ELSEVIER

Neurobiology of Aging 30 (2009) 646–655

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Spatial reference and working memory across the lifespan of male Fischer 344 rats

J.L. Bizon [a,*], C.L. LaSarge [a], K.S. Montgomery [a], A.N. McDermott [b], B. Setlow [a], W.H. Griffith [b]

[a] *Department of Psychology, Texas A&M University, College Station, TX 77843-4235, United States*
[b] *Department of Neuroscience and Experimental Therapeutics, Texas A&M Health Science Center College of Medicine, College Station, TX 77843-1114, United States*

Received 18 April 2007; received in revised form 7 July 2007; accepted 9 August 2007
Available online 21 September 2007

Abstract

Loss of mnemonic function is among the earliest and most disconcerting consequences of the aging process. This study was designed to provide a comprehensive profile of spatial mnemonic abilities in male Fischer 344 (F344) rats across the lifespan. Young, middle-aged, and aged F344 rats were trained in spatial reference and working memory versions of the water maze task. There was a progressive age-related decline in spatial reference memory across the lifespan. Reliable individual differences were observed among aged rats, with some aged rats performing as well as young cohorts and others performing outside this range. An age-related delay-dependent decline was observed on a working memory version of the water maze task although no relationship between performance on reference and working memory tasks was present. Notably, middle-aged rats were impaired relative to young on both tasks. Together these data demonstrate that individual differences in spatial reference memory exist among aged F344 rats and provide novel data demonstrating an unrelated decline in working memory across the lifespan, suggesting that age-related mnemonic dysfunction may occur across multiple brain systems.

Keywords: Aging; Hippocampus; Spatial learning; Working memory; Cognitive decline

1. Introduction

Declarative memory (memory for people, facts, and events, as well as for spatial locations) is dependent upon the medial temporal lobe system, including the hippocampal formation (Burke and Barnes, 2006; Della-Maggior et al., 2002; Squire et al., 2004; Wilson et al., 2004). Dysfunction of this system and concomitant loss of associated learning and memory functions are well documented at advanced ages. Indeed, both reference memory (memory for information that is held constant over time) and working memory (the ability to remember, usually for a relatively brief period of time, information that must be distinguished from previously learned similar information) are adversely affected during the aging process (Foos, 1989; Lindner et al., 1992; Lipman, 1991; Lynch et al., 2006; Missonnier et al., 2004; Park et al., 2002; Reuter-Lorenz et al., 2000; Rosenzweig and Barnes, 2003). Importantly, such impairments in medial temporal lobe functioning are not an inevitable consequence of the aging process (Bennett et al., 2002; Bizon and Gallagher, 2005; Gallagher et al., 1993; Wilson et al., 2002). At advanced chronological ages, a spectrum of mnemonic abilities exists such that some aged individuals perform on par with young adults whereas others experience deficits ranging from mild to profound.

Using a reference memory version of the Morris water maze, several naturalistic rat models of aging have been used to mimic the individual variability in mnemonic function associated with the medial temporal lobe system present in the human population (Gallagher et al., 1993; Lee et al.,

* Corresponding author at: Behavioral and Cellular Neuroscience, Department of Psychology, Texas A&M University, College Station, TX 77843-4235, United States. Tel.: +1 979 458 3653; fax: +1 979 845 4727.
E-mail address: jbizon@tamu.edu (J.L. Bizon).

0197-4580/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2007.08.004

1994; Shukitt-Hale et al., 1998). Moreover, a variety of functional deficits in these structures and changes in neuronal encoding properties correlate with individual differences in spatial learning abilities (Barnes et al., 1997; Brightwell et al., 2004; Colombo and Gallagher, 2002; Gallagher and Nicolle, 1993; Tanila et al., 1997). However, it has been reported that minimal individual variability exists among aged male F344 rats and that variability within this and potentially other rodent models of human cognitive aging may be a result of within-subject factors rather than reliable individual differences between aged rats (Barnes et al., 1997; Frick et al., 1995).

In the current study, young, middle-aged, and aged F344 rats were tested sequentially on a spatial reference memory and a delayed-match-to-place (working memory) version of the water maze (adapted from Baxter et al., 1995). The reference memory task was used to determine the age of onset of mnemonic deficits and whether reliable individual differences in performance could be observed during aging. The working memory task was used because it has been reported that working memory deficits may emerge at middle age in humans, and thus, we hypothesized that deficits in this task would be evident at middle age in the F344 rat population (Park et al., 2002). Early detection (at middle age) of mnemonic deficits might identify rats at risk for future cognitive decline, and thus allow studies of strategies to prevent such decline. The results demonstrate an age-related decline in both spatial reference and working memory across the lifespan, such that both middle-aged and aged rats were impaired relative to young, although the magnitude of these impairments was greater in aged rats. Reliable individual differences in spatial reference memory were also present among aged F344 rats. Somewhat surprisingly, no relationship was observed at any age between individual performance on the spatial reference and working memory tasks. These latter data suggest that neurobiological changes in independent brain systems can result in multiple domains of cognitive dysfunction in F344 rats across the lifespan.

2. Methods

2.1. Subjects

Young (6 months; $n = 35$), middle-aged (12 months; $n = 30$), and aged (22 months; $n = 60$) male Fischer 344 (F344) rats were used. Rats were obtained from the National Institute of Aging colony and housed in the vivarium in the Psychology Building at Texas A&M University for 2 weeks prior to the onset of behavioral testing. This AALAC-accredited vivarium was maintained at a consistent 25 °C with a 12:12 h light/dark cycle (lights on at 08:00), and rats had free access to food and water at all times. All rats in the study were screened daily for health problems including, but not limited to, cataracts, jaundice, food and water intake, and the appearance of tumors. Sentinel rats, housed alongside the rats used in this study, were routinely health screened and found to be negative for a range of pathogens. Finally, upon autopsy, each subject was screened for visible pituitary tumors that could potentially impair visual acuity by impinging on the optic nerve. Six aged rats were excluded from the study based on the health parameters described above. Thus, a total of $n = 35$ young, $n = 30$ middle-aged, and $n = 54$ aged rats completed behavioral protocols and were deemed healthy throughout all testing. All animal procedures were conducted in accordance with approved institutional animal care procedures and NIH guidelines.

2.2. Apparatus

Both the reference and working memory tasks were conducted in the same water maze apparatus. The maze consisted of a circular tank (diameter 183 cm, wall height 58 cm) painted white and filled with water (27 °C) made opaque with the addition of nontoxic white tempra paint. The maze was surrounded by curtains to which were affixed large geometric designs that provided extramaze cues. A video camera mounted above the center of the maze was connected to a DVD recorder and computer, which were used for data storage and analysis using a video tracking system (Water 2020, HVS Image, UK). In the reference memory task, the curtains were black with white cues, and a retractable escape platform (diameter = 12 cm, HVS Image, UK) was submerged 2 cm below the water's surface in the southwest quadrant of the maze. In the cued platform task a black platform (diameter = 12 cm) that protruded 2 cm above the water's surface was located in a different quadrant on each trial. In the working memory task, white curtains with black cues (that differed in shape and location from those used in the reference memory task) surrounded the maze. The platform (diameter = 12 cm) was submerged 2 cm below the water's surface and was located in a different position on each day that varied with respect to both distance from the maze wall and position in each quadrant. The southwest quadrant, used for the reference memory task, was not used in the working memory task.

2.3. Experimental design

Rats were tested on the reference memory task in four cohorts, with each cohort including $n = 8$–10 young and $n = 15$ aged rats at the outset of training. Two of the four cohorts also included $n = 15$ middle-aged rats. One week after completion of the reference memory task, including cued (visible platform) training, a subset of young ($n = 17$), middle-aged ($n = 29$), and aged ($n = 21$) rats were trained on the spatial working memory (delayed match-to-place) version of the water maze.

2.3.1. Spatial reference memory task

Rats were trained as described in LaSarge et al. (2007). In brief, rats received three training trials a day for eight consec-

utive days. On each trial, rats were placed into the water facing the wall of the maze at one of four equally spaced start positions (north, south, east, or west). The start positions were varied in a pseudorandom fashion such that all rats started from each of the locations the same number of times. Rats were allowed to swim for up to 90 s in order to locate the platform before they were guided to it by the experimenter. Rats remained on the platform for 30 s, and subsequently they were placed in a holding cage for a 30 s inter-trial interval. Every sixth trial was a probe trial, in which the platform was lowered to the bottom of the maze for the first 30 s of the trial, after which it was raised to allow rats to escape.

2.3.2. Cued (visible platform) task

Following training on the reference memory task, rats were given a single session with six trials of cue training. In this session, rats were trained to escape to a visible platform that was moved to a different maze quadrant on each trial. Rats were given 90 s to reach the platform and were allowed to remain there briefly before a 30 s inter-trial interval.

2.3.3. Spatial working memory (delayed match-to-place) task

One week following completion of the reference memory task, young ($n = 17$), middle-aged ($n = 29$), and aged ($n = 21$) rats were trained on a delayed-match-to-place version of the water maze (adapted from Baxter et al., 1995) to assess the effects of age on spatial working memory and to determine whether age-related deficits observed in reference memory generalized across spatial tasks. Over 12 consecutive days, rats received two trials a day with varying inter-trial intervals. On the first trial of each day (the information trial) the submerged platform was located in a novel position, different in quadrant and distance from the edge of the maze from the placement on the previous days. On the second trial (the retention trial), the submerged platform was located in the same position as the information trial. The start position was always distal from the platform, and the trials were otherwise conducted in the same manner as in the reference memory task. On the first 3 days of the task, a 30-s inter-trial interval was imposed between information and retention trials to acclimate the rats to the working memory task procedures. On the following 9 days, the inter-trial interval alternated between 30 min, 2 h, and 6 h, such that each delay was used three times. Note that only one cohort of rats received training with the 6 h delay ($n = 8$ young, $n = 15$ middle-aged, and $n = 12$ aged).

2.4. Behavioral and statistical analyses

For each task, data files created by the Water 2020 software (HVS Image, UK) were exported to Microsoft Excel and SPSS (v. 12.0.1) for analysis. In all statistical comparisons described below, p values less than 0.05 were considered significant.

2.4.1. Spatial reference memory task

Training trial data were averaged into four blocks consisting of the five trials preceding each probe trial, and performance was analyzed using both pathlength and cumulative search error measures. Pathlength is the total distance traveled from the start position to the platform and is reported in centimeters. To calculate cumulative search error, the rat's distance from the platform was sampled 10 times/s and these distances were averaged into 1 s bins. Cumulative search error is the sum of these 1 s bins minus the optimal path from the start location to the platform (Gallagher et al., 1993). Additional measures of performance (e.g. latency, swim speed) also were recorded. Interpolated probe trial data (i.e. every sixth trial) were analyzed using mean search error. This measure was derived by dividing the cumulative search error by 30 s (i.e. probe trial duration). Comparisons between age groups on both training trial blocks and probe trials were conducted using two-factor repeated measures ANOVAs (age × training trial block or probe trial) with Fisher's LSD post hoc tests conducted where appropriate.

In addition to the comparisons described above, a *Spatial Learning Index* score was derived for each rat using criteria established by Gallagher et al. (1993). The Spatial Learning Index is calculated by weighting and summing mean search error from the interpolated probe trials to provide an overall measure of spatial learning ability for each rat. Weights for each probe trial were derived by dividing the mean search error in the young group on probe trial 1 by the mean search errors on probe trials 2 (Gallagher et al., 1993). Using data from this F344 study population, the weights assigned to each probe trial were: probe trial 2: 1.25; probe trial 3: 1.60; probe trial 4: 1.70. Lower Spatial Learning Indices indicate better performance. One middle-aged rat with a Spatial Learning Index of 412 was excluded from all analyses. This outlier fell three standard deviations above the mean middle-aged group performance, and was higher than the worst performing rat in the aged group. With this exclusion, a total of $n = 29$ middle-aged rats were included in all analyses reported below. A one-factor ANOVA and Fisher's LSD post hoc tests were used to assess differences between age groups using the Spatial Learning Index measure.

Using the individual Spatial Learning Index as a basis for sub-grouping aged rats into "aged better-performers" (i.e. those aged rats that performed within the range of young subjects) and "aged worse-performers" (i.e. aged rats that performed outside this range), interactions between age and performance on the spatial reference memory water maze task were further investigated using a two-factor repeated measures ANOVA (cognitive age group × probe trial) and Fisher's LSD post hoc analyses.

Finally, to identify potential sources of variability in spatial reference memory among aged rats, the distributions of performance on probe trials using the mean search error measure were assessed using plots of frequency distributions, as

described in Barnes et al. (1997). These distributions were fitted to Gaussian and sum-of-two-Gaussian models using non-linear regression, and the relative fit of the two models was compared using Akaike's Information Criterion run in GraphPad Prism software (v. 4.0).

2.4.2. Cued (visible platform) task

Mean swim speed and pathlength were calculated from the six cued (visible platform) trials. Separate one-factor ANOVAs and Fisher's LSD post hoc analyses were used to compare age groups on each performance measure.

2.4.3. Spatial working memory (delayed match-to-place) task

Separate analyses were conducted for test trials at 30 min, 2 h and 6 h delays. One-factor ANOVAs were conducted to assess age differences on information trials. Test trial performance was calculated by subtracting the pathlength of each rat on the retention trial from its pathlength on the previous information trial (pathlength difference). Larger pathlength differences indicate better performance. These pathlength differences were averaged across the three trials at each delay. Due to uneven group sizes across the delays (only one cohort was tested at the 6 h delay), one-factor ANOVAs, followed by Fischer's LSD post hoc tests where appropriate, were used to compare age groups at each delay. A series of Pearson's correlation coefficients was used to compare individual performance of rats on the spatial reference memory task (Spatial Learning Index or mean pathlength on training trial block 4) with performance at each delay on the spatial working memory task (pathlength difference).

3. Results

3.1. Swim speed

Age-related differences in swim speed were assessed in three separate conditions minimally confounded by learning: the first trial of the spatial reference memory task, mean cue training to a visible platform, and mean information trials in the spatial working memory (delayed match-to-place) task. One-factor ANOVAs revealed a main effect of age on swim speed in each of these conditions: first trial of the reference memory task: ($F_{(2,110)} = 29.20$, $p < 0.01$); mean pathlength to the visible platform: ($F_{(2,110)} = 37.44$, $p < 0.001$); information trials in the delayed match-to-place task: ($F_{(2,66)} = 76.47$, $p < 0.001$). Fisher's LSD post hoc analyses indicated that aged rats swim at a significantly slower rate compared to young and middle-aged rats on each of these measures ($p < 0.001$ in all cases). Given that such differences in swim speed can confound some traditional measures of spatial memory performance such as latency to the escape platform, these data indicate that care must be taken in choosing performance measures in this aging model that are minimally confounded by or entirely independent of swim speed. Thus, in this paper, latency results are not reported. Pathlength (total distance swum to reach the escape platform) was used to confirm effects of age on training trial performance detected using cumulative search error. Despite being somewhat affected by swim speed, the latter measure is included as an assessment method because it was specifically developed to be sensitive to age-related impairment (Gallagher et al., 1993). An important goal of this study was to compare performance of young and middle-aged rats, between which swim speed did not differ. Although cumulative search error can be influenced to some degree by swim speed on training trials, note that mean search error on probe trials should be largely independent of swim speed differences given that these trials are of a fixed duration (i.e. 30 s, See Section 2 for more details).

3.2. Cued (visible platform) task

The cued (visible platform) water maze task was performed to control for sensorimotor and motivational deficits that might influence performance on the reference and working memory tasks, independent of mnemonic abilities. Middle-aged and aged rats that performed more than three standard deviations beyond young performance (assessed by mean pathlength across all six trials) were considered to have non-mnemonic or global mnemonic deficits that could interfere with the spatial memory assessment. Five aged rats met this exclusion criterion and were removed from all subsequent analyses. Thus, final group sizes for all subsequent statistical analyses were $n = 35$ young, $n = 29$ middle-aged, and $n = 49$ aged. Using this final data set, performance on mean pathlength on cue training trials was assessed. A one-factor ANOVA revealed a significant main effect of age on mean pathlength across all six trials ($F_{(2,110)} = 7.37$, $p < 0.001$). Subsequent Fisher's LSD post hoc comparisons showed that middle-aged rats had significantly shorter pathlengths on the cued task than both young and aged rats ($p < 0.05$ in both cases), whereas young and aged rats did not differ ($p = 0.39$). Note that despite the significant effect of age on cue training performance, these differences would not likely influence the age differences observed in the spatial reference and working memory tasks reported below. The pattern of between-group differences in the cued task (i.e. middle-aged rats having an overall shorter pathlength to reach the visible platform than young rats) would, if anything, minimize the ability to detect spatial learning deficits in middle-aged rats, as, if better performance by middle-aged rats in the cued task were also present in the spatial tasks, it would be expected to bias their performance in the direction of less impairment. However, as described below, deficits in both the reference and working memory tasks were observed in middle-aged relative to young rats, suggesting that the influence of any performance enhancements in middle-aged rats was minimal relative to the magnitude of the deficits (see Section 4 for further details).

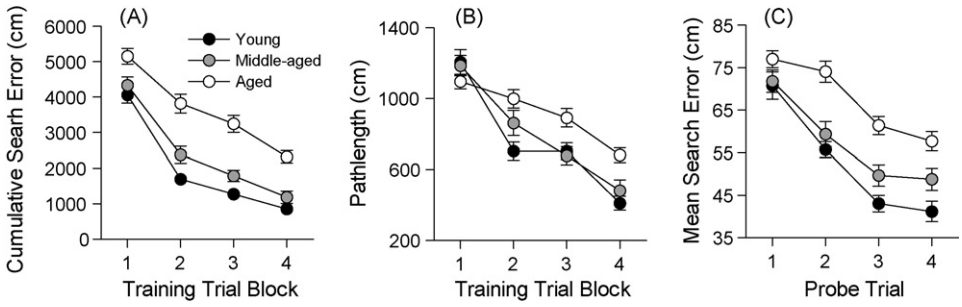

Fig. 1. Spatial reference memory performance of young, middle-aged and aged F344 rats. Cumulative search error ± S.E. (A) and pathlength ± S.E. (B) across the four training trial blocks is shown for young (black circles), middle-aged (gray circles) and aged (open circles) rats. Panel (C) shows mean search error ± S.E. on probe trials interpolated throughout the spatial learning protocol (every sixth trial). All age groups improved over the course of training although a significant interaction was observed such that aged rats did not improve their performance to the same degree as young and middle-aged rats (B and C). Fisher's LSD post hoc comparisons indicated that middle-aged rats were significantly impaired relative to young rats (A and C) and that aged rats were significantly impaired relative to both young and middle-aged rats (A–C). See text for statistical analyses.

3.3. Spatial reference memory task

3.3.1. Training trials

Fig. 1 shows cumulative search error (A) and pathlength (B) on training trials in young, middle-aged, and aged rats. A repeated measures ANOVA (age × training trial block) performed on both performance measures revealed that rats improved over the course of training (cumulative search error: ($F_{(3,330)} = 160.06$, $p < 0.001$); pathlength: ($F_{(3,330)} = 96.90$, $p < 0.001$)) and that there was a main effect of age on training trial performance (cumulative search error: ($F_{(2,110)} = 38.37$, $p < 0.001$); pathlength: ($F_{(2,110)} = 9.56$, $p < 0.001$)). Using the cumulative search error measure, there was a strong trend toward an interaction between age and trial block ($F_{(6,330)} = 2.07$, $p = 0.056$). This interaction reached significance using the pathlength measure ($F_{(6,330)} = 5.91$, $p < 0.001$), such that, as a group, aged rats improved to a lesser extent over training trials compared to young and middle-aged rats. Fisher's LSD post hoc comparisons on both measures further revealed that aged rats performed significantly worse than their young and middle-aged cohorts (cumulative search error: $p < 0.001$ in both cases; pathlength: $p < 0.05$ in both cases). A significant difference was also present between middle-aged and young rats using the cumulative search error measure such that middle-aged rats performed significantly worse than young rats ($p < 0.05$). This difference did not reach significance using the pathlength measure, although there was a trend in the same direction (i.e. middle-aged rats tended to perform worse than young rats; $p = 0.11$).

3.3.2. Probe trials

Spatial learning performance was also assessed by evaluating the mean proximity to the platform (mean search error) across probe trials interpolated throughout the training procedure. Fig. 1C shows performance of young, middle-aged, and aged rats across the four probe trials. A repeated measures ANOVA (age × probe trial) confirmed that, as observed during training trials, all rats improved over the course of training ($F_{(3,330)} = 71.04$, $p < .001$), and there was a main effect of age ($F_{(2,110)} = 30.36$, $p < .001$). Also, in agreement with training trial performance, a significant interaction was observed such that aged rats improved to a lesser extent than young and middle-aged rats over the course of training ($F_{(6,330)} = 2.13$, $p < 0.05$). Fisher's LSD post hoc comparisons indicated that middle-aged rats performed significantly worse than young ($p < 0.05$) and aged rats performed significantly worse than both young and middle-aged rats ($p < 0.001$ in both cases).

Using procedures described in Gallagher et al. (1993), a Spatial Learning Index was calculated for each individual rat. This measure, specifically designed to maximize individual differences in water maze performance within the context of aging, has been shown to correlate with age-related changes in numerous neurobiological substrates of spatial memory (Bizon et al., 2001; Colombo et al., 1997; Nicolle et al., 1999; Smith et al., 2000). Fig. 2A shows the distribution of Spatial

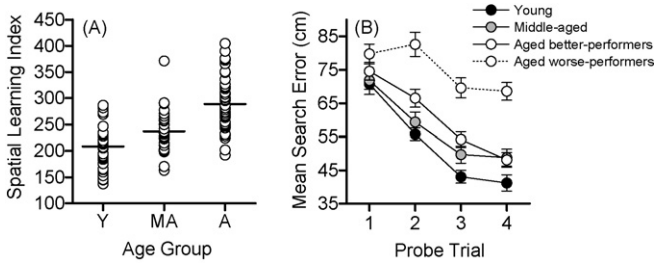

Fig. 2. Individual differences in spatial reference memory performance among young, middle-aged, and aged F344 rats. Panel (A) shows the distribution of individual Spatial Learning Indices for young (Y), middle-aged (MA), and aged (A) rats. Despite the fact that middle-aged rats were impaired relative to young rats, the majority of middle-aged rats performed within the range of young. Note the variability in performance among aged rats, however, such that some aged rats performed on par with young cohorts and others performed outside this range. Panel (B) shows mean search error ± S.E. on probe trials for young, middle-aged, and aged rats subgrouped into aged better-performers (those that fell within the range of young performance) and aged worse-performers (those that fell outside this range). Young, middle-aged and aged better-performers all improved to a similar degree across training, but aged worse-performers (open circles with dashed line) had a strongly attenuated learning curve.

Learning Indices for individual rats within each age group (means indicated by black horizontal lines). Lower Spatial Learning Indices indicate better learning. A one-factor ANOVA revealed a significant main effect of age on Spatial Learning Index scores ($F_{(2,110)} = 34.82$, $p < 0.001$). In agreement with previous measures, as a group, middle-aged rats were significantly impaired relative to young rats (Fisher's LSD post hoc comparison, $p < 0.05$). However, despite this difference and the shift toward higher individual Learning Indices (i.e. worse learning) among middle-aged relative to young rats, the vast majority of middle-aged rats still performed within the range of young cohorts. Fisher's LSD post hoc analyses also confirmed previous results indicating that aged rats as a group performed significantly worse than both young and middle-aged rats (post hoc comparisons, $p < 0.001$ in both cases). Moreover, among the aged rats, a large degree of variability in Spatial Learning Indices was observed such that 53% of the aged group ($n = 26$) performed within the range of their young cohorts (i.e. Spatial Learning Index < 285) whereas 47% ($n = 23$) performed outside this range (i.e. Spatial Learning Index > 285), demonstrating impairment on the task.

Given the large degree of variability in performance among aged rats and the interaction observed between learning performance and age (i.e. that aged rats improved to a lesser degree than young and middle-aged rats over the course of training), additional analyses were performed in order to determine whether this interaction was carried by aged rats that fell outside the range of young cohort performance. Aged rats were sub-grouped into "aged better-performers" (i.e. performed within the range of young) and "aged worse-performers" (i.e. performed outside the range of young). Fig. 2B shows probe trial performance of young, middle-aged, and aged sub-grouped rats. Note that although performance across young, middle-aged, and aged better-performers was similar, aged worse-performers were impaired relative to each of the other groups, demonstrating a dramatically attenuated learning curve. A two-factor repeated measures ANOVA (cognitive age group × probe trial) confirmed that, although all groups did improve over the course of training ($F_{(3,327)} = 66.43$, $p < 0.001$), there was a significant interaction between cognitive age group and probe trial performance ($F_{(9,327)} = 2.37$, $p < 0.05$). Fisher's LSD post hoc comparisons indicated that aged better-performers were significantly impaired relative to young (post hoc comparison, $p < 0.001$), but this aged sub-group did not perform significantly worse than middle-aged rats ($p > 0.05$). However, aged worse-performers were significantly impaired relative to all other groups ($p < 0.001$ in all cases). To confirm that the variability observed among aged rats was not due to subtle non-mnemonic or global mnemonic impairments, a Pearson's r correlation coefficient was calculated for aged rats using Spatial Learning Indices and mean pathlengths during cued (visible platform) training. No relationship was observed between these two variables ($r = 0.08$, n.s.).

It has been suggested that poor water maze performance among aged rats is related to a failure to consistently retrieve spatial maps (Barnes et al., 1997), and thus individual differences as described above may result from within-subject variability (i.e. individual aged rats perform poorly on some trials but not others), rather than between-subject variability (i.e. a subset of aged rats perform consistently poorly across trials). Such data demonstrate the need to verify whether the observed individual differences among the aged rats reported here are indeed reliable, or, alternatively, a consequence of unstable performance within individual aged subjects. This distinction is critical if spatial reference memory task performance is to be used to provide a legitimate cognitive context in which to deem age-related neurobiological changes relevant to mnemonic impairment. To address this issue, analyses were conducted on this dataset as originally described by Barnes et al. (1997), who found that the distribution of individual training trials (but not the means of these training trials) in aged rats was bimodal, indicating considerable within but not between-subject variability. The use of multiple interpolated probe trials in our water maze paradigm enabled us to conduct the same analyses on probe trial data, which are less subject to potential confounds than training trial data. Specifically, frequency histograms of probe trial performance were examined across young and aged rats to test for inter-trial consistency in performance across the spatial reference memory task. Note that in our analyses, differences in the distributions of young and middle-aged rats were minimal and thus only differences between young and aged rats are presented.

Fig. 3 shows histograms of individual probe trials plotted early in training (Probes 1 and 2) for young (Fig. 3A) and aged rats (Fig. 3B) and later in training (Probes 3 and 4) for young (Fig. 3C) and aged (Fig. 3D) rats. The distribution becomes narrower and shifts to the left (indicative of a more accurate search) later in training; however, bimodal distributions were not observed in rats of any age at any point during training using probe trial performance as the measure of spatial learning ability. Comparisons of the fit of unimodal (Gaussian) versus bimodal (sum of two Gaussians) distributions to the data using Akaike's Information Criterion revealed that the unimodal distribution had an overwhelmingly (>99%) greater probability of being the best-fitting model. These data strongly support the idea that the age-related differences in spatial learning reported here are indeed due to between- rather than within-subjects variability.

3.4. Spatial working memory (delayed match-to-place) task

The working memory task assessed rats' ability to retain trial-unique information about the platform location over 30 min, 2 h, and 6 h delays. A one-factor ANOVA revealed no difference in rats of any age in performance on information trials averaged across test delays ($F_{(2,66)} = 1.56$, n.s.). The difference in pathlength between the information and retention trials (information pathlength − retention pathlength)

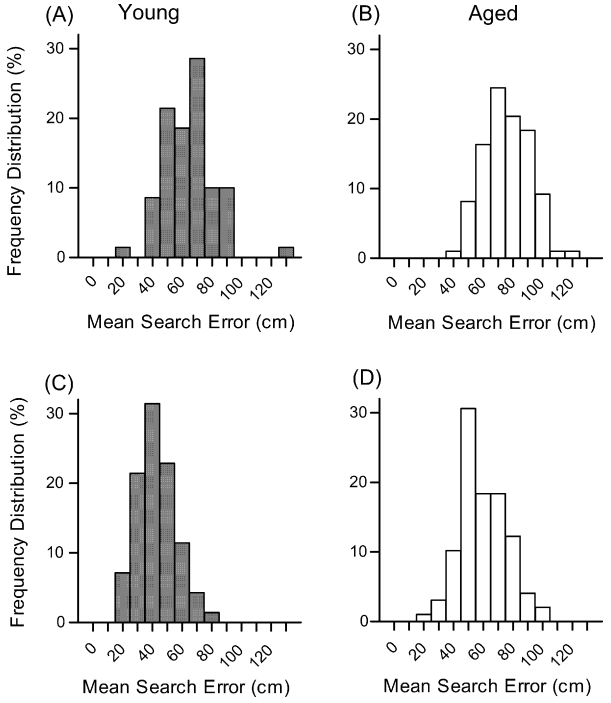

Fig. 3. Frequency histograms of individual probe trial performance in the spatial reference memory task for young and aged F344 rats. Frequency distributions of performance on individual probe trials for young (A and C) and aged (B and D) rats are shown for probe trials early in training (probe trials 1 and 2 (A and B)) and late in training (probe trials 3 and 4 (C and D)). Both age groups show improved performance over the course of training as indicated by a leftward shift in the distribution between probe trials 1 and 2 and 3 and 4. Note that unlike training trials, in which individual trial length can be confounded by non-mnemonic variables (e.g. circling strategy and start position), only unimodal distributions are observed across ages in the probe trial analysis. These data strongly support the contention that cognitive performance is consistent across trials, even at advanced age.

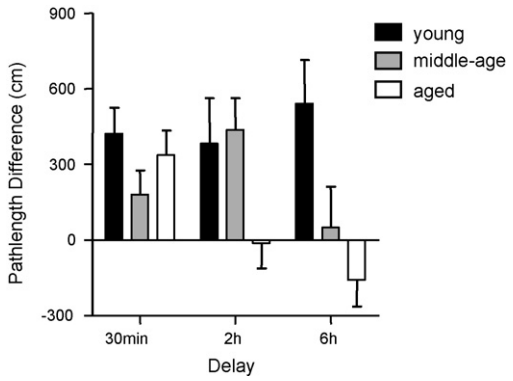

Fig. 4. Spatial working (delayed match-to-place) memory performance of young, middle-aged, and aged F344 rats. Bar graphs show mean pathlength difference ± S.E. for young (black bars), middle-aged (gray bars), and aged (open bars) rats on retention trials with varying delays. Although no differences among age groups were present with a 30 min delay, at both 2 and 6-h delays, Fisher's LSD post hoc comparisons indicated that aged rats performed significantly worse than the middle-aged rats. Additionally, at the 2 h delay there was a trend toward aged rats performing significantly worse than young rats, a difference which became significant at the 6 h delay, at which middle-aged and young rats also differed. Note that at 6 h, the mean pathlength difference for aged rats was near zero, indicating no retention of the platform location.

was used to compare performance of young, middle-aged, and aged rats at each retention interval using one-factor ANOVAs (Fig. 4). No main effect of age was observed with a 30 min delay ($F_{(2,66)} = 1.59$, n.s.); however, differences between age groups were observed with both 2 and 6 h delays ($F_{(2,66)} = 3.39$, $p < 0.05$ and $F_{(2,34)} = 4.47$, $p < 0.05$, respectively). At the 2-h delay, Fisher's LSD post hoc analyses revealed a strong trend toward a difference between young and aged rats ($p = 0.059$), and a significant difference between middle-aged and aged rats ($p < 0.05$). Young and middle-aged rats did not differ in performance at the 2-h delay. In contrast, at the 6-h delay, Fisher's LSD post hoc analyses indicated that middle-aged rats were impaired relative to young rats ($p < 0.05$), and that aged rats were impaired relative to both young and middle-aged rats ($p < 0.05$ in both cases). Together, these data suggest a progressive decline in spatial working memory with increased retention intervals across the lifespan in F344 rats.

To determine the relationship between performance on the reference and working memory tasks in young and aged rats, a series of Pearson's r correlations was performed between Spatial Learning Indices and mean pathlength on training trial block 4 of the reference memory task and mean pathlength differences at the 30 min, 2 h, and 6 h delays in the working memory task. No significant correlations were observed between reference and working memory performance at any delay at any age: Spatial Learning Index versus pathlength difference ($r = 0.02$–0.41, n.s.); mean pathlength on training trial block 4 versus pathlength difference ($r = 0.01$–0.42, n.s.).

4. Discussion

It is becoming widely recognized that medial temporal lobe dependent (declarative) memory declines progressively across the lifespan, generally reaching the detection threshold at middle-age (Albert, 1997; Ronnlund et al., 2005; Verhaeghen and Salthouse, 1997). Few rodent models have successfully modeled the typical human onset of cognitive dysfunction. Rather, in aged rodents, deficits in spatial reference memory are typically only observed at very advanced ages (Bizon and Gallagher, 2003; Rapp et al., 1987; Wyss et al., 2000). We demonstrate here, however, that middle-aged rats are impaired relative to young rats on both a spatial reference task and on a spatial working memory task (at long delays). In agreement with these findings, Shukitt-Hale et al. (1998) reported a decline in reference memory in male F344 rats at middle-age (12, 15, and 18 months). Together, these data demonstrate that the F344 model may be useful in detecting and investigating causative factors related to cognitive deficits at early stages of mnemonic impairment. The ability to detect subtle mnemonic deficits at middle-age is of particular value with respect to therapies aimed at slow-

ing or even halting the progression of age-related cognitive decline.

In addition to providing an in depth analysis of spatial mnemonic capabilities of F344 rats across the lifespan, this study suggests parameters for the use of this rat strain in aging research, including choices for optimal behavioral analyses. Notably, significant swim speed differences were observed between aged rats relative to both young and middle-aged rats. In agreement with previous studies, the cumulative search error measure appeared to be most sensitive for detecting age-related differences (particularly at middle-age). However, pathlength may be the optimal and most conservative choice for such analyses in aged F344 rats due to the lack of confounds with swim speed (Gallagher et al., 1993).

Barnes et al. (1997) reported that, early in training, when performance on individual training trials was plotted on a frequency histogram, both young and aged rats exhibited a strong bimodality in performance such that on some trials rats of both ages had short swim paths whereas on other trials they had long swim paths. Also reported in the same study were inconsistent place fields in individual aged rats, suggesting some trial-to-trial instability in retrieval of spatial information. However, analyses of data from individual training trials may be problematic, as within-subject variability may arise from sources other than spatial mnemonic ability (e.g. aged rats that employ a commonly recognized "circling" strategy to find the platform may encounter the platform by chance at different times in a trial depending upon their start location; Gallagher and Nicolle, 1993). Such potential confounds are eliminated during probe trials in which the platform is removed from the maze and rats' proximity to the platform location is recorded. The training protocol used here, in which multiple probe trials were given throughout training, afforded the opportunity to perform the same analyses described above using probe trial data. On the interpolated probe trials, although the distribution of performance becomes narrower and shifts to the left, which is indicative of a more accurate search later in training, bimodal distributions were not observed in rats of any age at any point during training. These data strongly support the idea that the differences in spatial learning ability reported here are indeed due to between rather than within-subjects variability.

This interpretation of the data is also consistent with previous research from our laboratory demonstrating consistency of aged rat performance across multiple cognitive domains. LaSarge et al. (2007) reported that spatial reference memory is correlated with olfactory discrimination learning ability, such that among aged rats, poor performance in the reference memory task described here is correlated with impaired odor discrimination abilities. These odor discrimination impairments were not due to anosmia nor were they reflective of global mnemonic impairments, as aged mnemonically impaired rats were able to learn non-olfactory discriminations as well as young rats. These cross-domain impairments provide further support for the idea that sub-populations are present among aged F344 rats such that some rats are severely cognitively impaired whereas others remain largely intact with respect to mnemonic function, even at advanced chronological ages.

In the present study, there were also age-related deficits on the working memory task, such that both middle-aged and aged rats were impaired relative to young rats in a delay-dependent manner. In agreement with results reported here, deficits in spatial working memory have been reported in aged (24 months) relative to young (4 months) F344 rats using delays of 3–4 min on a repeated acquisition task similar to that used in the current study (Frick et al., 1995). Similar deficits also have been reported in aged (22 months) and middle-aged (12, 15, and 18 months) F344 rats using a 10 min delay (Shukitt-Hale et al., 1998). Interestingly, the delays at which deficits in working memory were detected in the current study were much longer than the aforementioned studies. However, a novel feature of the current study is that the rats had 3 days of acquisition trials to learn the new task demands after finishing the reference memory task. This additional training may allow for a more accurate assessment of working memory ability because it separates the working memory component of the task from learning the parameters of the task (e.g. that the platform is located in a novel position each day). This difference in task design might contribute to the longer retention capabilities observed in middle-aged and aged rats in the current study in comparison to the previous reports, particularly when considering the attenuated learning curves characteristic of aged rats as demonstrated in the reference memory task.

Indeed, rats from other strains and primates, including humans, demonstrate a diminished capacity across the lifespan to retain trial-unique information with increased retention intervals (Ando and Ohashi, 1991; Bimonte et al., 2003; Foos, 1989; Means and Kennard, 1991; Missonnier et al., 2004; van der Staay et al., 1990). The hippocampus has been implicated in some forms of (particularly spatial) working memory; however, this form of memory also depends heavily upon extra-hippocampal structures, particularly prefrontal cortex (Davachi and Goldman-Rakic, 2001; Floresco et al., 1997; Hampson et al., 1999; Kesner and Giles, 1998; Winocur, 1992). Notably, there was no relationship between performance on the reference and working memory tasks in rats of any age. Previous studies have also reported a concurrent decline in reference and working memory in aged F344 rats (Frick et al., 1995; Lindner et al., 1992) and Brown Norway rats (van der Staay et al., 1990) that either loaded onto different factors during analysis or did not show a relationship. The lack of such relationships reported here, combined with this previous research, suggests the possibility of multiple loci of neural dysfunction among middle-aged and aged subjects.

In support of this hypothesis, Stern et al. (2001) reported differences in neural activation using functional magnetic resonance imaging in humans performing a "two-back" working memory task that required subjects to identify stim-

uli that were repeated after one intervening stimulus, using either novel or familiar visual stimuli. With the novel stimuli, increased signal was evident in medial temporal lobe structures, including the hippocampal and parahippocampal regions. In contrast, when familiar stimuli were used, greater activation was predominant in the prefrontal cortex. These data suggest that prefrontal cortical regions may be recruited to a greater degree in working memory under circumstances in which there is a possibility of interference from previously learned stimuli. Indeed, despite attempts to create a novel environment (using novel visual cues) for the working memory task in the current study, the same water maze apparatus was used for both the reference and working memory tasks. Therefore, we cannot rule out, and it is indeed likely, that rats' previous experience in the water maze during learning of the reference memory task influenced performance on the working memory task. Given the contingencies of our task design and evidence that prefrontal cortex may become more critical in situations with familiar stimuli, the age-related deficits in working memory observed here may be indicative of decline in prefrontal cortical function. In addition, it is notable that a recent study using transgenic mice exhibiting NMDA receptor dysfunction specifically in the dentate gyrus of the hippocampus reported dissociable effects on reference and working memory (Niewoehner et al., 2007). Results suggest that loss of functional integrity of sub-regions within the hippocampus itself may contribute to the lack of relationship observed between the age-related deficits on the two spatial tasks. Additional experiments using a non-water maze assessment for working memory and/or lesion studies of the prefrontal cortex and/or hippocampal sub-regions are needed to distinguish among these possibilities and to elucidate the neural substrates responsible for the working memory deficits reported here.

In summary, results from this study demonstrate progressive age-related impairments across the lifespan on both spatial reference and working memory tasks. The spatial reference memory task used here detects individual variability within this F344 population such that approximately half of the aged rats, while as a group were somewhat impaired relative to young rats, still performed within the range of young cohorts. In contrast, approximately half of the aged rats performed well outside the range of young, demonstrating pronounced spatial reference memory impairment. This latter subset of rats had a dramatically attenuated learning curve relative to younger rats and the other aged cohort. Analyses of performance on individual trials suggest that this variability was not solely due to within-subject factors. Finally, although an age-related impairment was detected with increasing delays in the spatial working memory task, there was no correlation between performance on the two spatial memory tasks. These latter data point to multiple loci of neural dysfunction in F344 rats across the lifespan and provide multiple contexts against which to uncover cognitively relevant neurobiological changes that occur with age.

Conflict of Interest

There are no actual or potential conflicts of interest.

Acknowledgements

We thank Simona Slaton, Atasi Bhavsar, and Deepa Ramamurthi for technical assistance, and Chris Jendro for suggestions on statistical analyses. Supported by: The Office of the Vice President of Research, Texas A&M University and R01 AG029421 (JLB), T32 MH65728 (ANM) and R01 AG007805 (WHG).

References

Albert, M.S., 1997. The ageing brain: normal and abnormal memory. Phil. Trans. Roy. Soc. B: Biol. Sci. 352 (1362), 1703–1709.

Ando, S., Ohashi, Y., 1991. Longitudinal study on age-related changes of working and reference memory in the rat. Neurosci. Lett. 128, 17–20.

Barnes, C.A., Suster, M.S., Shen, J., McNaughton, B.L., 1997. Multistability of cognitive maps in the hippocampus of old rats. Nature 388 (6639), 272–275.

Baxter, M.G., Bucci, D., Gorman, L., Wiley, R., Gallagher, M., 1995. Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats. Behav. Neurosci. 109 (4), 714–722.

Bennett, D., Wilson, C.J., Schneider, J.A., Evans, D.A., Beckett, L.A., Aggarwal, N.T., Barnes, L.L., Fox, J.H., Bach, J., 2002. Natural history of mild cognitive impairment in older persons. Neurology 59, 198–205.

Bimonte, H.A., Nelson, M.E., Granholm, A.E., 2003. Age-related deficits as working memory load increases: relationships with growth factors. Neurobiol. Aging 24, 37–48.

Bizon, J.L., Gallagher, M., 2003. Production of new cells in the rat dentate gyrus over the lifespan: relation to cognitive decline. Eur. J. Neurosci. 18 (1), 215–219.

Bizon, J.L., Gallagher, M., 2005. More is less: neurogenesis and age-related cognitive decline in Long-Evans rats. Sci. Aging Knowledge Environ. 7, re2.

Bizon, J.L., Helm, K.A., Han, J.-S., Chun, H.-J., Pucilowska, J., Lund, P.K., Gallagher, M., 2001. Hypothalamic–pituitary–adrenal axis function and corticosterone receptor expression in behaviourally characterized young and aged Long-Evans rats. Eur. J. Neurosci. 14 (10), 1739–1751.

Brightwell, J.J., Gallagher, M., Colombo, P.J., 2004. Hippocampal CREB1 but not CREB2 is decreased in aged rats with spatial memory impairments. Neurobiol. Learning Memory 81 (1), 19–26.

Burke, S.N., Barnes, C.A., 2006. Neural plasticity in the ageing brain. Nat. Rev. Neurosci. 7, 30–40.

Colombo, P.J., Gallagher, M.M., 2002. Individual differences in spatial memory among aged rats are related to hippocampal PKC immunoreactivity. Hippocampus 12 (2), 285–289.

Colombo, P.J., Wetsel, W.C., Gallagher, M., 1997. Spatial memory is related to hippocampal subcellular concentrations of calcium-dependent protein kinase C isoforms in young and aged rats. PNAS 94 (25), 14195–14199.

Davachi, L., Goldman-Rakic, P.S., 2001. Primate rhinal cortex participates in both visual recognition and working memory tasks: functional mapping with 2-DG. J. Neurophysiol. 85 (6), 2590–2601.

Della-Maggior, V., Grady, C.L., McIntosh, A.R., 2002. Dissecting the effect of aging on the neural substrates of memory: deterioration, preservation or functional reorganization. Rev. Neurosci. 13, 167–181.

Floresco, S.B., Seamans, J.K., Phillips, A.G., 1997. Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. J. Neurosci. 17 (5), 1880–1890.

Foos, P.W., 1989. Adult differences in working memory. Psychol. Aging 4 (3), 269–275.

Frick, K.M., Baxter, M.G., Markowska, A.L., Olton, D.S., Price, D.L., 1995. Age-related spatial reference and working memory deficits assessed in the water maze. Neurobiol. Aging 16 (2), 149–160.

Gallagher, M., Nicolle, M.M., 1993. Animal models of normal aging: relationship between cognitive decline and markers in hippocampal circuitry. Behavioural. Brain Res. 57 (2), 155–162.

Gallagher, M., Burwell, R.D., Burchinal, M., 1993. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav. Neurosci. 107 (4), 618–626.

Hampson, R.E., Jarrard, L.E., Deadwyler, S.A., 1999. Effects of Ibotenate hippocampal and extrahippocampal destruction on delayed-match and nonmatch-to-sample behavior in rats. J. Neurosci. 19 (4), 1492–1507.

Kesner, R.P., Giles, R., 1998. Neural circuit analysis of spatial working memory: role of pre- and parasubiculum, medial and lateral entorhinal cortex. Hippocampus 8 (4), 416–423.

LaSarge, C.L., Montgomery, K.S., Tucker, C., Slaton, G.S., Griffith, W.H., Bizon, J.L., 2007. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. Neurobiol. Aging 28, 928–936.

Lee, J.M., Ross, E.R., Gower, A., Paris, J.M., Martensson, R., Lorens, S.A., 1994. Spatial learning deficits in the aged rat: neuroanatomical and neurochemical correlates. Brain Res. Bull. 33 (5), 489–500.

Lindner, M.D., Balch, A.H., Vandermaelen, C.P., 1992. Short forms of the "reference-" and "working-memory" Morris water maze for assessing age-related deficits. Behav. Neural Biol. 58 (2), 94–102.

Lipman, P.D., 1991. Age and exposure differences in acquisition of route information. Psychol. Aging 6 (1), 128–133.

Lynch, G., Rex, C.S., Gall, C.M., 2006. Synaptic plasticity in early aging. Ageing Res. Rev. 5 (3), 255–280.

Means, L.W., Kennard, K.J.P., 1991. Working memory and the aged rat: deficient two-choice win-stay water-escape acquisition and retention. Physiol. Behav. 49 (2), 301–307.

Missonnier, P., Gold, G., Leonards, U., Costa-Fazio, L., Michel, J.P., Ibanez, V., Giannakopoulos, P., 2004. Aging and working memory: early deficits in EEG activation of posterior cortical areas. J. Neural Transm. 111, 1141–1154.

Nicolle, M.M., Colombo, P.J., Gallagher, M., McKinney, M., 1999. Metabotropic glutamate receptor-mediated hippocampal phosphoinositide turnover is blunted in spatial learning-impaired aged rats. J. Neurosci. 19 (21), 9604–9610.

Niewoehner, B., Single, F.N., Hvalby, O., Jensen, V., Meyer zum Alten Borgloh, S., Seeburg, P.H., Rawlins, J.N.P., Sprengel, R., Bannerman, D.M., 2007. Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. Eur. J. Neurosci. 25 (3), 837–846.

Park, D.C., Lautenschlager, G., Hedden, T., Davidson, N.S., Smith, A.D., Smith, P.K., 2002. Models of visuospatial and verbal memory across the adult life span. Psychol. Aging 17 (2), 299–320.

Rapp, P.R., Rosenberg, R.A., Gallagher, M., 1987. An evaluation of spatial information processing in aged rats. Behav. Neurosci. 101 (1), 3–12.

Reuter-Lorenz, P.A., Jonides, J., Smith, E.E., Hartley, A., Miller, A., Marshuetz, C., Koeppe, R.A., 2000. Age differences in the frontal lateralization of verbal and spatial working memory revealed by PET. J. Cogn. Neurosci. 12 (1), 174–187.

Ronnlund, M., Nyberg, L., Backman, L., Nilsson, L., 2005. Stability, growth, and decline in adult life span development of declarative memory: cross-sectional and longitudinal data from a population-based study. Psychol. Aging 20 (1), 3–18.

Rosenzweig, E.S., Barnes, C.A., 2003. Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Prog. Neurobiol. 69 (3), 143–179.

Shukitt-Hale, B., Mouzakis, G., Joseph, J.A., 1998. Psychomotor and spatial memory performance in aging male Fischer 344 rats. Exp. Gerontol. 33 (6), 615–624.

Smith, T.D., Adams, M.M., Gallagher, M., Morrison, J.H., Rapp, P.R., 2000. Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. J. Neurosci. 20 (17), 6587–6593.

Squire, L.R., Stark, C.S., Clark, R.E., 2004. The medial temporal lobe. Annu. Rev. Neurosci. 27, 279–306.

Stern, C.E., Sherman, S.J., Kirchhoff, B.A., Hasselmo, M.E., 2001. Medial temporal and prefrontal contributions to working memory tasks with novel and familiar stimuli. Hippocampus 11, 337–346.

Tanila, H., Shapiro, M., Gallagher, M., Eichenbaum, H., 1997. Brain aging: changes in the nature of information coding by the hippocampus. J. Neurosci. 17 (13), 5155–5166.

van der Staay, F.J., van Nies, J., Raaijmakers, W., 1990. The effects of aging in rats on working and reference memory performance in a spatial holeboard discrimination task. Behav. Neural Biol. 53 (3), 356–370.

Verhaeghen, P., Salthouse, T., 1997. Meta-analyses of age-cognition relations in adulthood: estimates of linear and nonlinear age effects and structural models. Psychol. Bull. 122, 231–249.

Wilson, R.S., Beckett, L.A., Barnes, L.L., Schneider, J.A., Bach, J., Evans, D.A., Bennett, D.A., 2002. Individual differences in rates of change in cognitive abilities of older persons. Psychol. Aging 17, 179–193.

Wilson, I.A., Ikonen, S., Gureviciene, I., McMahan, R.W., Gallagher, M., Eichenbaum, H., Tanila, H., 2004. Cognitive aging and the hippocampus: how old rats represent new environments. J. Neurosci. 24, 3870–3878.

Winocur, G., 1992. A comparison of normal old rats and young adult rats with lesions to the hippocampus or prefrontal cortex on a test of matching-to-sample. Neuropsychologia 30 (9), 769–781.

Wyss, J.M., Chambless, B.D., Kadish, I., van Groen, T., 2000. Age-related decline in water maze learning and memory in rats: strain differences. Neurobiol. Aging 21 (5), 671–681.