ESTABLISHING A RODENT (FISCHER 344 RAT) MODEL OF MILD

COGNITIVE IMPAIRMENT IN AGING

A Thesis

by

CANDI LYNN LASARGE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2007

Major Subject: Psychology

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Approved by:

Chair of Committee,   Jennifer L. Bizon
Committee Members,   Barry Setlow
                        William Griffth
Head of Department,   Leslie Morey

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ABSTRACT

Establishing a Rodent (Fischer 344 Rat) Model of Mild Cognitive

Impairment in Aging. (May 2007)

Candi Lynn LaSarge, B.S.; B.S., University of Washington

Chair of Advisory Committee: Dr. Jennifer L. Bizon

Mild Cognitive Impairment is characterized by age-related decline in a variety of cognitive domains, including reference and working memory and olfactory function. Importantly, declining age-related mnemonic abilities is not inevitable; learning and memory deficits emerge in some people by middle-age while others remain largely cognitively-intact even at advanced chronological ages. The goal of this thesis is to establish a Fischer 344 (F344) rat model with some features of human cognitive aging which can then be utilized to undercover the neurobiological underpinnings of age-related cognitive deficits.

Young (6 mo), middle-aged (11 mo), and aged (22 mo) F344 rats were behaviorally characterized in a well-established reference memory version of the Morris water maze task. Indeed, age-related impairments did occur across the lifespan. Moreover, the reference memory protocol used here was sufficiently sensitive to detect a difference in individual abilities among aged F344 rats such that approximately half of the rats performed on par with young while the other half performed outside this range, demonstrating impairment. These data mimic individual differences in declarative memory among aged humans.

Subsequently, subsets of rats initially characterized on the reference memory version of the water maze were tested on either a spatial working memory water maze task or an olfactory discrimination task. Despite detecting an age-related delay-dependent decline in spatial working memory, this impairment was not correlated with spatial reference memory. In contrast, a strong and significant relationship was observed among aged rats in the odor discrimination task such that aged rats with the worst spatial reference memory were also the most impaired in their ability to discriminate odors for a food reward. Importantly, this subset of cognitively-impaired rats was not impaired on digging media discrimination problems with identical task demands, nor were they anosmic.

These data are among the first to demonstrate a cross-domain cognitive deficit in a rodent model of human aging. Together, the current study both confirms the use of the naturalistic F344 rat model for the study of cognitive deficits within the context of aging and provides the most comprehensive cognitive profile of this rat population to date.

DEDICATION

To my mother, my daughter Chloe,

my husband Christopher, and my advisor Jennifer Bizon.

ACKNOWLEDGMENTS

I would like to extend a special thank you to my advisor, Jennifer Bizon, who was crucial in the development of this research. Her guidance and insight have greatly aided in my growth inside and out of the lab. She truly made my research experience enjoyable, and being in her lab is worth the sacrifice of living in BCS. I look forward to more years in her lab to continue learning from her vast experience and knowledge.

Thank you to my family and friends, especially Chloe and Christopher. They have helped to support me throughout my master's work and all other areas of my life. You two make me smile everyday.

To my committee, Barry Setlow and William Griffith, thank you for your guidance and flexibility to get through this thesis. I greatly appreciate the assistance with the animals and lab space to gather data and the input in my research development and presentation. Barry was extremely helpful in the behavioral experimentation, the data analysis, and finding the exciting results. I am thankful to have such great researchers as a part of my training.

Thank you to the other graduate students in the lab, Nick Simon, Ian Mendez, and Karienn Montgomery, who make lab an enjoyable place. I greatly appreciate all your help with the experimental set-up and running on this project, not to mention the four times you all got to listen to my talk and the countless times I had you read my rough drafts. Go set shifting on Fischers!!!

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CHAPTER I

INTRODUCTION: AGE-RELATED COGNITIVE DECLINE

Age-related cognitive decline is a growing problem in the United States and other developed nations. In the United States, the population over 65 years of age is increasing substantially; it is expected to escalate from 35 million in 2000 to 71.5 million by the year 2030, comprising about 20% of the US population [29]. Within this aged population, cognitive impairment is a considerable problem from both an individual and a public health perspective. Severe dementia resulting from pathological conditions such as Alzheimer's disease (AD) will impact 7 – 8% of aged individuals; however, a much larger number of the elderly will experience cognitive decline without age-related disease but that nevertheless substantially impacts their quality of life [33].   The term Mild Cognitive Impairment (MCI) is used to describe cognitive impairment at advanced ages prior to, or independent of, dementia associated with pathological conditions such as AD. In MCI, the characteristic pathology of AD (e.g. plaques, neurofibrillary tangles, and frank neural degeneration) is not present, but individuals nevertheless experience mild to profound deficits in cognitive function. It is estimated that up to 20% of people over age 65 suffer from MCI [59]. Although MCI is characterized by loss of a range of cognitive abilities, many of the deficits associated with this condition are known to depend

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This thesis follows the style of Neurobiology of Aging.

on the medial temporal lobe system [59].

Mild Cognitive Impairment is associated with a variety of deficits. The most noted deficit is the loss of declarative memory abilities, the ability to encode and recall factual information such as people, places, and things [23,25,36,59,62,81,104,115]. Two types of memory will be focused on in this thesis, namely reference and working memory. Reference memory uses information about a context that is continually held constant, such as a location in reference to spatial landmarks, whereas working memory is defined as the storage and manipulation of information, for a brief period of time, which is consistently changing [34,77]. Another deficit commonly seen in MCI is decreased olfactory function, as indicated by increased odor detection thresholds and odor discrimination deficits. Elderly people show compromised retrieval, encoding, and verbal mediation that facilitates odor identification [24,80,93,119].

Age-related cognitive decline is not an inevitable consequence of chronological age. Human studies show that substantial individual variability in mnemonic abilities in present among aged individuals; e.g. - some humans began to show decline in mnemonic abilities on a declarative memory task in their fourth decade, whereas others perform on par with young adults past their seventieth year of life [1,13,102]. Indeed, variance in memory abilities

significantly increases with age in the human population [1,13,102]. Thus, chronological age is not an indicator of declarative memory abilities.

Naturally occurring rodent models are useful to further delineate cognitive decline associated with the human aging process. Neuroanatomical structure and function has been largely conserved across species, and rats and humans have similarities in neurological changes associated with age, including those that relate to medial temporal lobe structures [13,46,55,85,102]. Many aging studies are difficult to conduct in humans, since many neurobiological markers that correlate with age-related decline can only be detected post mortem. In contrast, the use of rodent models in aging research affords the ability to conduct a variety of cognitive and neurobiological studies in a controlled environment (i.e. specific lesions, pharmacological manipulations, detect neurobiological markers, etc.). Additionally, due to their shorter lifespan, aging studies can be conducted in rodents in a reduced time period when compared to the human population. Indeed, the life expectancy of a F344 rat is 26.1 months, compared to humans at 77.9 years [40,72]. Thus, a rodent model of age-related cognitive decline seen in the human population would be of great utility to further characterize behavioral deficits and changes in underlying neurobiological structures.

The goal of this thesis was to establish a comprehensive cognitive rodent model of human aging that can be utilized to undercover the neurobiological underpinnings responsible for age-related mnemonic deficits. In order to model

some commons deficits observed among humans with Mild Cognitive Impairment, three tasks were used to assess cognition across the lifespan of F344 rats. Specifically, the aims of this Master's thesis were to determine 1): the integrity of spatial reference memory in young, middle-aged, and aged F344 rats, 2): if age-related differences in spatial working memory occur across the lifespan, and in relation to spatial reference memory in F344 rats, and 3): if spatially-impaired aged F344 rats are also impaired in olfactory functioning.

Spatial Reference Memory

As mentioned previously, the human population shows an age-related decline in declarative memory in which some individuals maintain mnemonic function on par with younger adults well into advanced ages, while others perform outside the range of the younger population, demonstrating impairment [1,13,102]. Past research on rodents has successfully modeled this individual variability in aging using a spatial reference memory task in the Long Evans rats, an outbred strain [37,39]. However, much neurobiological research on brain aging is conducted in F344 rats, as this is one of only two strains available from the National Institute of Aging. Information regarding the cognitive status of F344 rats, an inbred strain, is particularly limited and contradictory, including information on the individual variability in spatial reference memory abilities seen throughout the lifespan [34,43,47,89,101]. Further assessment of the spatial reference memory abilities in the F344 population, focusing on both age group

differences and individual variability, is needed to determine the cognitive profile of this commonly used rodent strain.

Declarative/ explicit memory is dependant upon the medial temporal lobe (MTL), and particularly the hippocampal formation. As seen in Fig. 1, information flows multi-directionally through MTL structures: the hippocampus communicates with cortical targets through the entorhinal cortex, which shares bi-directional projections with the parahippocampal gyrus (including perirhinal and postrhinal cortices).The parahippocampal gyrus, in turn, projects to the neocortex. Furthermore, hippocampus and entorhinal cortex receive direct projections from other regions, including subcortical projections from basal forebrain innervating all subfields of hippocampus and the entire neocortical mantle. Indeed, loss of basal forebrain projections to hippocampus and neocortex have been implicated in age-related cognitive impairment [19,60].

Neuropsychological studies and brain imaging in humans, using techniques such as Positron Emission Tomography (PET) and magnetic resonance imaging (MRI), have shown that the medial temporal lobe is one of the brain areas most susceptible to age-related decline [46,85,102]. Aged humans show impairments in declarative memory tasks that relate to changes in MTL functioning, such as remembering spatial relationships and landmarks [56,91]. The famous neuropsychological patient H. M., who suffered from severe bilateral epileptic seizures and consequently underwent removal of his medial
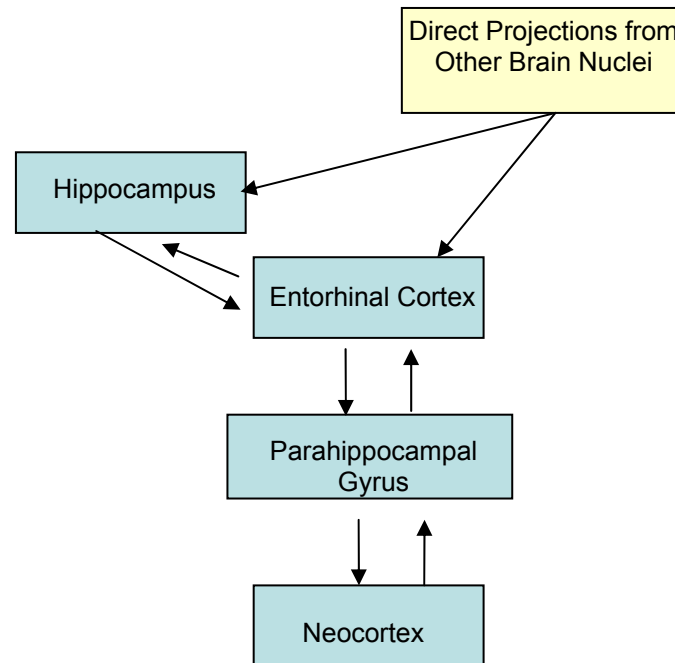
Fig. 1. Medial temporal lobe system. The hippocampus communicates with cortical targets through the entorhinal cortex, which shares bi-directional projections with the parahippocampal gyrus. The parahippocampal gyrus, in turn, has projections to and from the neocortex. Additional brain nuclei have direct projections to entorhinal cortex and hippocampus. Adapted from Bizon & Nicolle (2006).

temporal lobes, had severely impaired declarative memory. Deficits included complete anterograde amnesia (i.e. he was incapable of encoding declarative memories); however, H. M. retained his perceptual and reasoning abilities [26,99]. These studies implicate changes within the medial temporal lobe in impairments of declarative memory.

Many age-related deficits may specifically occur within the hippocampus, a brain structure within the medial temporal lobe that is particularly critical for declarative memory, including spatial reference memory. One task commonly used in the assessment of spatial reference memory abilities is the Morris water maze [68]. The water maze consists of a large, water-filled tank in which a platform is hidden beneath the waterline; the rat needs to learn the location of the platform in relation to extramaze cues in order to escape the water (Please see Methods in Chapter II for specific methodology). A visible platform training session, in which spatial location of a visible platform is irrelevant to the ability to escape, concludes training to test for sensorimotor and motivation deficits that can mimic a cognitive impairment. Researchers have used lesions studies in rodents to delineate the function and role of the hippocampus in memory. A seminal study from Morris, et al. (1982) demonstrated that animals with hippocampal lesions show deficits in performance in the Morris water maze such that, even after several training trials, they are severely compromised in their ability to retain information about platform location [69]. Thus, this study demonstrated that the hippocampus is critical for this spatial reference memory,

and that lesions in this area do not cause motor or sensory problems. Similar results have been observed in lesion studies in which other MTL structures have been specifically ablated (e.g., entorhinal cortex [41,94]). The water maze has also been shown to be particularly sensitive to age-related cognitive decline, implicating the hippocampus in this mnemonic impairment, such that aged rats perform like the hippocampal lesioned rats, demonstrating a more inaccurate search for the platform location compared to young, even after several days of training [37,55,57,69].

The Morris water maze will be used to assess F344 rats in a spatial reference memory task, to examine group and individual differences across the lifespan, in Chapter II.

Spatial Working Memory

Age-related impairment in declarative memory includes a decline in working memory such that the elderly population demonstrates a reduced memory storage capacity compared to young subjects [32]. A decrease in memory abilities may be related to multiple neurobiological changes, including differential activation of posterior cortical neurons [67]. PET has been used to further delineate neural substrates activated during spatial working memory problems. During a spatial matching task, in which subjects were required to discriminate between spatial locations after a 3 second delay, young adults (18-30 years old) showed activation in the dorsolateral prefrontal cortex,

concentrated in the left hemisphere, whereas elderly people (62-75 years old)

engaged both the right *and* left dorsolateral prefrontal cortex during the task [86].

Thus, it appears elderly people may recruit new brain systems during spatial

working memory tasks in an attempt to compensate for dysfunction.

Animal research has implicated both the prefrontal cortex (PFC) and

hippocampus in spatial working memory. In nonhuman primates, the prefrontal

cortex is activated during active retention on visuo-spatial working memory tasks

[35,66]. In rats, lesions of the prefrontal cortex or the ventral portion of the

hippocampus, to which it projects (See Fig. 2, [30,109,124]), impairs short-term

acquisition and retention of spatial information [30,100,112]. Thus, dysfunction in

either brain system may contribute to spatial working memory impairment.

Cognitive tasks also have shown impairments in spatial working memory in aged

rats, indicating that these neural systems may decline as a function of the aging

process. In a repeated acquisition discrimination, where an escape platform

varied in location for each session in a water maze, Frick, Baxter, Markowska,

Olton, & Price (1995) compared spatial working memory in young (4 mo), middle

aged (11 and 17 mo), and aged (24 mo) F344 rats with a 3 – 4 minute inter-trial

interval. The aged rats showed a significant decline in their ability to locate the

platform. Further studies also demonstrate that aged rats have greater difficulty

maintaining information pertaining to a spatial location when testing delays

increase [4,8,63]. However, even though spatial working and reference memory

both show age-related decline, and both involve hippocampus, the relationship
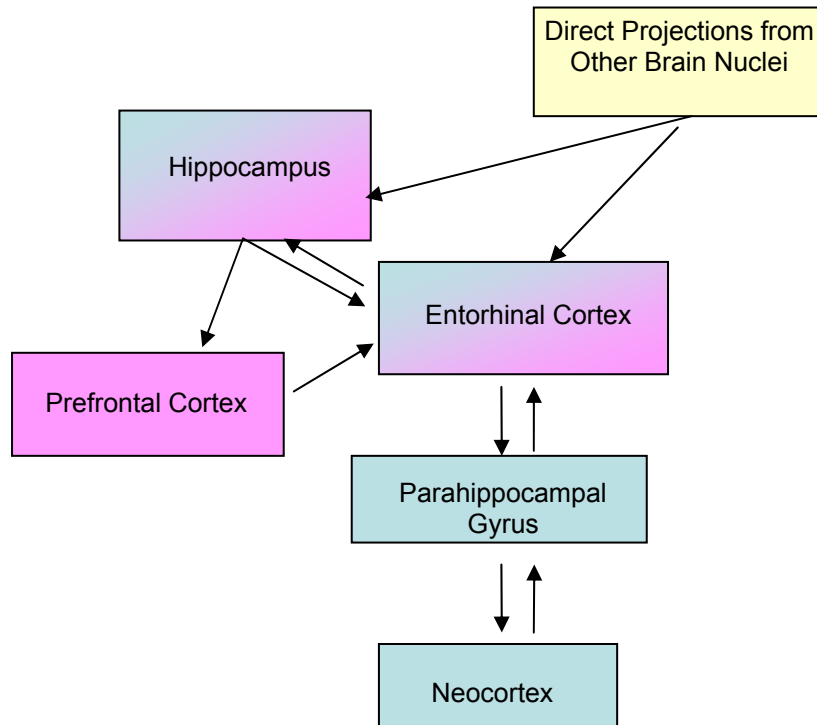
Fig. 2. Medial temporal lobe system and working memory. The prefrontal cortex receives input from the hippocampus and sends information back to hippocampus through the entorhinal cortex, with which it is reciprocally connected to.

between the two types of declarative memory is not clearly delineated.

Chapter III will assess the spatial working memory of F344 rats across the lifespan, and any observed changes will be compared to spatial reference memory abilities seen in Chapter II.

Olfactory Functioning

Using MRI, the damage responsible for H. M.'s amnesia was shown to primarily encompassed the medial temporal lobe system, and notably H.M. also exhibited odor identification and discrimination deficits [18]. The olfactory system projects to the medial temporal structures, providing the major source of olfactory sensory information to the hippocampus [27]. Odor information is initially processed in the olfactory bulb, which projects to both piriform cortex, which has connections to the entorhinal cortex, and the entorhinal cortex directly, which has reciprocal connections with the hippocampus, as seen in Fig. 3 [61,123]. Young people generally show uniformity in olfactory detection, whereas among elderly people a large degree of variation is present with respect to olfactory sensitivity [107]. Based on these findings and the anatomical connectivity, olfactory functioning may be dependent upon or at least related to age-related changes within the MTL and associated behaviors (e.g., spatial reference memory).

Olfactory discrimination learning is a convenient tool in research for the rodent population due to rodents' highly tuned sense of smell. After sampling an
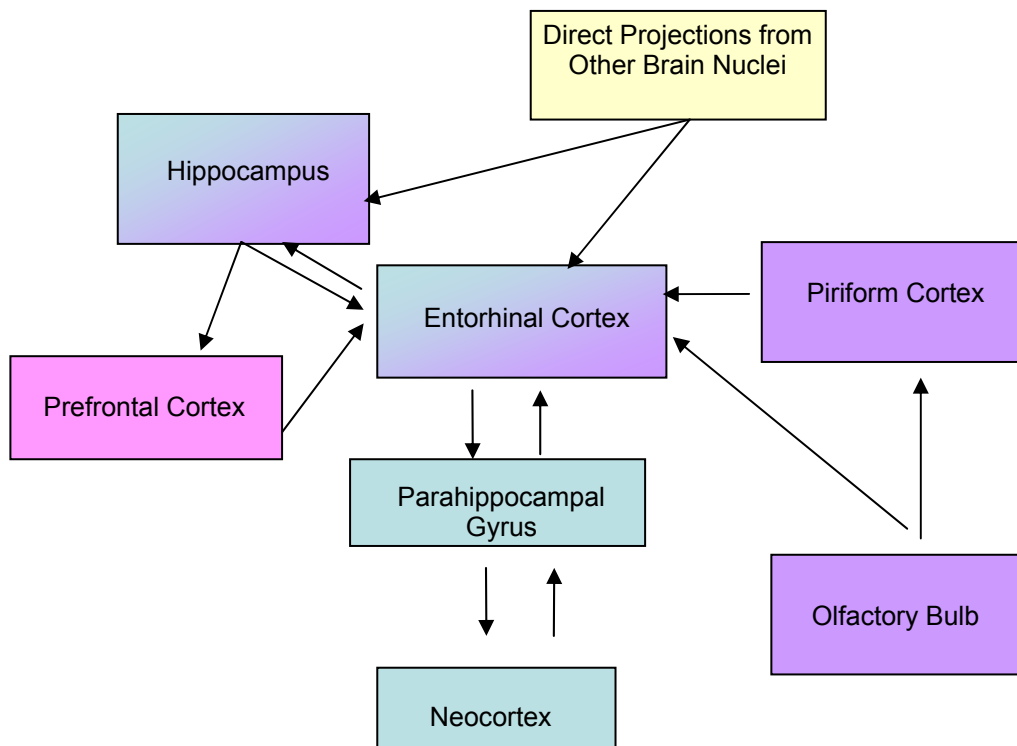
Fig. 3. Medial temporal lobe, working memory, and connections with olfactory function. Olfactory information is processed initially in the olfactory bulb. The bulb projects to the piriform cortex, which sends information to the entorhinal cortex, and directly to the entorhinal cortex. In turn, the entorhinal cortex is reciprocally connected with the hippocampus.

odor for only 200 ms, rats achieve maximum accuracy on odor problems in a two-alternative choice odor discrimination task [110]. Aged rats are significantly impaired in a Go No Go odor-reward association task, in which only 40% of aged F344 rats can learn at the same speed as young cohorts [89]. These data from rodents follow a pattern also observed in humans with MCI, such that some aged rats retain their cognitive abilities while the others show impairment [80].

Finally, it is notable that olfactory deficits have become recognized as an indicator of cognitive deficits and researchers have begun to use olfactory tasks as an early screening tool for MCI in humans [24,119]. One example involves the "Sniff N' Sticks" odor identification task, an olfactory assessment that utilizes twelve common odors presented in felt tip pens. Subjects are asked to identify an odor on a single odor stick from four written names. Impairment on this task is related to other cognitive assessments used to identify the presence and severity of MCI [24]. A separate study correlated performance on 19 cognitive batteries with the ability to perform odor identifications [119]. Thus a rodent olfactory function task may be of great utility in determining the underlying neural mechanisms of human cognition.

Chapter IV will examine olfactory function in young and aged rats through discrimination learning and odor threshold testing. Changes or impairments with respect to odor discrimination abilities will be correlated with spatial reference memory abilities assessed in Chapter II.

CHAPTER II

SPATIAL REFERENCE MEMORY ACROSS THE LIFESPAN OF MALE

FISCHER 344 RATS

Introduction

Declarative memory (memory for people, facts, and events, as well as of

spatial locations) is dependent upon the medial temporal lobe system, including

the hippocampal formation [15,21,104,118]. Dysfunction of the medial temporal

lobe system and concomitant loss of associated learning and memory functions

are well-documented at advanced ages [32,56,58,67,78,86,91]. Importantly,

however, such impairments in medial temporal lobe functioning are not an

inevitable consequence of the aging process [7,10,38,120]. At advanced

chronological ages, a spectrum of mnemonic abilities exists such that some

aged individuals perform on par with young adults in tests of declarative/

reference memory, 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 [38,54,101]. Moreover, a variety of functional deficits

in these structures [14,39,79] and changes in neuronal encoding properties

[6,108] correlate with individual differences in spatial learning abilities.

The goal of the current study was two-fold. The first objective was to determine if an age-related decline in spatial reference memory could be detected throughout the lifespan of F344 rats using a protocol that was specifically designed to be sensitive to age-related differences [38]. Past studies in F344 rats did detect middle-age and aged decline in performance on a spatial reference memory task; however, information on individual variability is inconsistent [34,47]. Thus, the second objective was to determine if individual differences were present within this inbred rat population. Specifically, young, middle-aged, and aged F344 rats were tested on a spatial reference memory task. The results demonstrate an age-related decline in spatial reference memory across the lifespan, and that reliable individual differences are present among aged F344 rats.

Methods

Subjects

Young (6 mo; n=35), middle-aged (12 mo; n=30), and aged (22 mo; n=60) male Fischer 344 (F344) rats were used in this study. 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 two weeks prior to the start of behavioral testing. This AALAC-accredited vivarium was maintained at a consistent 25º with a 12:12 hour light/dark cycle (lights on at 0800), 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 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. 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.

Apparatus

The reference task was conducted in the water maze. The maze consisted of a circular tank (diameter 183 cm, wall height 58 cm) painted white and filled with water (27 ^{o}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). The curtains were black with white cues. A retractable escape platform (diameter = 12 cm, HVS Image, UK) was

submerged two centimeters 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.

Experimental Design

Rats were tested 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.

Spatial Reference Memory Task

Rats received three trials a day for eight consecutive 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 there 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 the rat to escape.

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.

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.

Spatial Reference Memory Task

Training trial performance was analyzed using both cumulative search error and pathlength 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 per 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. Additional measures of performance (e.g., latency, swim speed) also were recorded. Interpolated probe trial data (i.e. every sixth trial) was

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 and probe trials were conducted using two-factor repeated measure ANOVAs (age X 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 individual rat. Weights for each probe trial were derived by dividing the mean search error in the young group on probe trial 1 (on which no age differences were observed) by the mean search errors on probe trials 2-4 (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 was 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 investigated using a two-factor repeated measures ANOVAs (cognitive age group X probe trial) and appropriate pos hoc analyses.

Cued (Visible Platform) Task

Mean swim speed and pathlength was calculated from the six cued (visible platform) trials. Separate one-factor ANOVAs were used to compare age groups on each performance measure.

Results

Swim Speed

Age-related differences in swim speed were assessed in two separate conditions minimally confounded by learning: the first trial of the spatial reference memory task and mean cued training to a visible platform. 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 cue training to a visible platform: ($F_{(2,\ 110)}$ = 37.44, p<0.001)). 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 or entirely independent of swim speed. Thus, in this thesis, latency data are not reported. Moreover, pathlength (total distance swum to reach the escape platform) was used to confirm significant effects involving the advanced age group 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 [38] and 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 Methods above (Chapter II) for more details).

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 memory task, independent of mnemonic abilities. Middle-aged and

aged rats that performed more than three standard deviations above young performance (assessed by mean pathlength across all 6 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 analysis were n=35 young, n=29 middle-aged, and n=49 aged. Using this final data set, performance of mean pathlength on cue training trials was assessed. A one-factor ANOVA revealed a significant main effect of age on mean pathlength across all 6 trials $(F_{(2, 110)}= 7.37, p<0.001)$. Subsequent 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 are not likely to influence the age differences observed in the spatial reference task reported below. The pattern of between-group differences in the cued task (i.e., middle-aged rats having an overall *shorter* pathlength to reach a visible platform than young rats) would, if anything, minimize the ability to detect spatial learning deficits in middle-aged relative to young rats. However, as described below, age-related deficits in the reference memory task were observed in both middle-aged and aged relative to young rats.

Spatial Reference Memory Task

Training Trials

Fig. 4 shows cumulative search error (A) and pathlength (B) on training trials in young, middle-aged, and aged rats. A repeated measures ANOVA (age X 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. 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).
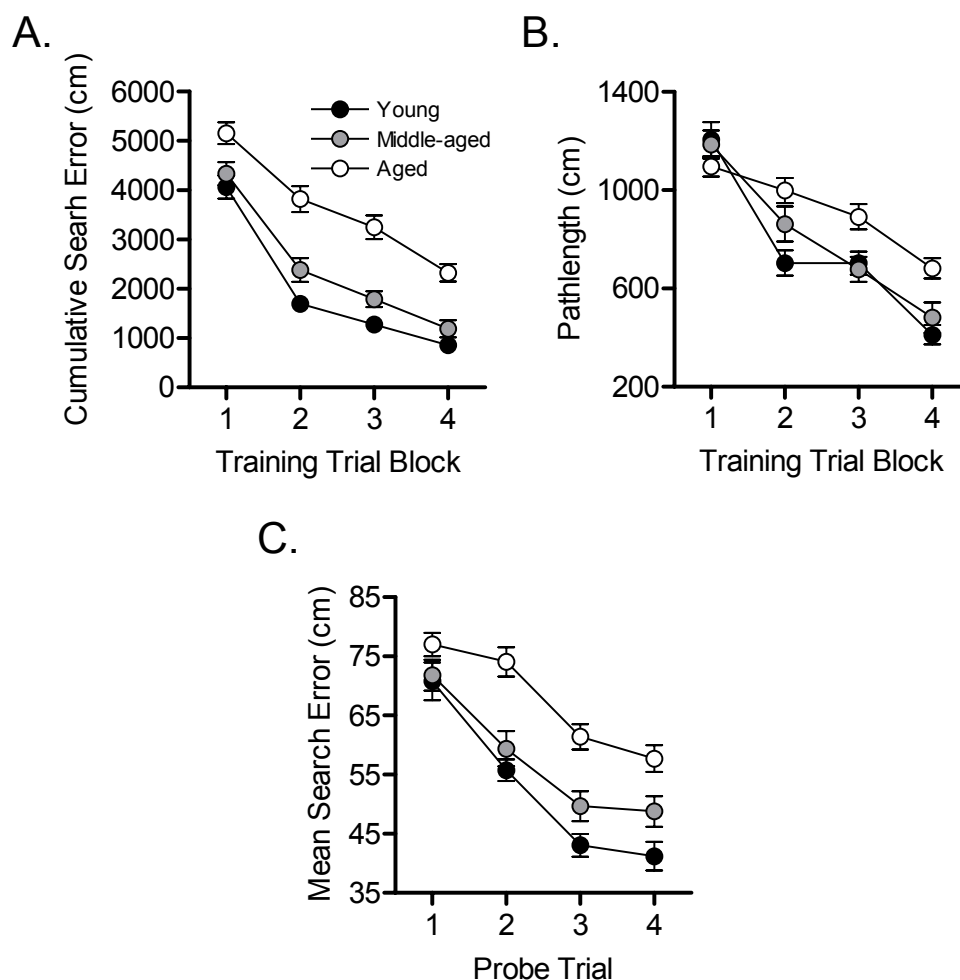
Fig. 4. Spatial reference memory performance of young, middle-aged, and aged F344 rats. Cumulative search error ± S.E. (A.) and pathlength ± S.E. (B.) averaged over the four training trial blocks is shown for young (black circle), middle-aged (gray circle) and aged (open circles) rats. Panel (C.) shows 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, C). Post hoc comparisons indicated that middle aged rats were significantly impaired relative to young rats (A, C) and that aged rats were significantly impaired relative to both young and middle-aged rats (A-C). See text for statistical analyses.

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. 4C shows performance of young, middle-aged, and aged rats across the four probe trials. A repeated measures ANOVA (age X 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 $(F_{(6,330)} = 2.13, p<0.05)$. 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 [12,17,73,103]. Fig. 5A shows the distribution of Spatial 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
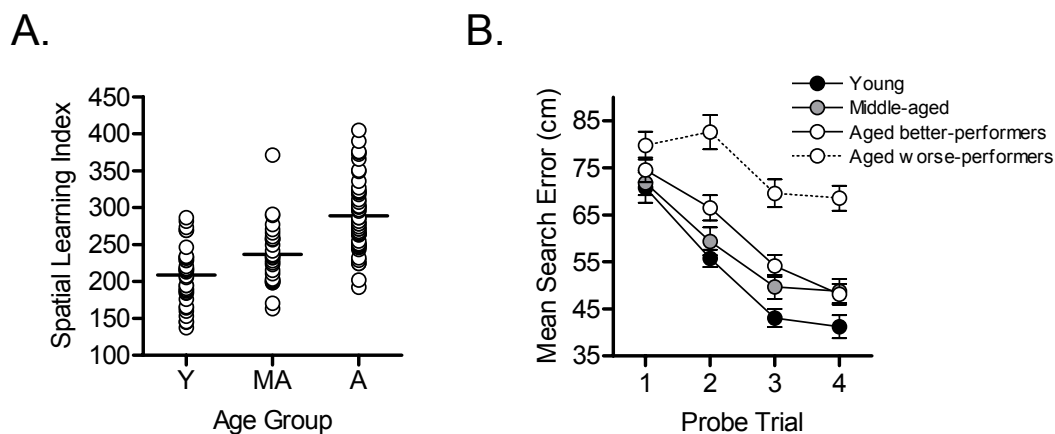
Fig. 5. Individual differences in spatial reference memory performance among young, middle-aged, and aged F344 rats. Panel (A.) shows the distribution of individual Spatial Learning Indices for young (Y), middle-aged (MA), and aged (A) rats. Despite a group impairment of middle-aged relative to young rats observed with mean Spatial Learning Indices, the majority of middle-aged subjects 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 sub-grouped 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.

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 (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 rats relative to younger subjects, the vast majority of middle-aged rats still performed within the range of young cohorts. 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 were observed such that 53 percent of the aged group (n= 26) performed within the range of their young cohorts (i.e.- Spatial Learning Index < 285) whereas 47 percent (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 we sub-grouped into "aged better-performers" (i.e. performed within the range of young) and "aged worse-performers" (i.e. performed worse than young rats). Fig. 5B shows probe trial

performance of young, middle-aged, and aged sub-grouped rats. Note that while 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 X probe trial) confirms that while overall, 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). Post hoc comparisons indicated that while aged better-performers were significantly impaired relative to young (post-hoc comparison, p<0.001), this aged sub-group did not differ significantly from 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=.08, n.s.).

Discussion

Results from this study demonstrate progressive age-related impairments in male F344 rats across the lifespan on a spatial reference memory task. The spatial reference memory task used here detected individual variability among the rats within this naturalistic population such that about half

of the aged rats were still performing within the range of young, whereas the other half performed outside the range of young, demonstrating impairment on the task. Indeed, the latter subset of rats had a dramatically attenuated learning curve relative to younger rats and other aged cohorts.

It is becoming widely recognized that declarative/reference memory declines progressively across the lifespan, generally reaching the detection threshold at middle age [2,90,116]. 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 [11,83,122]. It is demonstrated here, however, that middle-aged rats are impaired relative to young rats on a spatial reference task. Middle-age impairment is of particular interest since detection of initial cognitive changes may be useful, not only in the identification of early neurobiological changes, but also in prevention and treatment strategies for future cognitive decline.

Moreover, the current data indicate that the F344 rodent model of cognitive aging described here may be useful in detecting individual variability throughout the lifespan. This variability was of interest due to the inbreeding in this particular rodent strain, as the more uniform genetic population could have resulted in homogeneity across the aging process. In contrast, the present data rather definitely demonstrates that among aged F344 rats, there are subsets of subjects which differ dramatically in their ability to learn a spatial reference memory task in the Morris water maze. A subset of aged rats were impaired

relative to young and aged-better performing cohorts in that these "aged- poor performers" not only failed to acquire the task at the same rate, but these rats also never reached the same level of performance, at least with the amount of training used in the current study. Both the ability to observe such individual variability and to detect middle-age impairment in F344 aged rats demonstrate that two characteristics of human aging are also present in the F344 rats. These data suggest this rat model (i.e.-naturally occurring aged male F344 rats trained on the reference version of the Morris water maze) should have significant utility with respect to investigating causative factors of age-related declining cognition in aging.

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. Most notably, significant swim speed differences were observed between aged F344 male rats relative to both young and middle-aged rats. In agreement with previous studies, the cumulative search error appeared to be the most sensitive measure for detecting age-related mnemonic differences, particularly at middle-age; however, pathlength may indeed be the optimal choice for analyses in aged F344 rats due to the persisting confound of group swim speed differences [38].

Due to the individual variability seen in the population of F344 rodents, this model can be useful in the examination of neurobiological changes

associated with age-related decline. Spatial Learning Indices of age worse- and better-performers can be correlated with neural markers to assess age-related neurobiological differences between rats that perform on par with young and those that perform outside the range of the younger cohorts. Additionally, the Spatial Learning Indices of the middle-age population show that most rats perform in the range of the younger cohort, but as a group their scores were shifted towards higher numbers, indicating impairment. Further studies on middle-age rats would be useful to determine if, as suggested here, the entire population declines as a function of age, or alternatively, if using more sensitive methodology, some individual rats begin to experience progressive age-related decline at middle-age while others continue to maintain mnemonic function throughout all ages . Such studies will be of great utility in the understanding of age-related cognitive decline and the development of therapeutic interventions that prevent mnemonic impairment.

CHAPTER III

SPATIAL WORKING MEMORY ACROSS THE LIFESPAN IN MALE

FISCHER 344 RATS

Introduction

Age-related cognitive impairment is not confined to reference memory,

but also encompasses working memory [4,8,32,63,67]. Working memory is used

to retain trial-unique information over a time delay. Aged human and rodent

subjects show impairment in working memory, compared to young, such that

they have difficulty retaining information over a delay period [4,8,32,63,67].

However, information about working memory has been inconsistent in the F344

rat strain, especially in the middle-aged population [34,101].

Working memory is dependant on the prefrontal cortex and the

hippocampus (See Fig. 2 for schematic of neural systems implicated in working

memory) [35,66,86]. Lesions of both the ventral portion of the hippocampus and

the prefrontal cortex cause impairment in working memory tasks in rats

[30,100,112]. Additional studies indicate that some neural systems are

differentially activated during working memory problems, possibly in an attempt

to compensate for impairment in function [67,86]. Indeed, those aged individuals

that show increased neural activation relative to young subjects generally

perform better on working memory problems relative to aged subjects who

recruit brain regions in a similar manner to young cohorts.

The goal of the current study was to examine spatial working memory abilities of F344 rats across the lifespan and to determine, given some overlapping circuitry responsible for spatial reference and working memory, if any age-related deficits observed in a spatial working memory task would be related to and/or precede those observed on the spatial reference memory task described in Chapter II. Specifically, young, middle-aged, and aged F344 rats were tested on a spatial working memory task. The results demonstrate an age-related decline in spatial working memory across the lifespan of F344 rats that is, perhaps surprisingly, independent of age-related deficits in spatial reference memory ability.

Methods

Subjects

Young (6 mo; n=17), middle-aged (12 mo; n=29), and aged (22 mo; n=21) rats with previous training on the spatial reference water maze task, tested in Chapter II, were used in this study. As mentioned in previously, rats were obtained from the National Institute of Aging colony and then housed and screened for health in the same manner as described in Chapter II. All animal procedures were conducted in accordance with approved institutional animal care procedures and NIH guidelines.

Apparatus

The spatial working memory task was conducted in the same water maze used in the spatial reference memory task, described in Chapter II. The maze consisted of a circular tank (diameter 183 cm, wall height 58 cm) painted white and filled with water (27 ^{o}C) made opaque with the addition of nontoxic white tempra paint. The maze was now surrounded by white curtains (instead of the black curtains from the spatial reference memory task) to which were affixed large geometric designs that provided extramaze cues (that differed in shape and location from those used in the previous task) surrounded the maze. 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). An escape platform (diameter = 12 cm) was submerged two centimeters 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.

Experimental Design

One week following completion of the reference memory task, a subset of 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 twelve 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 to the edge of the tank from the placement on the previous days. On the second trial (the retention trial), the submerged platform was located in the same position as on the first trial. The start position was always distal from the platform, and the trials were otherwise conducted in the same manner as the reference memory task (see Chapter II). On the first three 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 nine 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).

Behavioral and Statistical Analyses

Separate analyses were conducted for acclimation (30 s delay) and testing (30 min, 2 h and 6 h delays) trials. 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. 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 PLSD post hoc tests where appropriate, were used to compare age groups at each delay. A series of Pearson's correlation coefficients were used to compare individual performance of rats on the spatial reference memory (Spatial Learning Index or mean pathlength on training trial block 4, from data obtained in Chapter II spatial reference memory task) with performance at each delay on the spatial working memory task (Pathlength Difference).

Results

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) was used to compare performance of young, middle-aged, and aged rats at each retention interval using one-factor ANOVAs (Fig. 6). 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 h and 6 h delays $(F_{(2, 66)} = 3.39$, $p<0.05$ and $F_{(2, 34)} = 4.47$, $p<0.05$, respectively). At
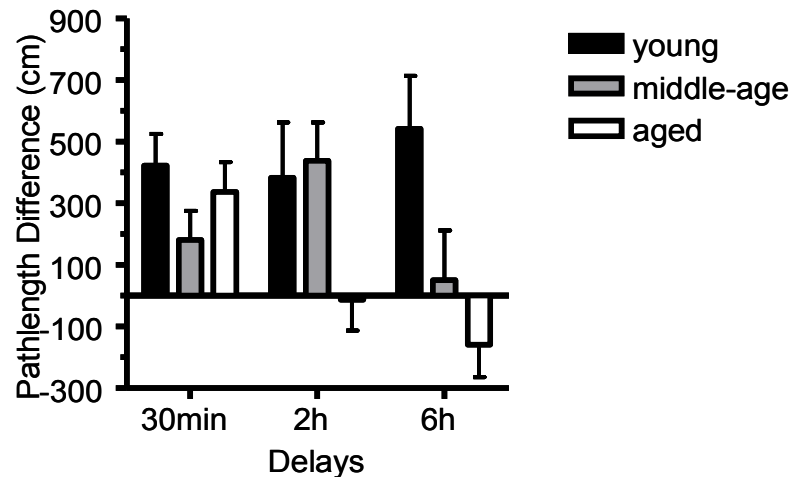
Fig. 6. Spatial working (delayed match-to-place) memory performance of young, middle-aged, and aged F344 rats. Bar graphs show Mean Pathlength Difference ± S.E. for young (black bars), middle-aged (gray bars), and aged (open bars) rats for retention trials with varying delays. Although no differences between age groups are seen with a 30 min delay, at both 2 and 6 hour delays the aged perform significantly worse than the middle-aged group. Additionally, at the 2 h delay there is a trend toward significance between the aged and young groups, which becomes a significant difference at the 6 h delay, where middle-aged and young also differ. Note that at 6 h, aged rats perform below 0, indicating that, as a group, aged rats perform worse on retention trials than information trials.

the 2 h delay, post hoc analyses revealed a strong trend toward a difference between young and aged rats (p= 0.059), and a significant difference between aged and middle-aged rats (p<0.05). Young and middle-aged groups did not differ in performance at the 2 h delay. In contrast, at the 6 h delay, post hoc analyses indicated that middle-aged were impaired relative to young rats (p<0.05), and that aged 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 delay increases across the lifespan in F344 rats. To determine the relationship between performance on the reference and working memory tasks, a series of Pearson's r correlations were 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 vs. pathlength difference (r= 0.02 to r= 0.41, n.s.); mean pathlength on training trial block 4 vs. pathlength difference (r= 0.01 to r= 0.42, n.s.).

Discussion

The present data show that age-related memory decline is not only confined to reference memory; age also impacts working memory (i.e., memory associated with transient information [34,77]). In the spatial working memory

task, aged F344 rats showed impairment in performance with a two hour delay between the information and retention trials. The middle-aged group showed impairment in performance with an increased delay, at 6 hours, even though the young group could retain spatial information with increasing delays throughout testing. Although age-related cognitive decline was seen in both spatial reference and working memory tasks in this subset of F344 rats, there was no correlation between the two tasks.

In agreement with results reported here, deficits in spatial working memory have been reported in aged (24 mo) relative to young (4 mo.) rats using delays of 3 – 4 minutes on a repeated acquisition task, where a hidden platform was located in a novel position each day so that the second trial measured working memory related to the position of the platform on the first trial of the day [34]. In a similar task using a 10 min delay, aged (22 mo) and middle-aged (12, 15, and 18 mo) demonstrated impairment [101]. Indeed, rats from other strains and primates, including humans, have a diminished capacity across the lifespan to retain relevant but transient information with increased retention intervals [4,8,32,63,67]. Although hippocampus has been implicated in some forms of working memory (particularly spatial), this form of memory also depends heavily upon extra-hippocampal structures [20,31,49,121]. The lack of relationship between the age-related deficits on spatial reference and spatial working memory tasks in the current study suggest the possibility of multiple loci of neural dysfunction among middle-aged and aged F344 rats.

Previous studies have shown that lesions of the medial temporal lobe system in rats, particularly entorhinal cortex, can impair performance on a delayed match-to-place task and non-match-to-sample task [42]. Interestingly, however, Stern and colleagues (2001) reported differences in neural activation in humans performing a two-back working memory task, requiring the subject to identify any stimuli that were repeated after one intervening stimulus, using either novel or familiar visual scenes. With the novel stimuli, increased signal was apparent in medial temporal lobe structures, including the hippocampal and parahippocampal regions. In contrast, when familiar stimuli were utilized, 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.

Such findings are relevant to the current results because, despite attempts to create a novel environment for the delayed-match-to-place test in the current study, the same water maze was utilized for both the reference and working memory tasks. Thus, 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. Thus, given the contingencies of our task design and the evidence from the Stern study, the age-related deficits in working memory observed here may be indicative of an age-related decline in prefrontal cortical function. On a related note, a recent study,

using a genetically altered mouse exhibiting the complete loss of the NR1

subunit of NMDA receptors in the granule cell layer of the dentate gyrus,

provided evidence that changes or differences in subregions within the

hippocampus itself may result in impairment of working memory abilities [74].

Additional experiments including using a non-water maze assessment for

working memory in addition to lesion studies of the prefrontal cortex and/ or

hippocampal subregions would prove useful in distinguishing among potential

causes for the lack of correlation with the decline in spatial reference memory

and elucidate the neural substrates responsible for the working memory deficits

reported here.

CHAPTER IV

DEFICITS ACROSS MULTIPLE COGNITIVE DOMAINS IN A SUBSET OF

AGED FISCHER 344 RATS*

Introduction

Cognitive decline observed in normal and pathological aging is not a unitary phenomenon, as a variety of deficits are observed among distinct and overlapping elderly populations [44,52,76,87]. Among the most prominent and debilitating consequences of aging is loss of cognitive functions dependent upon medial temporal lobe structures, including the ability to encode and recall facts and events. Importantly, the emergence of such deficits is not inevitable as some humans maintain these and other cognitive abilities on par with young adults well into advanced age. To investigate relevant neurobiological factors, rodent models have been developed using the Morris water maze, a task that is both dependent on medial temporal lobe structures and that reliably detects individual variability in performance among aged rodents (see Chapter II for further description) [36,38,62,114]. Other age-related cognitive deficits have been detected in humans and rodents [5,95]; however, given the widespread nature of deficits associated with medial temporal lobe dysfunction in humans, there are surprisingly few instances of non-spatial age-related impairments that

correlate with spatial learning impairment in rodent aging models. In addition, the extent to which such multi-dimensional deficits across have been observed, the reliability of performance across multiple test sessions and thus the utility of these behavioral tasks for understanding human mnemonic dysfunction is not clear [37,92,125].

Olfactory detection and discrimination deficits occur in human aging, and medial temporal structures receive major afferents from the olfactory system (see Fig. 3) [27,52,76]. In addition, a limited literature has described deficits in odor discrimination learning in some strains of aged rats [53] but not others [82,89,95]. The neural substrates and specificity of such learning deficits are not clearly defined, although some evidence suggests that medial temporal lobe structures are involved [27,125]. In the current study, young and aged Fischer 344 rats, characterized in the Morris water maze task in Chapter II, were trained on a series of two-item discrimination problems in a naturalistic task involving digging for a food reward [9]. We found striking impairments across multiple olfactory but not non-olfactory discrimination problems only in the subset of aged rats that was spatially impaired relative to young cohorts in the Morris water maze task. These deficits were not due to impaired olfaction as aged worse-performers were not impaired relative to young and aged better-performing cohorts on an odor detection threshold test. These are among the first data to demonstrate a reliable learning deficit that is related to spatial learning impairments in a rodent model of cognitive aging. These data provide the

foundation for a model that should prove useful in neurobiological and preclinical investigations of human age-related cognitive impairments.

Methods

Subjects

A subset of young (n= 8) and aged (n= 12) male F344 rats from the spatial reference memory task (Chapter II) were also assessed in this odor discrimination and threshold procedure. Rats were obtained at 6 (young) and 22 (aged) months of age (National Institute of Aging) and trained sequentially in the spatial reference memory task (Chapter II) and discrimination learning. Rats were given free access to food and water except during discrimination testing, when they were food-restricted to 85% of their free-feeding weight. All rats in the study were housed and screened for health as described in Chapter II, and all animal procedures were conducted in accordance with approved institutional animal care procedures and NIH guidelines.

Apparatus

Water-maze Testing

Young and aged rats were assessed for spatial learning abilities on the Morris water maze task using a protocol modified from Gallagher et al., as described in Chapter II [38].

Discrimination Testing and Odor Detection Threshold Testing

Discrimination learning was conducted in an open-topped translucent plastic box (49 x 33 x 28 cm). The front and side walls of the box were made opaque with black paper affixed to the outside of the box, whereas the back wall was left translucent. A video camera mounted outside of the box and connected to a TV monitor allowed viewing of the rats through the translucent wall. The box was divided into a start (16 cm) and test (33 cm) compartment by an opaque sliding Plexiglas barrier. Two terra cotta flower pots (11 cm in diameter at the top, 10 cm high) were placed side by side against the back wall of the box and affixed to the box floor with Velcro pads. A top-down schematic of this apparatus setup is seen in Fig. 7.

Experimental Design

Water-maze Testing

Rats were assessed in a spatial reference memory task utilizing the water maze for hidden platform and cued training, described in Chapter II.

Discrimination Testing

The procedure was modeled after Barense et al. [5]. Following the completion of cue training in the water maze, rats were food-restricted to 85% of their free-feeding weight over 5 days. Pots identical to those used in the test apparatus and filled with clean home cage bedding in which several rewards

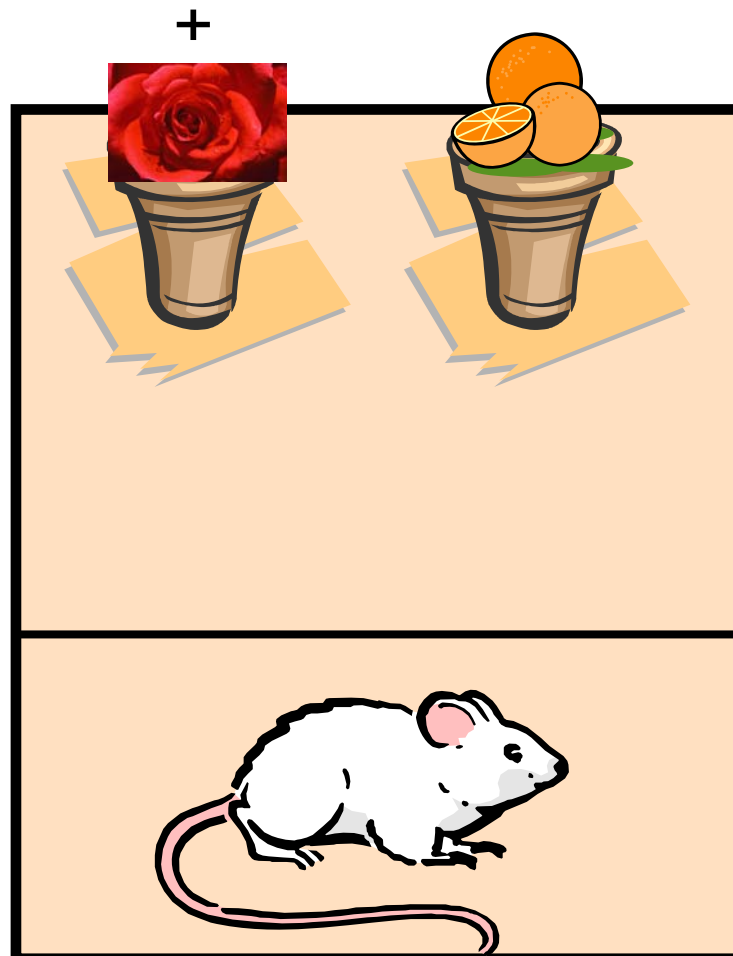**Fig. 7. Odor discrimination testing apparatus**. For odor discrimination testing, rats were placed in a translucent box with a plexiglass barrier between the start and test compartments. The rats had a choice of two pots that differed in either odor, such as rose and citrus, or digging medium, such as sequins and styrofoam. The rat must learn to associate the positive stimulus with a food reward.

were buried were placed in the rats' home cages on the night before shaping began, to reduce neophobia to the pots and food reward. Shaping took place in the test apparatus and consisted of training the rats to dig in two pots, each filled with clean home cage bedding and a food reward buried 2 cm below the surface of the bedding (1/4 of a Froot Loop, Kellogg's, Battle Creek, MI). On each trial, a rat was placed in the start compartment and the barrier was raised to allow access to the pots. Rats were considered shaped when they would reliably retrieve both rewards in less than one minute.

On the day following shaping, rats began the discrimination problems. On these problems, only one pot contained a food reward, and its odor was disguised by the addition of several crushed Froot Loops sprinkled over the surface of each pot. The position (left or right) of the rewarded pot was varied pseudorandomly across trials. For the first four trials of every new discrimination problem, rats were allowed to dig in both pots until they obtained the reward (i.e. - they were allowed to self-correct if they dug in the incorrect pot). On these trials, only their first choice was scored (as correct or incorrect). On trials thereafter, rats were removed from the test chamber after only one dig (either correct or incorrect). A dig in a pot was scored if a rat displaced the digging medium with either its paws or nose.

On odor discrimination problems, both pots were filled with clean home cage bedding. A small drop of odorant (20 μl) was applied to the rim of each pot, and the reward was consistently associated with only one of the odors. The

odorants used were perfume oils obtained from The Bath Junkie and The Body Shop. On non-olfactory discrimination problems, rats were trained to discriminate between two different substances (digging media) filling the pots, and the reward was associated with only one of these substances. The following discrimination pairs were used for each rat: odor: rose and citrus, hazelnut and peppermint; digging medium: styrofoam and sequins, shredded latex gloves and shredded tissue paper. The positive and negative stimulus in each pair of discriminada and the sequence of discrimination problems were randomized across rats, although each rat received alternating odor- and medium-discrimination problems (i.e. – either odor-medium-odor-medium, or medium-odor-medium-odor). There were no effects of sequence of discrimination problems on task performance. Rats were considered to have acquired a discrimination problem when they achieved 6 consecutive choices of the correct (baited) pot, after which they immediately began the next problem in the sequence. Both number of trials and number of errors to criterion were recorded.

Odor Detection Threshold Testing

Following completion of discrimination testing, rats were tested for their ability to detect and respond to decreasing concentrations of odorants. One aged better-performing rat died prior to odor threshold testing. The same apparatus used for discrimination testing was used. This test began with a new odor discrimination problem using 20 µl of full-strength odorant ("ocean mist")

vs. 20 μl of mineral oil applied directly to the rims of two pots filled with clean home cage bedding. The food reward was in the pot with the odorant, and rats were trained until they reached criterion performance (i.e., 6 consecutive correct trials). Trials and errors to criterion were recorded. Testing continued with 3 further discrimination problems using decreasing concentrations of the same odorant (diluted 1:10, 1:100, or 1:1000 in mineral oil) vs. mineral oil alone. New pairs of pots were used for each of these discrimination problems. Rats were given 16 trials at each dilution, and the number of correct choices out of 16 was recorded.

Behavioral and Statistical Analyses

Water-maze Testing

In the hidden platform task from Chapter II, accuracy of performance was assessed using two proximity measurements. A cumulative search error measurement was computed from training trials, and a Spatial Learning Index was calculated from probe trials. Lower Spatial Learning Indices indicate a more accurate search. In this study, aged rats are referred to as "aged better-performers" or "aged worse-performers". Aged worse-performers had learning indices outside the range of young rat performance (>250), whereas aged better-performers had indices within the range of young rats (<250). A two-way ANOVA (Spatial Learning Group X Trial Block) was performed *post-hoc* on groups using the aforementioned grouping criterion among aged rats to confirm

that the aged worse-performers and aged better-performers were different as a group with respect to spatial learning.

Discrimination Testing

Performance measures (trials and errors to criterion) of age and spatial learning groups were compared by two-way repeated measures ANOVAs (discrimination problem X age or spatial learning group). Separate analyses were performed for trials and errors to criterion on both odor and medium discrimination problems. Repeated measures ANOVAs were also used to compare trials and errors to criterion across odor and medium discrimination problems (to assess relative difficulty). When warranted, Fischer's PLSD post hoc analyses were used to compare performance of age and spatial learning groups. In all cased $p<0.05$ was considered significant.

Odor Detection Threshold Testing

Trials and errors to criterion were recorded during the odor detection threshold testing and analyzed using separate one-factor ANOVAs. During the 16 dilution problems, the number of correct choices out of 16 was recorded. A group x odorant concentration repeated measures ANOVA was used to compare performance of spatial learning groups and $p<0.05$ was considered significant.

Correlational Analyses

As one rat died just prior to odor threshold testing, all correlations were performed with an n=19 (n=8 young; n=11 aged). To assess test-retest reliability of performance on the odor discrimination task, Pearson's correlations were performed on both trials and errors to criterion across the three odor discrimination problems in aged rats. In order to directly compare performance on spatial learning and odor discrimination tasks, a Pearson's partial correlation was conducted on corrected data (spatial learning index on the water maze task and trials and errors to criterion on the odor discrimination task). For each rat, performance values were zeroed to their respective age-group means (i.e. – the group mean was subtracted from their individual value), thus controlling for the age differences present across groups. Correlations were performed on these corrected data.

Results

Spatial Learning Performance

The results of the spatial learning assessment in young and aged rats are described in depth in Chapter II. This subset of young and aged rats had spatial reference memory performances representative of the larger dataset. In brief, some aged rats had learning index scores comparable to young rats (hereon referred to as aged better-performers , n=6) whereas others fell outside that range, demonstrating impairment on the task (hereon referred to as aged worse-

performers, n=6). The mean spatial learning indices of each of these groups were as follows: young = 189 ± 13; aged better-performers = 214.8 ± 8.7; and aged worse-performers = 311 ± 13.5. As expected, using these selection criteria, a one-way ANOVA demonstrated a significant difference in spatial learning indices among these groups ($F_{(2, 17)}$ = 26.51, p<0.0001) and *post-hoc* analyses demonstrated that aged worse-performers were significantly different from both young (p<0.0001) and aged better-performers (p<0.0001). There was no significant difference between young and aged better-performers (p=0.2).

Discrimination Learning

As described above, aged rats were split into better- and worse-performing subgroups based on their spatial learning index scores. Fig. 8 shows trials (A) and errors (C) to criterion on odor discrimination problems. Two-way ANOVAs (odor discrimination problem X spatial learning group) revealed main effects of spatial learning group on both trials ($F_{(2, 17)}$ = 12.25, p<.0005) and errors ($F_{(2, 17)}$ = 9.98, p<.005) to criterion. Post-hoc tests revealed that aged worse-performers took significantly more trials with more errors to reach criterion in comparison to both young and aged better-performers (p< .05 in both cases). Although aged better-performers had numerically more trials and errors to criterion than young rats, these differences never reached significance. Also shown in Fig. 8 are trials (B) and errors (D) to criterion on digging medium discrimination problems. Two-way ANOVAs revealed no main effects of spatial
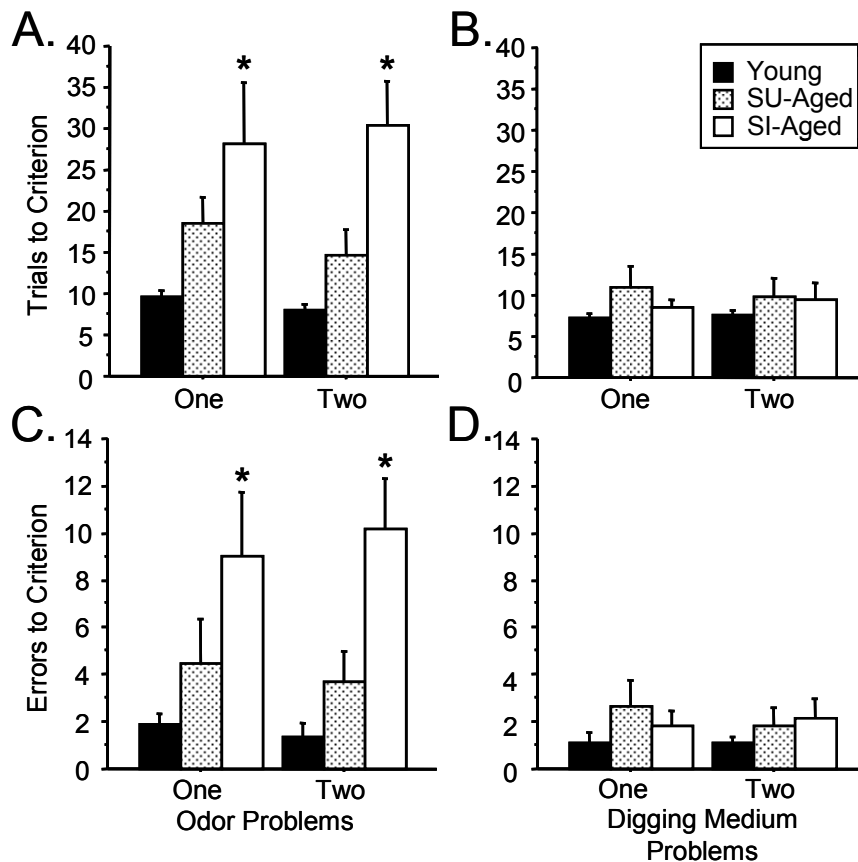
Fig. 8. Odor and digging medium discrimination performance in young and aged Fischer 344 rats. (A.) and (B.) show trials to criterion on odor and medium discrimination problems, respectively. aged better-performers (SI) took more trials to reach criterion performance than both young and aged worse-performers (SU) on odor discrimination problems but did not differ from either group on medium discrimination problems. Young rats had comparable performance on both odor and medium discrimination problems. (C.) and (D.) show errors to criterion performance on odor and medium discrimination problems. Deficits evident in the spatially impaired aged rats using trial to criterion were even more prominent using this measure of performance. * = significantly different from young and aged better-performers.

learning group on trials ($F_{(2, 17)}$ = 1.39, ns), or errors ($F_{(2,17)}$ = 1.68, ns) to criterion on the digging medium discrimination problems.

Comparisons between performance on odor and medium discrimination problems revealed main effects of spatial learning group ($F_{(2,17)}$ = 9.6, p<0.005) and a main effect of discrimination type ($F_{(1,17)}$ = 31.9, p<0.0001) as well as a significant interaction between spatial learning group and discrimination type ($F_{(2, 17)}$ = 12.15, p< .0005). Further analyses revealed that young rats did not differ in the number of trials needed to reach criterion on odor and medium discrimination problems, whereas aged better-performers (p<0.05) and aged worse-performers (p<0.005) took significantly more trials to reach criterion on odor compared to medium discrimination problems.

Odor Threshold Testing

Fig. 9 shows trials (A) and errors (B) to criterion during acquisition of the full strength odor discrimination problem used for odor threshold testing. In agreement with performance on the previous odor-discrimination problems, a one-factor ANOVA revealed a main effect of spatial learning group on both trials ($F_{(2, 16)}$ = 19.0, p<0.0001) and errors ($F_{(2,16)}$ = 19.9, p<0.0001) to criterion. Fischer PLSD post hoc analyses revealed that aged worse-performers were significantly different from young and aged better-performers on both measures (p<0.05 in both cases). Performance of aged better-performers did not differ significantly from young on either measure. Fig. 9 (C) shows percent accuracy
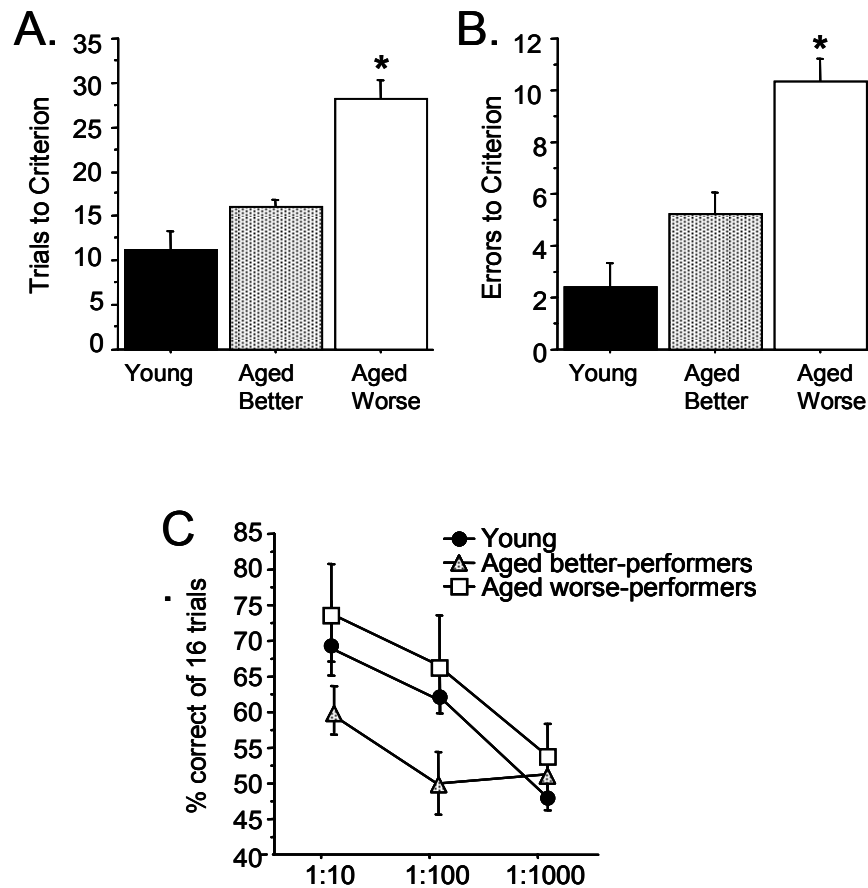
Fig. 9. Odor detection threshold performance in young and aged Fischer 344 rats. Bar graphs show trials (A.) and errors (B.) to reach criterion performance on the full strength odor discrimination problem used for threshold testing. As with the initial odor discrimination problems, aged worse-performers were impaired on both trials and errors to reach criterion performance compared to both young and aged better-performers. (C.) shows however, that upon learning the discrimination problem, aged worse-performers did not differ from the other groups in their ability to detect and discriminate descending concentrations of the odorant.

on the same discrimination problem at descending odor concentrations. A repeated measures ANOVA revealed that performance worsened with decreasing odor concentrations ($F_{(2,16)}$ = 14.5, $p<0.0001$) but that there was neither a main effect of spatial learning group ($F_{(2,16)}$ = 1.3, ns) nor an interaction between odor concentration and spatial learning group ($F_{(4,32)}$ = 0.72, ns).

Test-retest Reliability of Odor Discrimination Deficits

Intercorrelations performed among criterion measures from aged rats on the full-strength odor discrimination problem used during odor detection threshold testing and the initial two odor discrimination problems ranged from r = 0.54 to 0.83, with all p values reaching significance except in one case in which p = 0.08. These latter data demonstrate a high degree of reliability in performance across the odor discrimination problems among the aged Fischer 344 aged rats.

Relationship Between Spatial Learning and Odor Discrimination Problems

Individual values for spatial learning index scores and mean trials and errors to criterion across all three odor discrimination problems were normalized to the mean for each age group and a Pearson's partial correlation was conducted to directly evaluate the relationship between behavioral performance on both tasks across all subjects in the study (n = 19). The Pearson's partial correlation (Fig. 10) revealed a significant positive relationship between spatial
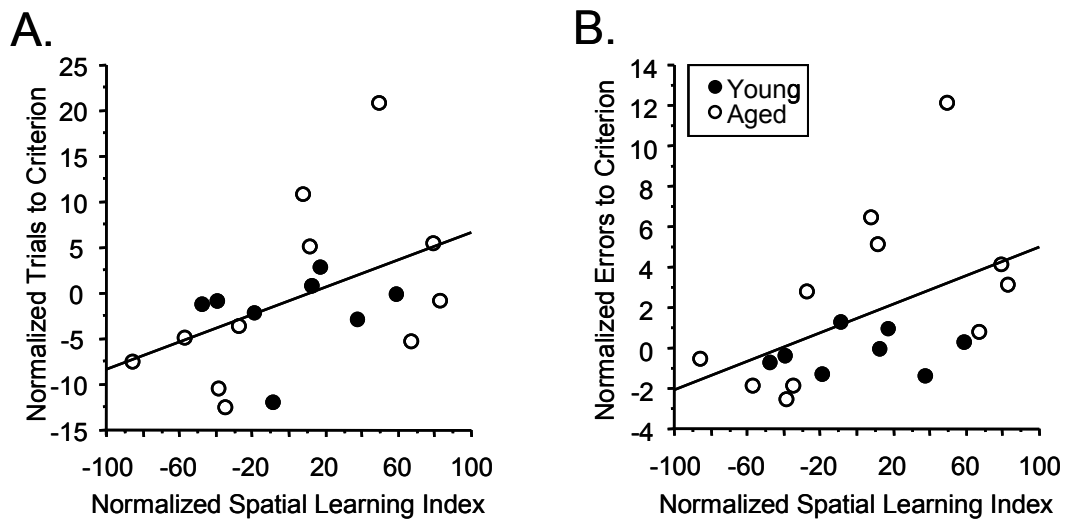
Fig. 10. Scatterplots of individual rat performance, normalized by the mean of each group (young and aged). (A.) Spatial learning index on the water maze vs. mean trials-to-criterion across all three odor discrimination problems. (B.) Spatial learning index on the water maze vs. mean errors-to-criterion across all three odor discrimination problems.

learning performance and odor discrimination abilities (r=0.47 and 0.48 for trials and errors to criterion, respectively; p<0.05 in both cases). There were no reliable correlations between performance measures on the cue training task and either the spatial learning index or measures of odor discrimination learning.

Discussion

The current results are among the first to demonstrate cognitive deficits in a subset of aged rodents characterized on a spatial learning task that extend to a distinct behavioral domain. The odor discrimination task used here has no spatial component and odors are largely irrelevant to performance in the water maze task. The odor detection abilities of the aged worse-performing rats were not different from young or aged better-performing rats, demonstrating that the deficits on odor discrimination problems indeed reflect a learning impairment. Moreover, the aged worse-performers did not exhibit deficits on digging medium discrimination problems, suggesting that performance on different types of discrimination problems are supported, at least partially, by independent neural substrates that are differentially affected by age. The relationships observed here between spatial learning and odor discrimination deficits suggest that performance on these tasks may be indicative of profound deficiencies in medial temporal lobe structures and/or broad neural deficits that extend across two or more brain systems in a subset of aged Fischer 344 rats.

The fact that the aged worse-performers were not deficient on the digging medium discrimination problems clearly demonstrates that the observed odor discrimination deficits are not due to a global learning impairment. Moreover, the fact that young rats took the same number of trials to reach criterion performance on odor and medium discriminations problems indicates that the odor discrimination deficit detected in the aged worse-performers was not a consequence of an increased difficulty in the odor discriminations compared to medium discriminations. Indeed, all aged worse-performers did reach criterion performance on all odor discrimination problems, indicating that these rats are able to learn odor discrimination problems, albeit with much greater difficulty than young and aged better-performers. Once the aged worse-performers had acquired the odor discrimination problem used for threshold testing, there were no group differences in detecting or responding to descending odor concentrations. These data demonstrate that the odor discrimination deficits were not due to the inability of aged worse-performers to detect the odors.

Notably, in comparison to young rats, aged better-performers took more trials, and, to a lesser extent, made more errors, before reaching criterion performance on odor discrimination problems. This observation, in addition to the fact that unlike young rats, aged better-performers were significantly worse on odor compared to digging medium discrimination problems, suggests a subtle overall age-related impairment in odor discrimination learning. Importantly, none of the numerical differences between aged better-performing and young groups

observed on the three odor discrimination problems reached significance, whereas aged worse-performers performed significantly worse compared to both young and aged better-performers on both measures across each of the three discrimination problems. Although a modest impairment in odor discrimination learning may occur across the lifespan in Fischer 344 rats, there appears to be a strong relationship between the severity of this learning impairment and spatial learning dysfunction. This relationship is supported by the fact that, across all rats in the study population, there was a significant correlation between spatial learning performance and performance on the odor discrimination task.

To our knowledge, the only other non-spatial deficit previously associated with age-related deficits in water maze performance is in recovery from gustatory neophobia in Long Evans rats [37,92]. Deficiencies related to memory for novel stimuli do not likely account for the learning impairments observed here, as the rats had no experience with the odors prior to the discrimination problems, and aged worse-performers were able to discriminate between digging media as well as young rats. Memory for previously learned odors, which would be more akin to the recovery from gustatory neophobia tested in the Long-Evans model, was not tested in this report but would be an interesting topic of future exploration.

It is generally accepted that performance in the water maze is dependent upon medial temporal lobe structures. Lesions of the hippocampus and multiple parahippocampal brain regions can impair water maze performance

[16,69,71,117]. In addition, numerous neurobiological and neurophsyiological

changes in hippocampus and other medial temporal lobe structures correlate

with spatial memory impairment in aged rodents [36,91,118]. In contrast, the

neural circuitry underlying odor discrimination performance is less clearly

defined. Primary and association cortices that encode olfactory information are

highly interconnected with the medial temporal system in rodents (Fig. 3). Odor

information is initially processed by the olfactory bulb and then in the piriform

and orbitofrontal cortices, which in turn have heavy reciprocal connections with

perirhinal and entorhinal cortices (reviewed in [27]). There are also direct

connections from the olfactory bulb to the entorhinal cortex [61,123]. In support

for a direct role of medial temporal lobe structures in odor discrimination

learning, the hippocampus is activated during odor discrimination learning in

rodents and humans [45,48]. Moreover, electrophysiological recordings indicate

that the parahippocampal cortices contribute to memory processing associated

with odor learning, although activity in these structures is primarily associated

with the persistence of learned discriminations rather than their acquisition [27].

In agreement with the latter findings, direct damage to the hippocampus or

parahippocampal cortices does not impair acquisition of simple odor

discrimination problems in some contexts, suggesting that these structures are

not critical for odor discrimination learning [27]. In contrast, lesions of the

orbitofrontal cortex can produce such deficits in some circumstances [28], and

encoding of odor-reward associative information in orbitofrontal cortex during

odor discrimination learning is attenuated in aged rats [97]. Damage to orbitofrontal cortex function may also produce deficits in water maze performance under some conditions, although the existing data on this subject are contradictory [50,51,111]. Additional experiments are needed to determine whether the relationships observed here between impairments in spatial learning and odor discrimination performance is a result of age-related deficits in a single neural system (e.g. – medial temporal or orbitofrontal cortex) or rather a result of coordinated loss of function across two independent neural systems.

It is notable that odor discrimination deficits are not characteristic of other rat strains in which individual variability in spatial learning performance is reliably observed [5,53,64,95], suggesting that aging may affect odor discrimination learning and its underlying neural substrates differently across rat strains. Task design does not likely account for the reported differences, as another group using a very different task also observed odor discrimination deficits in a subset of aged Fischer 344 rats [89]. In addition, using the same task parameters as those employed in the current study, Long-Evans rats were not impaired on odor discrimination problems, despite a subset of those rats demonstrating spatial impairment in the water maze [5]. It is notable, however, that neural activity in orbitofrontal cortex encodes odor-reward associative information [88,96], and that encoding of such information is attenuated in a subset of aged Long-Evans rats [98]. These latter data suggest that although there may be age-related neurobiological changes that relate to odor learning in Long Evans rats (and

possibly other rat strains), these changes may not reach a threshold sufficient to produce behavioral deficits. Thus, a promising hypothesis is that, during the aging process, a subset of Fischer 344 rats develop neural deficits that are either more widespread or more severe than those observed in other rat models of cognitive aging (e.g., Long Evans rats) and as such might provide an excellent model for investigating neural substrates related to more severe forms of age-related dementia observed in the human population.

A final point to note regarding the olfactory discrimination deficit is that although the aged worse-performers did eventually acquire each odor discrimination problem, they never improved in their ability to acquire new odor discrimination problems. These rats were impaired to the same degree on the first and second problems and continued to show impairment on the third problem given just prior to odor threshold testing. These data suggest that the impairment is enduring, and that the odor discrimination task reliably identifies a set of aged rats with cognitive deficits. Indeed, performance across the three discrimination problems was highly correlated. The reliability and endurance of this deficit could prove very useful, not only for identifying underlying neurobiological causes of age-related cognitive impairments but also for testing pharmacological agents designed to enhance cognitive function in aging. It is notoriously difficult to test the efficacy of cognitive enhancing drugs on the water maze task as even aged worse-performers improve performance with multiple exposures to this task [83]. Future studies in young and aged Fischer 344 rats

should prove informative for determining the substrate for the olfactory learning deficit observed here and contribute significantly to understanding and treatment of age-related cognitive deficits.

CHAPTER V

SUMMARY AND CONCLUSIONS

Multiple cognitive domains, including reference memory, working memory, and olfactory functioning, are affected in humans suffering from Mild Cognitive Impairment (MCI). Currently the factors that distinguish aged individuals who develop MCI and other forms of dementia from aged individuals who maintain cognitive capacities are not well-understood. Variability between rodent strains and their ability to model human deficits, along with inconsistencies in the literature within individual strains, have made it difficult to relate neurobiological changes and therapeutic interventions in animal models to cognitive changes in humans. The current study provides a carefully characterized rodent model in which young, middle-aged, and aged rats were assessed on three separate learning and memory behavioral tasks that show age-related mnemonic impairment. This model should prove useful for future studies investigating the causative neurobiological factors that contribute to age-related cognitive dysfunction.

Young, middle-aged, and aged male Fischer 344 (F344) rats were assessed in three distinct behavioral tasks: spatial reference memory (Chapter II), spatial working memory (Chapter III), and odor discrimination learning (Chapter IV). Although aged rats showed an attenuated learning curve in all behavioral tasks, all rats were able to satisfy the requirements of each of the

tasks utilized in this study. Aged worse-performing rats required additional training trials in the spatial reference memory task to achieve stable performance in which, as a group, the rats demonstrated accuracy in their search for a hidden platform. Moreover, aged rats required significantly more trials than young rats to reach criterion on odor discrimination task, and performed significantly worse than the young or middle-aged groups during the initial acquisition (the first three days with a 30 s delay) in the spatial working memory task. These data suggest that aged rats can acquire a range of mnemonic tasks, including those with a hippocampal component, when provided with sufficient training. This slower learning rate has also been reported in aged humans on an odor recall task and declarative memory tests using word recall [70,75]. Our data suggests that humans with MCI may benefit from additional exposure/training across a variety of learning domains.

The age-dependent deficits in the F344 rodent population are not a result of a global learning deficit. Although all three behavioral assessments showed an age-related cognitive decline, tasks such as digging media discriminations and cued training showed no differences in performance between age groups. This model implicates a neurobiological decline in either a common system important for olfactory learning and reference memory, or dysfunction in two systems that decline in concert. There also may be an additional, independent system involved in working memory that is vulnerable to age-related decline. Importantly, these systems are not completely compromised, particularly since

aged rats can learn each task with extended training and they learn several

tasks as well as young.

As seen in the human population, only a subset of aged F344 rats decline

in their cognitive capacities in the spatial reference memory and odor

discrimination tasks (Chapters II and IV). Some individual aged rats, even at 22

months, perform on par with young rats 6 months of age. In this population, the

within-group variability does not differ between age groups; in contrast, in the

human population there is an age-dependant increase of within-group variability

in both declarative memory and olfactory functioning [1,107]. The differences

between the human data and the current study may be the result of differences

in sample size. In the spatial reference memory task a large number of animals

was used, but the 49 aged and 29 middle-aged rats in this study is nevertheless

much less than the 200 aged and middle-aged used by Albert (1993), or the 345

humans used by Park et al. (2002), who reported significant increases in

variability among human subjects in declarative memory abilities. Additionally, in

the human population, there is an enormous number of factors that may affect

memory capacity, including diet, exercise, and disease, all of which can increase

variability within the population [65,84,105]. In the inbred F344 population, the

normal aging process is not strongly influenced by environmental factors. All rats

experience the same diet, housing conditions, and environmental stimuli with

minimal external factors that may affect development or performance. Therefore,

individual differences detected in mnemonic function in the F344 population can

be attributed largely to the normal aging process. The increased degree of environmental and genetic variability inherent to the human population likely contributes to increased within-group variability observed in the human population that is absent in the F344 model.

Impairment in working memory has been previously reported in aging in both humans and rodents [4,32,34,63,67]. However, the delay at which this impairment has been detected varies across studies. In the current study, no differences were observed between young, middle-aged, and aged groups at a 30 min delay; aged and middle-aged rats demonstrated impaired performance compared to young at 2 h and 6 h respectively (Chapter III). The working memory capabilities reported here for the middle-aged and aged groups are much greater than previously reported for F344 rats. Frick et al. (1995) reported a significant difference between young (4 mo) and aged (24 mo) performance after a 3-4 minute delay using a repeated acquisition task, in which a hidden platform was located in a novel position each session so that the second trial was a demonstration of working memory. Shukitt-Hale and colleagues (1998) observed a working memory deficit after a 10 minute delay in middle-aged (12, 15, and 18 mo) and aged (22 mo) rats in a spatial working memory task using the water maze, similar to the spatial working memory task used in this study. However, novel to the current study, all rats had three days of acquisition trials to learn the task. This additional training may allow for a more accurate assessment of working memory ability because it separates the working memory

task from learning the parameters of the task (e.g. a novel platform 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 previous reports, as rats in the aforementioned studies may not have sufficiently acquired the task rules, particularly when considering the attenuated learning curves characteristic of aged rats.

It is becoming recognized that olfactory functioning is correlated with cognitive decline in the human population, and odor identification abilities are starting to be utilized as a screening tool for early cognitive decline [24,75,119]. Thus, the finding of correlated impairments in olfactory functioning and spatial reference memory in aged F344 rats is not only novel, but is also an advantageous characteristic of this model. Using the spatial reference memory task, middle-aged rats showed a group impairment compared to young (Chapter II). However, their population was almost completely performing in the range of the young cohorts. Thus, the odor discrimination task may be able to detect impairment in middle-aged rats that are at risk of further mnemonic decline. This at-risk population can be used to develop and test pharmacological agents aimed at reversing cognitive impairment or preventing further decline.

One novel and notable feature regarding the odor discrimination test is the test-retest reliability across individual test problems. In many behavioral tasks, including water maze, rats demonstrate savings in learning that prevents the ability to retest animals in the same paradigm. After previous water maze

training, even impaired rats show preservation of learning such that the previous experience influences future training [3,22,113]. Unfortunately, this savings makes manipulation (i.e. lesion or pharmacological studies) or longitudinal studies difficult to conduct, even though within-subjects assessment allows more control of variables, such as mnemonic impairment, and is therefore more conclusive. Data from the odor discriminations showed that there is not a learning curve in this task, such that even after rats reach criterion on one odor discrimination they perform similarly on subsequent problems with novel odor pairs. The task is therefore an optimal behavioral assessment to be used in situations requiring a test-retest design.

This F344 model provides a cognitive context in which neurobiological studies can be used to delineate the causes of age-related mnemonic impairment. Dysfunction in medial temporal lobe structures has been linked to decline in spatial reference memory. However, the neural mechanisms on which odor discrimination learning and spatial working memory are dependent remain unclear. Although the odor discrimination task is correlated with the spatial reference memory task, damage to the hippocampus or parahippocampal cortices does not induce odor discrimination impairment [27]. Thus, lesion studies that damage other areas involved in olfactory function, namely the orbitofrontal cortex, may be useful in determining the neural substrate that is vulnerable to age-related decline [28]. Lesions also may be effective in elucidating the region responsible for age-related decline seen in the spatial

working memory task. This task was not correlated with the spatial reference

memory, indicating that it is mostly likely not hippocampal dependent within the

parameters used in the current study. However, as indicated recently in the

literature, prefrontal cortical regions appear to be a logical target in which lesions

and/or pharmacological manipulations could be used to distinguish brain

system(s) critical for this spatial working memory task [106]. Determining the

areas of dysfunction in the olfactory discrimination and the spatial working

memory could aid in development of future therapies to treat patients suffering

from or at risk for developing age-related mnemonic impairments.

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Selected Publication:
1. LaSarge, C.L., Montgomery, K.S., Tucker, C., Slaton, G.S., Griffith, W.H., Setlow, B., & Bizon, J. L. (in press). Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. *Neurobiology of Aging.*

Abstracts:
1. LaSarge, C.L., Montgomery, K.S., Griffith, W.H., and Bizon, J.L. (2006). Reference and working memory across the lifespan of Fischer 344 rats. *Soc. Neurosci. Abst.*
2. Montgomery, K. S., LaSarge, C. L., Slaton, G. S., Griffith, W. H., Setlow, B. & Bizon, J. L. (2006). Cross-domain cognitive impairments in Fischer 344 rats across the lifespan; improvement with donepezil. *Soc. Neurosci. Abst.*
3. Mendez, I.A., De Souza, K.A., LaSarge, C.L., Simon, N.W., Bizon, J.L. & Setlow, B. (2006). Long-term effects of cocaine exposure on spatial learning and memory. *Soc. Neurosci. Abst.*
4. LaSarge, C.L., De Souza, K. A., Tucker, C., Slaton, S., Setlow, B. & Bizon, J. L. (2006). Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. *Eighth Conference on the Neurobiology of Learning and Memory.*
5. De Souza, K., LaSarge, C. L., Tucker, C., Slaton, S., Mieckowski, A.N., Griffith, W.H., Bizon, J.L. (2006) Spatial learning impairment in middle-aged and aged fisher 344 rats. *Eighth Conference on the Neurobiology of Learning and Memory.*
6. Mendez, I.A., De Souza, K.A., LaSarge, C. L., Simon, N.W., Bizon, J.L., Setlow, B. (2006) Long-term effects of prior cocaine exposure on spatial learning. *Eighth Conference on the Neurobiology of Learning and Memory.*
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