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Teaching old rats new tricks: Age-related impairments in olfactory reversal learning

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Abstract

Recent work suggests that normal aging may be associated with decline in different brain systems. In the present study, young and aged Long-Evans rats were tested in a spatial version of the Morris water maze dependent on medial temporal lobe function and also on an odor discrimination reversal task previously used to investigate orbitofrontal function. Aged rats acquired the odor discrimination problems normally but were impaired in acquiring subsequent reversals of the problems. A subset of the aged rats also exhibited impaired spatial learning in the water maze. There was no correlation between reversal performance and spatial learning in the aged rats, indicating that the reversal learning impairment was not related to decline in medial temporal lobe function. Instead the performance of the aged rats on the odor discrimination task resembled that of young rats with neurotoxic lesions of orbitofrontal cortex. These data indicate that rats show independent decline of different brain systems during normal aging and suggest orbitofrontal cortex as one prefrontal area where changes may be localized for further study. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

The development of a rat model for the effects of aging on hippocampus and the medial temporal lobe system has provided considerable information relevant to understanding cognitive decline in normal aging. This approach has assessed medial temporal lobe function using a standardized spatial version of the Morris water maze in which performance is sensitive to hippocampal lesions in young rats. Repeated experiments have demonstrated that a proportion of aged Long-Evans rats perform poorly in this task relative to controls, exhibiting prolonged and non-spatial search strategies when required to remember the location of a submerged platform relative to distal cues placed around the perimeter of the pool [15]. By providing a standardized index of function, such testing has allowed rigorous examination of the underlying neural substrate to determine the etiology of the age-related decline in medial temporal lobe function [14,17]. Contrary to expectations, these studies indicate that functional decline is not the result of neuronal

loss [35,37] but rather is associated with a complex set of alterations including changes in synaptic connectivity and function, gene expression and signal transduction [1,6,7,18,29,48]. These findings are correlated with deficits in spatial cognition and accompanied by changes in encoding and neural representations in the hippocampus [49,50].

At the same time, additional models are needed to capture the effects of aging on other aspects of cognitive function in humans. For example, normal aging in humans can be associated with poor judgment, perseveration and impulsivity, impaired recall of source information and information for temporal order [8,36,44]. These symptoms of age-related cognitive decline closely resemble, at least in mild form, those found in younger individuals with prefrontal lesions [24,25,32,38,45]. Altered prefrontal function is also evident in functional imaging studies that show age-related declines and changes in blood flow and activation patterns during the performance of certain “prefrontal” tasks [20]. Notably, such prefrontal symptoms often occur in the absence of the declarative memory deficits associated with medial temporal lobe impairment and appear to reflect a differential and somewhat independent decline with aging. For example, when aged subjects were formally evaluated on a battery of neuropsychological tests, impaired perfor-

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mance was accounted for by independent factors related to the assessment of prefrontal or medial temporal lobe function by different groups of tests [19]. Like medial temporal lobe deficits, deficits linked to prefrontal dysfunction appear to occur against a background of preserved neuron number in these areas [30], ruling out this simple explanation for the cognitive decline. As a result, an understanding of the role of prefrontal cortex in the pathology of normal aging is likely to depend heavily on the development of adequate animal models. Such animal models would allow researchers to carefully examine the structural, molecular, and neurophysiological markers associated with the age-related decline as has been done in medial temporal lobe structures.

Although previous work to develop a prefrontal model of normal aging in rats has focused on medial wall, the orbitofrontal region (OFC) within prefrontal cortex may provide a model in rats that closely parallels a homologous region in the primate brain. Neuroanatomical connectivity and key functions determined by lesion and electrophysiological recording studies are remarkably similar between rats and primates [31,33,43]. Importantly this region is less closely related to medial temporal lobe structures than medial wall in the rat, and its role in spatial tasks appears to be limited [11]. Thus age-related deficits that involve OFC would be less prone to contributions from effects of aging on medial temporal lobe structures.

One function that appears to be particularly sensitive to OFC damage is the ability to use information regarding the current incentive value of cues to guide goal-directed or adaptive behavior. Early reports noted that primates with damage to prefrontal cortex [13,23], and OFC in particular [21,26], exhibited impulsive or disinhibited behavior. These symptoms typically result when otherwise appropriate responses are made in inappropriate circumstances and are particularly evident in controlled settings involving extinction or devaluation testing and in rapid reversal learning. For example, both humans [38] and non-human primates [27] with lesions in the orbitofrontal area are able to learn simple discrimination problems normally but exhibit impaired acquisition of those same discrimination problems if the response contingencies associated with the items are subsequently reversed, such that the previously positive item becomes negative and vice versa. Rather than altering their responses to reflect the new contingencies, lesioned subjects continue to perseverate in the old pattern of responding. Although such deficits may be found after damage elsewhere in the brain, reversal learning appears to be particularly sensitive to OFC damage. Recently we have found that rats show a similar deficit on olfactory discriminations and reversals after OFC lesions; rats learn the initial discriminations normally but are impaired in subsequent reversal training [34].

In the present study, we have used this olfactory reversal paradigm to test for cognitive deficits in aged rats. Young and aged rats were characterized on a series of odor discriminations followed by several new problems with rever-

sals after each problem was acquired. The same rats were also tested in the spatial version of the Morris water maze to provide an index of medial temporal lobe function. As discussed above, previous studies have shown that a subpopulation of aged Long-Evans rats is impaired in this task, exhibiting spatial learning scores significantly outside the range of young rats, and this impairment has been related to changes in structure and function in medial temporal lobe structures. In the current study, performance of aged rats in this task was compared to performance in the odor discrimination reversal task.

2. Methods

2.1. Subjects

The subjects consisted of 9 young and 22 aged pathogen-free Long-Evans male rats. Aged rats were obtained as retired breeders at 8–9 months of age from Charles River Laboratories, Wilmington, MA. Young rats were obtained from the same company at the start of the experiment. Young rats were 4 months old at the start of odor discrimination training and 5 months old at the start of water maze testing. Aged rats were 21–22 months old at the start of odor discrimination training and 22–23 months old at the start of water maze testing. Rats were housed individually on a 24-h light/dark cycle with ad libitum access to food and water except during odor discrimination training. During odor discrimination training, the rats continued to have ad libitum access to food but were only allowed access to water for 30–60 min at the end of the day after the training sessions were completed. The health of all subjects during this phase was monitored to ensure adequate hydration. All testing was performed during the light phase of the cycle. During the course of the study, five aged rats died, and data from these rats were excluded from the study. Viral screening and necropsies performed on the remaining 17 aged rats indicated that they were healthy at the end of the study.

2.2. Odor discrimination testing

Odor discrimination testing procedures were similar to those employed by us previously. These procedures were adapted from those developed for olfactory studies by other labs [11,12,46].

2.2.1. Apparatus

Odor discrimination training was conducted using a set of 4 identical operant chambers similar to that described elsewhere [40]. Each operant chamber was constructed of aluminum and measured approximately 18" on each side but with sloping walls narrowing to an area of 12" × 12" at the bottom. An exhaust fan was located on the upper back wall of the operant chamber, and the front wall was hinged to open outward and provide access to the interior of the

training box. Two panel lights were located on the right wall of the chamber. The set of 4 training boxes were located in a 10' × 10' testing room that was closed during the training sessions. Two speakers located in the corners of the room broadcast output from a white noise generator to mask noises in the room.

Each behavioral box was paired with a Pentium II 266 MHz computer for behavioral control and data acquisition. Sessions were conducted using a program written in C++ and running in DOS. This program used i/o registers located on a DT2817 I/O board (Data Translation) to control equipment and to detect behavioral events. All events (behavioral and computer-initiated) were time-stamped using values from a CIO-DIO-CTR3 clock timer board (Computer Boards), capable of microsecond resolution. These data were saved to the hard drive for later analysis of performance and response latency.

Odors were selected from a set of compounds obtained from International Flavors and Fragrances (New York, NY) and identified as iso-propyl hydratropic ald para, verbena oliffac, petinerol, ald C-8 orange fraction florex, cedryl acet trubek, vanoris, geranyl formate, auralva, camekol DH, dimeth phen eth carb acetate, hexenol B gamma extra, celeriax, cyclemone A, and phenoxanol. These odor compounds were classified subjectively by smell according to a published classification system [47]. Discrimination problems consisted of odors from different categories (fruity, spicy, herbal, etc), and categories did not repeat in sequentially presented discriminations. These odor compounds were diluted 1:20 in propylene glycol. The set of diluted odors used in the discrimination problems in this experiment were isolated on a removable cartridge connected to a system of solenoids and flowmeters to allow each odor to be individually delivered to the training chamber. All tubing and valves associated with an odor were dedicated to that odor to prevent any cross-contamination between cues, and each training chamber had an identical set of odor cues.

Odors were presented at an odor delivery port located in a polycarbonate panel bolted into an opening below the panel lights in the right wall of each operant box. The odor sampling port consisted of a 2.5 cm diameter opening. Responses at the odor port were detected by a photobeam passing across this opening. Behind this port was a small hemicylinder where odorized air streams could be presented when a rat nosed at the odor port. Odors were delivered through tubing connected to the base of the hemicylinder behind the sampling port.

Before each trial, an odor was selected for delivery by opening a solenoid valve that allowed a clean air stream to pass over one of the odor solutions on the removable cartridge. The odorized air stream at 1.5 L/min was brought to a vacuum dump behind the odor port drawing at 2.0 L/min. The vacuum was also attached to the hemicylinder behind the odor port by a short (1 cm) length of tubing, resulting in a negative flow of 0.5 L/min out of the hemicylinder between trials and during trials when the odor port was not

occupied. Upon detection of nosed at the odor port, odor delivery was initiated by closing a solenoid valve controlling this vacuum, causing the odorized air stream to be diverted into the hemicylinder behind the port with an onset latency of approximately 25 ms. Odor delivery was terminated by re-opening the vacuum line when the rat left the odor port. During odor presentation, odor was prevented from entering the training chamber by a second vacuum line drawing at 2.0 L/min from the top of the hemicylinder. This vacuum was always on, thus ensuring a constant airflow out of the training chamber through the odor port. The net flow from the chamber was 0.5 L/min during odor sampling and 2.5 L/min at all other times.

Fluids were delivered to a depression located in a ledge just below the odor sampling port. Responses at the fluid well were detected by a photobeam passing parallel to the ledge approximately 1 mm above the well depression. Water was delivered to the well through activation of a solenoid controlling a water line concealed in the bottom of the fluid well.

2.2.2. Training procedure

Before the start of discrimination training, the rats were first trained to nosed at the odor port to receive a water reward. Initially any response at the odor port resulted in the delivery of a water reward to the well, then the rats were gradually shaped to hold their snout in the odor port for a period of 250 ms before odor delivery and 500 ms after odor delivery and to make a response to the well within 3000 ms to trigger fluid delivery. Once shaped to this procedure, odor discrimination training was begun. In all subsequent sessions, trials were signaled to the rat by illumination of the panel lights inside the box. When these lights were on, nosed into the odor port resulted in delivery of the pre-selected odor cue. The rat terminated odor sampling by leaving the odor port. The rat then had 3 s to make a go response at the fluid well. If a response was made after sampling a positive odor, then a 0.05 ml bolus of water was delivered to the well. If the same response was made after sampling a negative odor, no fluid was delivered and instead the panel lights were extinguished. On rewarded trials, the panel lights remained on until the rat left the fluid well, then the lights were extinguished to end the trial. If the rat did not respond within 3 s of exiting the odor port, the trial was counted as a no-go, and the panel lights were extinguished. Inter-trial intervals were 4 s after correct responses and 9 s after incorrect responses.

Odor discrimination problems consisted of 2 odors. One odor was positive, indicating that a response after sampling would result in delivery of a water reward. The other odor was negative, indicating that the same response after sampling would result in termination of the trial and a prolonged inter-trial interval. Rats began each new discrimination problem by responding to both odors and then learned to not respond on negative trials. Rats were trained on this discrimination until they met a criterion of 18 correct responses

in a moving block of 20 trials. Rats were run for approximately an hour each day (~150 trials) or until they met criterion.

After shaping on the initial odor pair, the rats were presented with 2 new discrimination problems. These discrimination problems were the same as the original problem except that they involved two new odors. The odors were chosen from different categories [47] so that they were dissimilar from each other and also from the two other discriminations. Again rats were run for approximately an hour each day or until they met a criterion of 18 correct responses in a moving block of 20 trials. When a rat met criterion on the first of these two problems, training was begun on the second problem in the next training session.

The reversal phase of training began after all of the rats had acquired the initial set of three discrimination problems. In the reversal phase, the rats were presented with 4 new two-odor discrimination problems. The rats were required to reach the performance criterion on each problem, and then the session was terminated. The following day, the problem was presented again. If the rat met the performance criterion a second time, the associations between the odors and the water reward were reversed during this session. During acquisition of reversed problems, the rats were run for approximately 90 min each day. Training on a reversal was continued until the rat met the performance criterion. The following day training would begin on the next problem.

At the completion of reversal training, odor detection thresholds were assessed. Rats were trained to discriminate a new odor from a clean air stream at a similar flow rate. The odor was presented using the same procedure and at the standard concentration used in the discrimination training. After each rat reached criterion, the proportion the 1.5 L/min air stream diverted through the odor was progressively reduced from 50% to less than 3%, and performance was assessed in 20 trial blocks at each new concentration.

2.2.3. Data analysis

Acquisition on each discrimination problem was evaluated by calculating the trials required to reach the performance criterion (TTC) for each animal. Data from the first three discrimination problems were analyzed by ANOVA with repeated measures (age \times discrimination); data from the reversal training phase were analyzed by ANOVA with repeated measures (age \times reversal \times discrimination) followed by step-down ANOVA's. In addition, changes in response latency during the acquisition of the second and third discrimination problems were examined. For this analysis, acquisition of each discrimination problem was divided into an early and a late phase according to when the rat made the sixth error in the session. We have used this approach previously to analyze behavior and neural activity in rats acquiring new odor discriminations [41,42]. Latency to respond at the fluid well after odor sampling was calculated for positive go (S+R+) and negative go (S-R+)

trials in each phase. Response latency on each trial was calculated as the time between unpoke from the odor port and the response at the fluid well. No-go trials (primarily S-R- trials) were not considered in the analysis of latency data. The difference in latency in each phase was analyzed by ANOVA with repeated measures (age \times discrimination \times phase), and latencies on positive and negative trials were also evaluated similarly in a separate analysis with valence as an additional factor. Performance and the number of trials within each phase were also examined to ensure that choice behavior was similar between lesioned and control groups in each phase. All statistical analyses were performed using routines in Statistica (Statsoft, Tulsa OK) at a significance level of $P < 0.05$.

2.3. Water maze testing

2.3.1. Apparatus

After completion of the odor discrimination training, the rats were trained on a spatial learning task in the Morris water maze. The maze consisted of a large, circular tank (diameter 183 cm; wall height 58 cm) filled with water (27°C) made opaque by the addition of powdered milk (0.9 kg). A retractable white escape platform (height 34.5 cm) was located 1 cm beneath the water surface near the center of one of four maze quadrants. White curtains affixed with large black geometric designs provided extramaze cues. Data were analyzed using an HVS Image Analyzing VP-116 video tracking system and an IBM PC with software developed by HVS Imaging (Hampton, UK).

2.3.2. Training procedure

The spatial training protocol has been described in detail previously [15]. Briefly, rats received three trials per day for eight consecutive days using a 60 sec intertrial interval. On each training trial, rats were placed in the water and allowed to swim until finding the platform or for 90 s at which time they were placed on the platform by the experimenter. Rats remained on the platform for 30 s before removal from the maze and the start of the intertrial interval. For spatial learning assessment, the location of the platform remained constant in one quadrant of the maze and the starting position for each trial was varied among four equally spaced positions around the perimeter of the maze. Every sixth trial was a probe trial, during which the platform was retracted to the bottom of the pool for the first 30 s of the 90 s trial. Training and probe trials assessed acquisition and search strategy, respectively [15]. In addition, rats received one session with six trials of cue training after the last day of spatial training to assess sensorimotor skills and motivation to escape independent of spatial learning abilities. In this session, rats were trained to escape to a visible black platform located 2 cm above the water surface that varied in position from trial to trial. Each rat was given 30 s to reach the platform and was allowed to remain there briefly before a 30 s intertrial interval.

2.3.3. Data analysis

Accuracy of performance in the water maze was assessed using two proximity measures: a cumulative search error computed from training trials and a learning index score computed from probe trials. As detailed previously [15], the distance of the rat from the platform was sampled 10 times per second during each trial and these distances were averaged into 1 s bins. Cumulative search error is the sum of these 1 s averages across training trials corrected for start and platform location. The learning index is derived from average proximity (cumulative search error divided by the length of the probe trial) on the second, third, and fourth probe trials. Scores from these trials are weighted and summed to provide an overall measure of spatial learning ability. Lower scores on the learning index indicate a more accurate search. More traditional measures of escape latency and pathlength during place and cue training were also taken.

The effect of age on acquisition was assessed by a two-factor ANOVA with repeated measures (age \times trial block) comparing cumulative search error in 5 trial blocks. The effect of age on search strategy was assessed using a one factor ANOVA to compare index scores computed from the probe trials. Latency to escape to a visible platform during cue training was also inspected to ensure no aged rats exhibited abnormal scores indicative of sensorimotor or motivational deficits. Latencies less than 30 s were considered within the range of normal performance. Correlations between odor discrimination measures and water maze performance were computed using the learning index for individual rats. In some analyses or comparisons the performance of aged rats is referred to as “unimpaired” or “impaired.” Aged-impaired rats possessed learning indices outside the range of young rats (>240), whereas aged-unimpaired rats had scores within the range of young rats (<240). All statistical analyses were performed using routines in Statistica (Statsoft, Tulsa OK) at a significance level of $P < 0.05$.

3. Results

3.1. Odor discrimination performance

3.1.1. Rate of acquisition and choice performance on new discriminations

The acquisition of each discrimination problem is shown in Fig. 1. All rats acquired each of the 3 discriminations, and there were no differences between young and aged rats. A 2-factor ANOVA (age \times discrimination) revealed that there was no effect of age on acquisition [$F(1,24) = 0.36$, $P = 0.55$] nor was there any interaction between age and discrimination problem [$F(2,48) = 0.52$, $P = 0.60$], indicating that aging was not associated with impairment in the rats' ability to acquire odor discriminations at a rate comparable to that of young rats. As expected, there was a significant

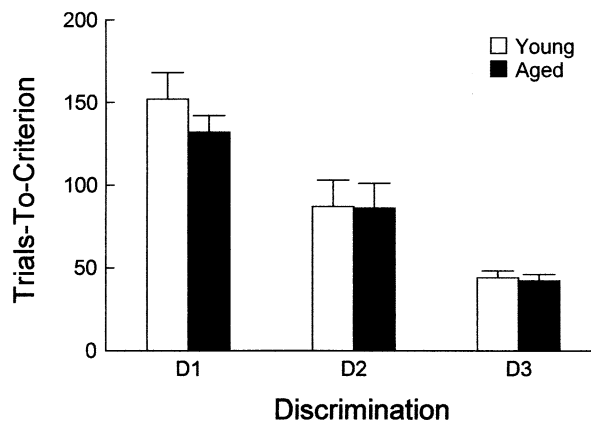


Fig. 1. Acquisition of the initial odor discrimination problems (D1, D2, D3) by aged and young rats. Trials-to-criterion represents the number of trials required to meet a criterion of 18 correct responses in a moving block of 20 trials. Both groups acquired each discrimination problem and improved across successive problems; there was no effect of age.

main effect of discrimination problem [$F(2,48) = 46.9$, $P < 0.001$]. Contrast testing revealed that rats in each group required significantly more trials to acquire the first discrimination problem than either the second or third problems. Subsequent analyses of performance measures during acquisition excluded data from this first shaping problem.

3.1.2. Rate of acquisition and response latency during acquisition of new discriminations

The performance of the aged and young rats was similar on the second and third discrimination problems. As noted above, there were no differences between young and aged rats in the rate of acquisition of these problems. In addition, the rats in each group made similar numbers of errors on these two problems. This similarity was evident when the pre-criterion trials were divided into early and late blocks as we have done in previous electrophysiological recording experiments [41,42]. The numbers of trials in each block did not differ significantly between groups. Aged rats averaged 13.2 trials in the early block and 51.6 trials in the late block on these two discrimination problems, whereas young rats averaged 11.4 trials in the early block and 48.9 trials in the late block. Rats also performed at a similar level of accuracy across the different trial blocks. Aged rats performed at 56% correct and 71% correct in the early and late blocks respectively, whereas young rats performed at 53% correct in the early block and 73% correct in the late block.

In addition to the similarities in measures of choice behavior on these two discriminations, both groups developed a difference in response latency on positive versus negative trials during the late phase of pre-criterion training. These data are shown in Fig. 2. A 3-factor ANOVA (age \times discrimination \times phase) indicated that this difference increased significantly between the early and late phase of training [$F(1,24) = 13.2$, $P = 0.001$], but there was no main effect of age [$F(1,24) = 0.28$, $P = 0.59$] or any interactions

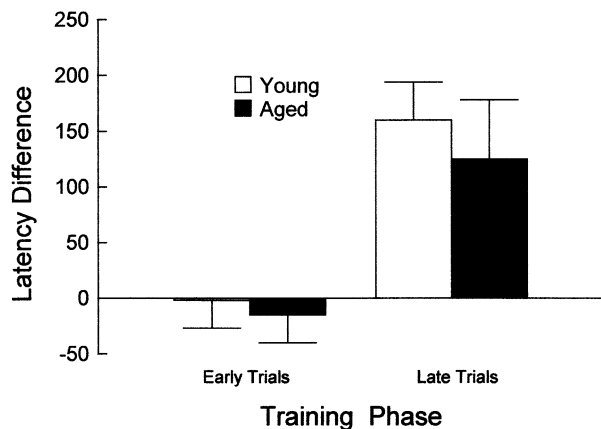


Fig. 2. Changes in response latency during acquisition of new odor discrimination problems (D2 and D3) in aged and young rats. Acquisition was divided into an early and late phase, and response latency was calculated as the time (ms) between unpoke from the odor port and response at the fluid well for each trial. Trials in which no response was made (R-trials) within 3000 ms were not considered. The difference in latency on positive go (S+R+) and negative go (S-R+) trials (neg-pos) is shown for trials in a given phase. Both groups developed a positive difference in response latency in the late phase; there was no effect of age.

between age and the other factors. When latencies from positive (S+R+) and negative (S-R+) trials were evaluated separately in a second analysis (age \times discrimination \times phase \times valence), there was a main effect of age [$F(1,24) = 10.77, P = 0.003$], however there were no significant interactions between age and the other factors. Subsequent contrast testing indicated that aged rats had slower response latencies overall (489 ms for aged and 349 ms for young rats) but exhibited the same the pattern of change in response latency with learning as young rats.

3.1.3. Acquisition of reversals

All rats successfully completed reversal phase training, acquiring and reversing each of the four discrimination problems. Reversal training typically required 1–5 sessions for a given problem. The data of interest are shown in Fig. 3. As a group, aged rats exhibited a moderate impairment in acquiring reversals. This impairment was evident in a 3-factor ANOVA (age \times discrimination \times reversal), which revealed a significant main effect of age [$F(1,24) = 4.88, P = 0.037$] and a significant interaction between age and reversal [$F(1,24) = 4.34, P = 0.047$], consistent with the effect illustrated in Fig. 3. As expected there were significant main effects of discrimination [$F(3,72) = 4.40, P = 0.007$], and reversal [$F(1,24) = 171.8, P < 0.001$], and a significant interaction between discrimination and reversal consistent with improvement in performance on reversal but not acquisition by both groups across the four problems [$F(3,72) = 5.37, P = 0.002$]. There was no interaction between age and discrimination. Separate analyses of acquisition and reversal confirmed aged rats did not differ from controls in acquiring these four discrimination prob-

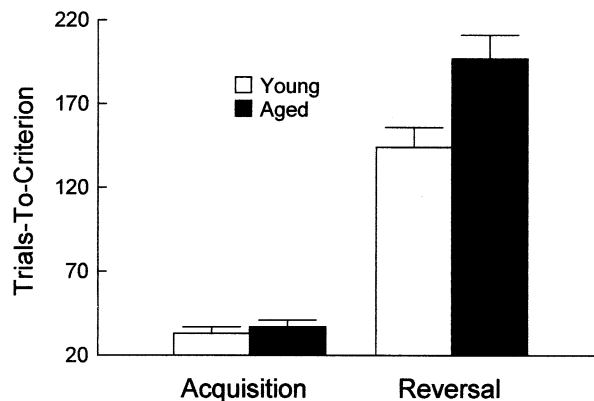


Fig. 3. Average performance on acquisition and reversal of the new odor discrimination problems by aged and young rats during the reversal training phase. Trials-to-criterion represents the number of trials required to meet a criterion of 18 correct responses in a moving block of 20 trials. Both groups acquired and reversed each discrimination problem. There was no effect of age on acquisition, however the aged group exhibited moderate impairment on reversals.

lems, however they did require significantly more trials than young rats to reach criterion again after reversal [$F(1,24) = 4.87, P = 0.037$]. Again there was no interaction between age and discrimination across the four problems in either analysis.

3.1.4. Odor detection

Aged and young rats performed similarly at discriminating between a new odor and a clean air stream. Both groups reached criterion at the same rate (53 trials for aged rats; 60 trials for young rats) and showed similar rates of decline in performance as the odor concentration was reduced (Fig. 4). A 2-factor ANOVA (age \times odor concentration) confirmed that there was neither an effect of age nor any interaction between age and the rate of decline in performance with decreasing odor concentration.

3.2. Water maze performance

The data of interest are shown in Fig. 5. Assessment of spatial learning revealed a significant effect of age on acquisition and probe-trial performance. As illustrated in Fig. 5A, aged and young rats performed similarly on the first training trial [$F(1,24) = 1.71, P = 0.20$], but over the course of subsequent training trials the aged rats exhibited a higher mean search error [$F(1,24) = 5.35, P = 0.029$], indicating that they performed more poorly than controls at locating the hidden platform during these trials. Impairment is also evident in the spatial learning index scores calculated from performance on the probe trials when the platform was not present. Aged rats exhibited higher learning index scores than young rats when the platform was removed [$F(1,24) = 10.11, P = 0.004$], indicating a search pattern less closely related to the platform location than young controls. The distribution of these scores is shown in Fig. 5B. These data

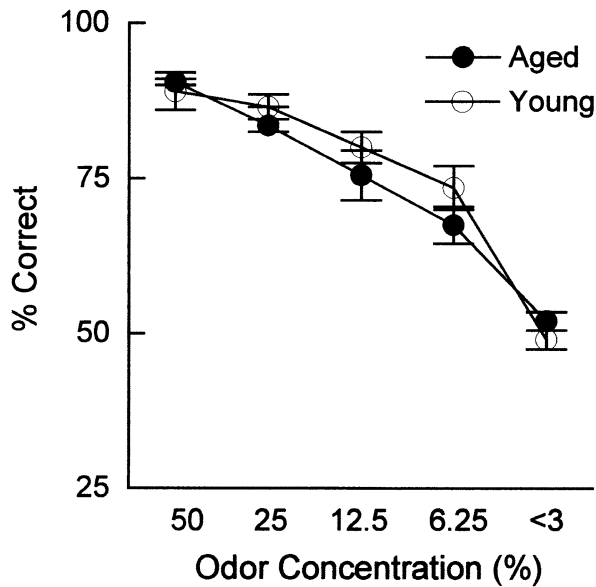


Fig. 4. Odor detection threshold. Performance of aged and young rats at discriminating a target odor from clean air was assessed at various decreasing odor concentrations. The assessment began after the rats acquired the odor/air discrimination, then the concentration of the odor was progressively decreased from the training concentration (50%) without changing the flow rate. Performance was assessed in 20 trial blocks at each concentration. Rats in both groups exhibited accurate performance (>80%) at higher odor concentrations and exhibited chance performance (50%) at low odor concentrations (<3%). There was no effect of age.

indicate that some aged rats performed as well as young rats while others fell outside this range. As in prior studies, aged rats with learning index scores above 240 were classified as impaired [15].

During subsequent cue training with a visible platform, the aged and young rats exhibited similar escape latencies, indicating that the two groups did not differ with respect to sensorimotor abilities or motivation to escape from the water. Young rats had an average latency of 9.53 s, whereas aged rats had an average latency of 8.59 s. These data did not differ significantly [$F(1,24) = 0.23$, $P = 0.63$]. In addition, all of the individual rats had escape latencies of less than 30 s.

3.3. Comparisons between odor discrimination and water maze performance in aged rats

The spatial learning index calculated from performance on probe trials in the water maze provides a reliable index of medial temporal lobe function. The distribution of these scores in Fig. 5B suggests that a many of the aged rats in this study had hippocampal impairment. To determine whether there was any relationship between hippocampal function and performance on reversals, we compared the spatial learning index and reversal performance in the aged rats. Reversal performance was quantified by the average trials to reach criterion across the four reversal problems. As

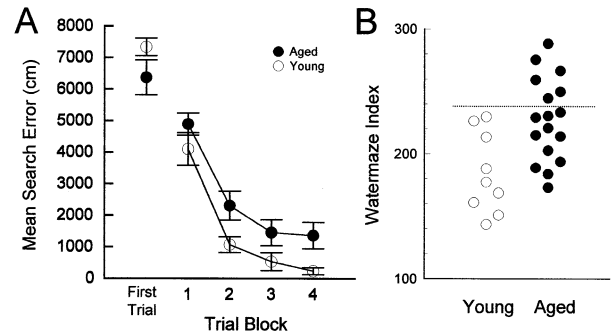


Fig. 5. Water maze performance. A. Performance of young (open circles) and aged (closed circles) rats during training trials in the spatial version of the water maze as assessed by cumulative search error. Cumulative search error measures the proximity of the rat to the platform during the course of a training trial. The figure shows the average cumulative search error for 4 blocks of 5 trials (1–4). In addition, the search error from the first training trial is shown. Young and aged rats did not differ on the first training trial, but over the course of training aged rats were reliably impaired relative to young rats in acquiring the task. B. Distribution of individual learning index scores for young (open circles) and aged (closed circles) rats. As in prior studies, a subpopulation of aged rats exhibited abnormally elevated index scores (>240), indicating an impaired spatial learning ability relative to young rats.

illustrated in Fig. 6A, there was no difference in the performance of aged-impaired (learning index > 240) and aged-unimpaired (learning index < 240) rats in the odor discrimination reversals. In addition, there was no correlation between performance in the water maze and reversal performance when the scores of the individual rats were examined ($r = 0.05$, $P = 0.85$) as illustrated in Fig. 6B.

Regression analyses comparing trials-to-criterion by aged rats on the four reversal problems found a significant correlation between performance on the second and third reversal problems ($r = 0.65$, $P = 0.004$), indicating that performance on these reversals showed test-retest reliability similar to that documented for the spatial learning index [15]. Comparisons restricted to performance on these two reversal problems confirmed that there was no correlation between performance in the water maze and reversal learning.

4. Discussion

In the present experiment, we have compared the performance of young and aged rats in two different tasks designed to test the function of different brain systems. Rats were first tested in an odor discrimination reversal paradigm designed to reveal deficits related to OFC dysfunction and then on a spatial version of the Morris water maze recognized as a reliable assay for medial temporal lobe dysfunction. We found that rats in the aged group were impaired on both tasks, exhibiting deficits in spatial and reversal learning relative to young controls. Importantly performance on the spatial task did not predict performance in reversal

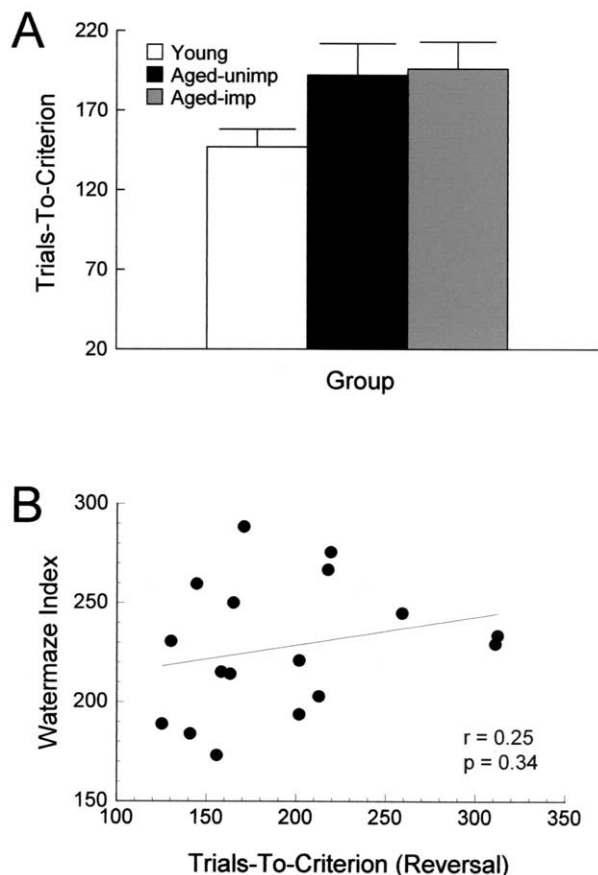


Fig. 6. Relationship between maze performance and odor discrimination reversal for aged rats. A. Performance of aged rats during reversal training is shown separately for rats classified as impaired (gray bars) or unimpaired (black bars) by probe testing in the water maze. Impaired rats were those animals with learning index scores >240 on the water maze. Young rats are shown for comparison (open bars). The average trials-to-criterion on the reversals is shown as in Fig. 3. B. Comparison of performance of individual aged rats across the two tasks. Maze and reversal performance were quantified by the learning index scores and averaged trials-to-criterion on reversals respectively. Scores for individual aged rats are plotted, and the line indicates the linear relationship between them for the aged group. The results from a regression analysis are shown. No distinction was made in this analysis between impaired and unimpaired rats. There was no relationship between the two performance measures.

training (or any other measure of discrimination performance) either for individual rats or when aged rats classified as impaired and unimpaired on the water maze were evaluated separately. These results are consistent with a contribution to age-related cognitive decline attributable to structures outside the medial temporal lobe system in rats and suggest that changes in OFC (or related structures) may mediate some of these performance decrements.

4.1. Reversal as an assay of orbitofrontal function

Impairment of reversal learning has long been a hallmark of prefrontal damage and in particular of orbitofrontal damage (orbitofrontal syndrome). These reports were first made

as long ago as the mid-1800's with the finding that damage to primate prefrontal regions resulted in impulsive or disinhibited behavior [13,23], as manifest in the clinical case of Phineas Gage [21]. More recently, these so-called deficits in response inhibition have been localized to the orbitofrontal region within prefrontal cortex [4,26,38]. In controlled settings, these tendencies are evident as perseverative responding during discriminations, typically in reversal training where the outcomes associated with cues are switched [27, 38,39]. Notably impairments are often not seen when the animals are trained on the initial discriminations, despite the requirement for response inhibition, suggesting that alternative explanations may better account for the deficit. Recent work indicates that OFC is part of a circuit important for the ability to effectively use acquired incentive value to organize response choices [3,16,22], and it appears to be crucially involved when new responses must be acquired to cues within a single stimulus dimension [10], as is the case in reversal training used in the current research.

Here we have used olfactory reversals as an assay of OFC function in aged rats based on the effects of OFC lesions in young rats on performance in a similar paradigm [39]. In that study, we found that rats with bilateral neurotoxic lesions of OFC made before the start of discrimination training were able to acquire discrimination problems normally and improve across problems similar to intact controls, but they were markedly impaired in acquiring subsequent reversals. Notably all lesioned rats were able to acquire the reversals with sufficient training and exhibited similar performance on the discrimination during subsequent testing, thus the deficit appeared to be specific to the acquisition of reversal learning. The current findings in aged rats closely resembled these results. The aged rats exhibited normal performance when learning a set of new discrimination problems but were impaired during subsequent reversal training. Like OFC lesioned rats, this impairment was evident only upon reversal of previously learned discriminations; aged rats continued to perform normally in acquiring new problems during reversal training.

It is important to note that other structures have been implicated in reversal learning, most notably medial temporal lobe regions. It is not our intent to review the role of hippocampal structures in reversal learning here except to note that, unlike results from OFC, reports of reversal impairment after medial temporal lobe lesions differ widely. Various accounts have found impairment, facilitation or no effect of lesions of these regions on spatial, object or olfactory reversal learning [5,12,28,51,54,55]. These reports differ in their specific experimental design and also in the specific site of the lesions. However in the current investigation it is notable that aged rats with hippocampal-dependent learning impairment did not differ in reversal learning from aged cohorts that had preserved spatial learning ability. The lack of correlation along with the similarity between the results in this study and our earlier report of the effect of OFC lesions on reversal learning in this paradigm

provide support for the contention that reversal impairment in this task reflects cognitive decline outside the hippocampal system.

4.2. Orbitofrontal function in aging

As recent reports have pointed out, cognitive decline with normal aging is likely a heterogeneous process [14,17,19,36,44]. Previously animal models of normal aging have focused heavily on tasks sensitive to medial temporal lobe dysfunction. This approach has been productive, correlating behavioral impairment across species and revealing relationships between behavioral impairments and a number of markers in the medial temporal lobe system [17]. This approach has also been employed to examine prefrontal function using tasks identified in primates as sensitive to damage in dorsolateral prefrontal cortex [17,34,53]. Yet prefrontal cortex is characterized by functional heterogeneity whereby different regions play different roles in the strategic use of information in guiding behavior. This functional heterogeneity may also be evident in the effects of aging.

In particular, a constellation of symptoms noted in the literature to be associated with prefrontal decline in aged individuals is reminiscent of orbitofrontal dysfunction. For example, impulsive or disinhibited behavior and poor judgment are often prominent features of age-related decline, and perseverative errors are frequently noted in behavioral testing [9]. These symptoms closely resemble the so-called “orbitofrontal syndrome” observed in primates and rats following damage to OFC [26,27,38,39]. This similarity suggests that within prefrontal cortex there may be changes associated with normal aging in the orbitofrontal subdivision. Indeed prior work indicates that declines in OFC function are evident in aged rats [56], and here we report that aged-rats also exhibit impaired reversal learning, a hallmark of the orbitofrontal syndrome across species. Although the report of reversal impairment associated with normal aging is not unique [2], the current data show that impairments in reversal learning may be dissociated from medial temporal lobe dysfunction and instead point to OFC as a likely locus for the age-related changes.

Rat OFC and related structures may provide a particularly useful system in which to investigate the effect of aging on prefrontal function. Renewed interest in primate orbitofrontal function has revealed much new information regarding this region, and key functions appear to be conserved across species [43]. In particular, OFC in both rats and primates is crucial to the processing of information regarding incentive value [3,16,22], and experimental studies in rats and monkeys have shown that in associative learning tasks neurons in OFC fire differentially to cues based on the incentive value of the events predicted by those cues [42,52]. In addition, when delays intervene between the presentation of informative cues and reward, OFC neurons are differentially active during these delays and appear

to prospectively encode the value of the impending reinforcer [41,52]. These neural correlates provide an associative map that may be critical for guiding behavioral responses. For example, damage involving OFC eliminates the ability to adjust responding based on changes in the value of a reinforcer in both rats and primates [3,16,22]. This role may also provide a basis for the well-documented effects of OFC damage on reversal learning. While animals can acquire an initial behavioral response, possibly via alternate stimulus-response learning mechanisms, the ability to modify initial learning when contingencies change is likely to require an associative mapping mechanism that links cues to representations of the events they predict. Our results suggest that this cognitive function is compromised during the aging process.

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