	-	+	-	+	++	++	+	++	+	++	+	+	+	+	++	+	+
AG8	22	+	+	+	-	+	-	+	++	+	++	+	++	-	-	+	+	++	-	+
AG9	19	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	++	-
AG11	19	+	+	-	+	+	-	++	++	+	++	+	+	+	+	-	-	+	-	-
AG12	19	+	+	Fail	+	+	+	++	+	++	+	+	-	-	-	+	+	+	+	+
AG13	22	+	+	+	+	+	+	++	++	+	++	+	++	+	+	+	+	++	-	+
AG14	22	+	+	-	-	+	-	++	+	++	+	+	-	++	+	++	+	+	++	+
AG15	20	++	++	Fail	-	-	-	++	-	++	-	+	-							
AG16	18	-						-	+	+	+	+	+							
AG17	20	-	-					-	++	-	++	-	-							
AG18	18	+	+	+	++	+	+	++	+	++	+	+	+							
AG19	19	++						-	-	+	+	+	+							
Avg	19.71	33.71	95.67	4.91	4.31	3.27	3.00	11.87	1.47	7.67	12.19	27.53	25.17	31.90	53.34	25.81	38.75	72.82		
SD	1.69	19.28	2.90	2.60	2.41	1.86	1.31	16.76	1.40	14.16	5.41	17.97	7.51	28.74	42.37	9.84	24.94	32.67		

Symbols: ++ denotes 1 or more standard deviation above group average, + denotes between -1 and 1 standard deviation of group average, and - denotes more than 1 standard deviation below group average; Com - commissions; Om - omissions; SD - simple discrimination; VDRL - visual discrimination reversal learning; CPT - continuous performance task; ATSS - attention set shifting task. Blank box indicates animal did not perform that task.