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Hippocampal place cells as a window into cognitive aging

Doctoral Dissertation

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Abstract

As scientific and medical advances allow humans to live longer and physically healthier lives, it becomes increasingly important to maintain mental well-being in old age. Among the normal aging population, or those spared from the devastation of neurodegenerative diseases, there exists considerable variability in the cognitive ability to learn and remember new information. This study investigates the mechanisms underlying why some people suffer from age-associated memory impairments, whereas others remain as cognitively adept as young people.

This study examined the hippocampal spatial representations of a rat model of cognitive aging characterized by heterogeneous abilities on learning and memory tasks. The spatial memory capacity of young and aged rats was first characterized on the Morris water maze task. Then the firing patterns of hippocampal CA1 and CA3 pyramidal cells were recorded as the rats explored both familiar and novel environments. These "place cells" are highly active when a rat occupies particular places within an environment, and they thus serve as a window into spatial information processing.

The neurons of young and memory-intact aged rats used different spatial firing patterns to represent the familiar and the visually novel environments, and their cells rotated with rotations of the arena landmarks on every occasion. The aged memory-impaired rats, on the other hand, initially used the same place cell firing patterns to represent both the familiar and visually novel arenas, and even after new spatial representations were created, their cells failed to rotate with the landmarks on many but not all occasions, including the first rotation experiment. Furthermore, the extent of these hippocampal failures correlated with the degree of memory impairment in the rats. In subsequent analyses the place cells of the aged CA3 subregion, in particular, had higher firing rates and failed to rapidly encode changes to the external environment in comparison to young CA3 cells. CA1 place cell properties, on the other hand, were similar for aged and young rats.

In a further experiment the same rats walked between two visually identical environments, pitting self-motion cues that indicated change against visual inputs that indicated no differences between environments. Now place cells of young and aged rats were equally likely to create new spatial representations in second compartment, suggesting that the hippocampus of aged rats is able to process changes in internally-generated cues without rigidity.

These results suggest that age-related spatial memory impairments may arise from incomplete processing of external visual landmarks, coinciding with a proclivity towards self-motion cues, and a hyperactivity and a weakened encoding of novelty specific to the CA3 subregion.

National Library of Medicine Classification: WT104; WL314
Medical Subject Headings: hippocampus; aging / physiology; memory / physiology; spatial behavior; rats; maze learning; cholinergic fibers; models, animal; cognition

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Kuopio, May 2005

Iain Wilson

Abbreviations

AChE	Acetylcholinesterase
AMPA	D, L-α-amino-3-hydroxy-5-methyl-5-isoxalone proprionic acid
Ca^{2+}	Calcium ions
CA1	Cornu ammonis 1 subregion of the hippocampus
CA2	Cornu ammonis 2 subregion of the hippocampus
CA3	Cornu ammonis 3 subregion of the hippocampus
ChAT	Choline acteyltransferase
GABA	Gamma-aminobutyric acid
IgG	Immunoglobulin G
i.p.	intraperitoneal injections
i.m.	intramuscular injections
LTD	long-term (synaptic) depression
LTP	long-term (synaptic) potentiation
NMDA	n-methyl-d-aspartate, a glutamate receptor

List of original publications

This thesis is based on the following original publications, referred to by their Roman numerals in the text.

I **Wilson I.A.**, Ikonen S., McMahan R.W., Gallagher M., Eichenbaum H., Tanila H. Place cell rigidity correlates with impaired spatial learning in aged rats. Neurobiology of Aging, 24(2): 297-305, March-April 2003.

II **Wilson I.A.**, Ikonen S., Gureviciene, I., McMahan R.W., Gallagher M., Eichenbaum H., Tanila H. Cognitive Aging and the Hippocampus: How Old Rats Represent New Environemnts. Journal of Neuroscience, 24(15): 3870-3878, 14 April 2004.

III **Wilson I.A.**, Ikonen S., Gurevicius, K., McMahan R.W., Gallagher M., Eichenbaum H., Tanila H. Place cell of aged rats in two visually identical compartments. Neurobiology of Aging, 26(7): 1099-1106, July 2005.

IV **Wilson I.A.**, Ikonen S., Gallagher M., Eichenbaum H., Tanila H. Age-associated alterations of hippocampal place cells are subregion specific. Journal of Neuroscience, in press.

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"My own brain is to me the most unaccountable of machinery –
always buzzing, humming, soaring roaring diving, and then buried in mud. And why?"
– Virginia Woolf, 1932

1. Introduction

Aging brings grey hair to humans, dogs and even rats. Indeed no species escapes the grasp of time. Unfortunately, grey hair and wrinkles are not the only evidence of aging upon the head. Many aged individuals suffer cognitive impairments in addition to the physical challenges of old age. Humans today, thanks to the progress of science and medicine, can expect to live longer and physically healthier lives than ever before. As it becomes possible to live longer, it becomes increasingly important to maintain mental well-being into old age and to avoid the mud described above by Virginia Woolf.

Similar to the rest of the body, the brain experiences a general slowing with aging. Manifestations of this include slower reaction times, and more importantly for function in society, memory impairments which afflict the aged population. The most pronounced age-associated cognitive deficits are seen in difficulties in forming new memories of events. Through studies of amnesic patients and functional imaging, it is now clear that the rapid creation of memories of life's events is critically dependent upon the hippocampus. The study of anatomic and mechanistic details of age-related memory impairments is not possible with human subjects, but much insight has been gained through studies of other animals.

While much human aging research has examined memory impairments through verbal recall, language tasks are not possible in animal experiments designed to investigate the mechanisms of age-related impairments. Instead as a representative of episodic memory common to all animals, memory for places has been used by researchers to study the neurobiological basis of age-related memory impairment. Spatial learning and memory play significant roles in the daily functioning of both humans and other species of mammals, and they are easily tested in many different species.

Aged rats have proven a particularly fitting model to study the mechanisms underlying human age-associated memory impairments for three reasons. First, like

aged humans, some aged rats are impaired on spatial tasks whereas others perform normally. Second, the hippocampus region of the brain is similar in its anatomical structure and its function in memory formation in both rats and humans. Third, the hippocampus of rats, like that of humans, undergoes age-associated changes and degenerations to its circuits. The goal of this study has been to understand why the aged memory-impaired brain often fails to encode new information. To this end, we have investigated how spatial information is processed differently by the neurons of the hippocampus of young memory-intact rats, aged memory-intact rats, and aged memory-impaired rats.

2. Literature Review

Introduction to memory

One of the major advances in behavioral neuroscience over the last 40 years is the uncovering of multiple memory systems in the brain. Memory falls into many different categories: the memory for how to cross-country ski is essentially different from the memory of the moment you first skied without falling. These different aspects of memory are accomplished by different learning systems in different regions of the brain; damage to a particular region can impair certain types of memory while leaving other types intact (Cohen and Eichenbaum, 1993). Figure 1 illustrates the organization of memory systems and the brain regions involved in each one.

The simplest classification of memory separates the short-term memory from the long-term memory systems. Short-term memory, or working memory, is used for temporary maintenance and storage of information. The most widely accepted model of working memory by A. Baddeley branches it into three subsystems: the central executive, the visuospatial sketchpad, and the verbal storage system (Baddeley, 2003). Working memory is carried out by multiple brain regions in the frontal, parietal and cingulate areas, with the prefrontal cortex controlling the storage as the central executive (Baddeley, 2003).

Long-term memory refers to a more permanent storage of information and can be classified into declarative and procedural memories, based on the types of learning required by specific brain areas (Cohen and Eichenbaum, 1993; Milner et al., 1998). Declarative memory refers to facts, events or relationships which one can declare, such as what was eaten for dessert last night. Such episodes can be engrained into memory with only one exposure, largely thanks to the medial temporal lobe (including the hippocampus) (Milner et al., 1998). Declarative memory is further divided into memory for facts (semantic) and events (episodic) (Tulving and Markowitsch, 1998). Facts can be remembered independently from the situation in which they were learned, whereas episodic memories carry a specific spatio-temporal context, or information about "what", "when" and "where" personal events occurred (Clayton and Dickinson, 1998).

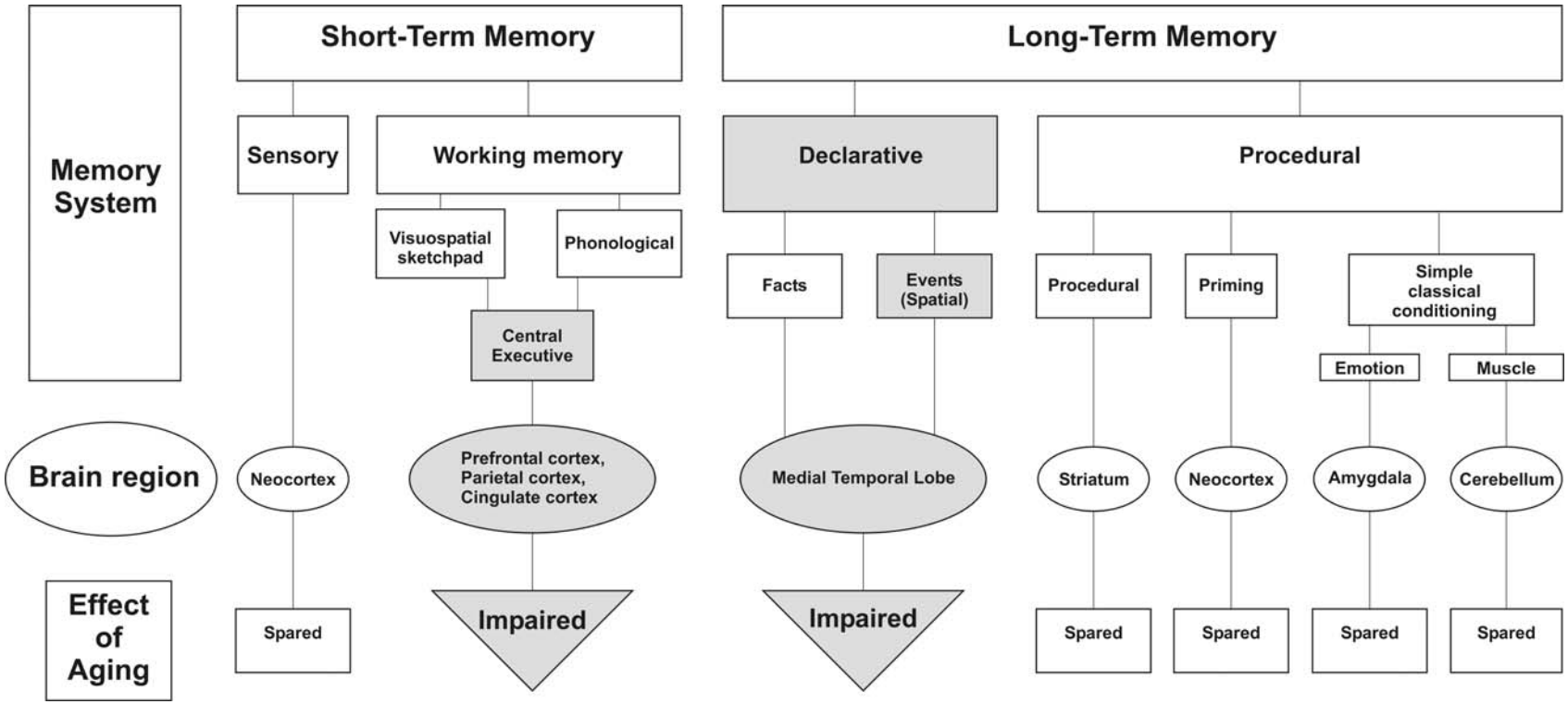

Figure 1
Memory systems and the effects of aging (adapted from Milner, Squire and Kandel, 1998).
Classifications of memories are shown in the top tier by rectangular boxes. The second tier shows the brain region responsible for each kind of memory. The bottom tier shows whether the memory system is impaired (down triangles) or spared (rectangles) during normal aging. The grey highlighting denotes age-associated memory impairments in declarative memory and the central executive of working memory.

Procedural memories are skills or patterns of behavior which are learned through practice and repetition of trials and expressed by improved performance on repeated trials, rather than conscious declaration. They are accomplished by tuning the specific circuits involved, such as those neuronal circuits involved in coordinating the cross-country skiing muscles. The tuned circuits are highly specific and inflexible for use under other situations (Cohen and Eichenbaum, 1993); knowing how to cross-country ski skating style does not mean classic style skiing comes easily. The cerebellum and the striatum are responsible for encoding complex patterns of muscle coordination or behavioral responses (Milner et al., 1998). Procedural learning also occurs with sensory perception as the ability to discriminate between sounds of a foreign language improves with increased exposure. Indeed, in priming, the simplest form of procedural memory, mere exposure to a word, for example, causes it to be processed faster by the sensory neocortices.

What are the age-associated cognitive impairments?

In normal human aging, although there is evidence for a general "cognitive slowing" throughout the brain and body (reaction times are slower, evoked potential latencies are longer) (Fleischmann, 1994; Gilmore, 1995; Kugler et al., 1996), specific memory systems are spared from functional decline, whereas other systems become noticeably impaired (see Figure 1) (Gallagher and Rapp, 1997). Procedural memory remains intact throughout the lifespan, whereas the most robust functional declines in cognitive function due to aging occur in two categories: episodic memory and working memory (Hedden and Gabrieli, 2004).

<u>Procedural and recognition memories are unimpaired</u>

Memories which are recalled unconsciously, namely the procedural memories which were learned over the course of much practice, are not forgotten and new ones can be made throughout the lifespan (Hedden and Gabrieli, 2004). Priming (Lustig and Buckner, 2004), emotional memories (Denburg et al., 2003), and skills (Grady and Craik, 2000) remain intact with aging. It is not, however, only the ability to make unconscious memories which is spared during aging; much evidence indicates that the elderly are able to accomplish the learning and recall of facts, especially when tests tax

recognition memory aspects of the declarative memory system rather than the free recall aspects (Nyberg et al., 1996; Piolino et al., 2002).

Working memory and free recall are impaired

Normal aging is associated with declines in ability on working memory tasks such as recalling word and number lists, particularly amidst interference from other non-required information (Grady and Craik, 2000; Hedden and Gabrieli, 2004). The most severe age-associated memory impairments span across both short-term and long-term memory through declines in free recall ability (Harvey and Mohs, 2001). Recent autobiographical events are remembered less by the elderly (Piolino et al., 2002), supporting the view that the declarative memory system is impaired with aging (Gallagher and Rapp, 1997). Memories of actual events, especially remote ones, are difficult to evaluate and quantify in the laboratory setting so tests of human declarative memory have been specifically designed for the controlled research setting. One of the most common requires paired-associate learning in which two random words or objects (strawberry and umbrella) are united. The aged population shows distinct declines in the ability to recall paired-associates (Gallagher and Rapp, 1997; Keefover, 1998; Rabbitt and Lowe, 2000). The impairment is particularly pronounced when a delay is introduced between learning and recall (Flicker et al., 1984; Golomb et al., 1993), suggesting the deleterious effects of interference in the aged or the insufficient storage of the information in the first place. Furthermore, not all aspects of a piece of information are encoded equally. Aged subjects are capable of recognizing a fact, but they have impaired memory of the source or context in which they learned that fact (Spencer and Raz, 1995; Trott et al., 1997; Wegesin et al., 2000). These impairments in free recall suggest that it is task demands requiring flexible access to information which are most impaired with aging.

Spatial memory is impaired

The majority of research in human declarative memory has used experiments based on language, but with verbal tests it is not possible to use other species to examine the mechanisms of age-related memory decline. Spatial memory, on the other hand, is a fundamental cross-species function and therefore represents a key means of

studying age-associated impairments through animal models. The memory for places is an ecologically important aspect of episodic declarative memory; the relationships of spatial landmarks are stored in a manner that permits flexible manipulation, retrieval and use (Cohen and Eichenbaum, 1993). In other words, with a cognitive map (based on spatial relationships) of a town, people can navigate to and from any two points (flexible) within the town (Burgess et al., 2002).

As with the impairments in the flexible use of memory for free recall, human aging is also associated with declines in the ability to remember spatial relationships among landmarks (Caplan and Lipman, 1995; Evans et al., 1984; Kirasic, 2000; Lemay and Proteau, 2003; Moffat et al., 2001; Monacelli et al., 2003; Newman and Kaszniak, 2000; Sharps and Gollin, 1987; Uttl and Graf, 1993; Wilkniss et al., 1997). Specifically in experiments, aged subjects recalled fewer locations of items after walking through a museum exhibit (Uttl and Graf, 1993). These aged subjects were able to recognize objects they had passed along the route, but were unable to recall the order of seeing the objects. Furthermore, aged subjects were impaired at drawing from memory a studied map, and then they had difficulties navigating the route memorized from that studied map (Wilkniss et al., 1997). Aged people are significantly impaired on human versions of the most widely-used rodent test of spatial learning, the water maze (see section below: The rat - *Rattus norvegicus*). The water maze requires the use of spatial landmarks to navigate in an open-field for a hidden target; for rats and mice the target is a rescue platform hidden under the water surface. In a dry land version, aged human subjects were impaired relative to young ones in identifying the target location based on the relationship of spatial cues (Newman and Kaszniak, 2000). In a virtual computer version of the water maze the young subjects focused their searches nearer to the platform than the aged subjects did. Furthermore the aged subjects successfully used objects proximal to the target but not distal spatial cues to aid navigation (Moffat and Resnick, 2002). These results all point to an age-related deficit among humans in spatial learning.

Caveats

Human aging is associated with deficits in working memory and episodic memory, which are particularly pronounced in tests requiring flexible and free recall

(such as required by spatial memory). From these results it is important to note two caveats. Firstly, impairment in recall is only the performance measure; the cause of the impairment could equally well be due to poor retrieval or to poor encoding of the information (Gallagher and Rapp, 1997). This question is best addressed by taking advantage of animal models of cognitive aging to examine specific neurobiological mechanisms.

The second caveat notes that within the aging human population considerable individual differences exist in cognitive ability. For example, Monacelli and colleagues (Monacelli et al., 2003) found an interesting continuum across ages in the tendency to get lost when tested in a familiar hospital. Almost all Alzheimer's Disease patients and 38% of normal aged subjects made errors of navigation, but other normal aged subjects, as well as all middle-aged and younger subjects had no navigation difficulties. Factors influencing the course of cognitive aging may be education, genetics, and lifestyle (Gallagher and Rapp, 1997; Nyberg et al., 1996; Rapp and Amaral, 1992; Shimamura et al., 1995). It is unclear at present why some suffer early from cognitive decline, while others remain cognitively sharp well into old age. Individual differences are therefore an important variable to keep in mind when considering animal models of aging.

Why do we need animal models of human cognitive aging?

All animals, including humans, have been exposed to many of the same environmental problems and, through a common evolution, have developed many of the same solutions to the problems. Indeed, the brain structures across the mammalian phylogenetic tree are remarkably similar; from mouse to human all possess similar anatomy from the amygdala to the visual cortex. Furthermore, aging is a prime example of the similar ecological problems which all species face. The clock ticks inside the body of every animal. Although it is apparent that the same pathological processes of human disease (such as Alzheimer's or Parkinson's Diseases) do not naturally manifest in identical ways in other species, normal aging appears to affect the behavior of many species in similar ways. Monkeys, apes, dogs, rats, and mice all show signs of age-associated memory impairments (Bach et al., 1999; Baxter, 2001; Erwin et al., 2001; Gallagher and Burwell, 1989; Head et al., 2001; Ingram, 2001).

One of the dilemmas of human aging research is the confounding effect of pathological diseases upon any subject sample. The elderly population falls into several cognitive categories, each with their own neurobiological characteristics: the population which suffers from neurodegenerative diseases (such as Alzheimer's or Parkinson's Disease), the normal aging cognitively-impaired population, and the normal aging cognitively-intact population. It is important to understand how the normal aging brain functions in order to provide effective therapy for those elderly with pathological brain states. Currently, it is difficult to identify and separate individuals in the earliest stages of Alzheimer's Disease from individuals with normal aging, even among non-demented elderly (Small et al., 2004). Thus, assessment of human normal cognitive aging is confounded by neurodegenerative diseases. Because animals, such as rats and monkeys, do not develop neurodegenerative diseases, they provide a useful means of testing the effects upon cognition of normal aging alone.

The great advantage of an animal model is that it allows us to get to the actual workings of the animal's brain, allowing study of the molecules and the neuronal networks involved in the cognitive impairments. An appropriate animal model for human cognitive aging is one that possesses age-related impairments in similar memory domains (in this case spatial learning and memory), similar basic neuroanatomy, and perhaps also individual differences within the group of aged animals.

Spatial memory impairments in aged animals

Non-human primates - M*acaca mulatta*

The rhesus monkey is a natural choice as an animal model because phylogenetically it is one of humans' closest relatives (among the animals used in laboratories). Aging monkeys are impaired on two tests of spatial memory. In the delayed response test of spatial working memory, monkeys view food being placed underneath one of two containers. As in humans, aged monkeys are impaired at choosing the correct the container when a delay is introduced between the initial setup and the response (Bachevalier et al., 1991; Rapp and Amaral, 1989; Roberts et al., 1997; Voytko, 1993). Importantly, some aged monkeys are able to solve the task as well as young monkeys, whereas other aged monkeys are spatially impaired (Baxter, 2001; Rapp and Amaral, 1991; Rapp and Amaral, 1992).

A drawback of this response task is that it cannot distinguish between whether the monkey truly remembers the spatial location in the room of the correct container (an allocentric strategy) or whether it is using an egocentric response strategy ("the container is on the left"). To test the use of allocentric versus egocentric spatial information processing, Rapp and colleagues (Rapp et al., 1997) cleverly adapted the classic radial-arm maze for rodents to primate use in a large open room. Both aged and young monkeys readily learned to collect food rewards from the end of eight arms and to visit the common center in between entries to a new arm. Interestingly, the young and aged monkeys used different strategies to locate the next arm. Aged rhesus macaques simply entered adjacent arms one after another, whereas young monkeys used a more random search. In addition, delays between the initial four and final four entries caused the aged monkeys to have difficulties in remembering which arms remained with food. Finally, a probe trial in which the spatial cues on the walls were shuffled caused poor performance in the young monkeys but had little effect upon the aged monkeys. These three results clearly suggest that the young macaques successfully used allocentric spatial information from the landmarks to navigate, whereas the aged macaques used an egocentric response strategy. This parallels the results in human studies which show that spatial navigation (episodic memory) is impaired with aging, whereas procedural memory is spared.

Despite the evolutionarily close relationship and similar behavioral impairments to aged humans, rhesus monkeys are not a common animal model of human aging. The reasons are largely practical: monkeys are expensive and difficult to keep. A monkey must reach 25 years of age before cognitive impairments become clear. This and the space required to house a monkey limit the numbers of animals possible per group and the cost-effectiveness of experimental manipulations.

The rat - *Rattus norvegicus*

The most studied animal model of cognitive aging is the rodent, specifically in this case, the rat. The rat is easier and cheaper to use experimentally than the monkey since it reaches old age at about two years. Importantly, the rat is adept at spatial tasks - at least, until it reaches old age. Furthermore, the brain anatomy of rats grossly resembles that of humans. Certainly the rat possesses a proportionately smaller

neocortex and a larger olfactory bulb, but areas such as the hippocampus and amygdala have similar connectivity across the human and rat species. Lastly, certain strains of rats have pronounced individual differences among the old aged in spatial memory (such as the Long-Evans strain used in these experiments), which enables correlations between behavior and age-associated neurobiological findings.

Behavioral studies of rats have repeatedly found that aged rats perform poorly in comparison to young rats on a wide variety of tests of spatial memory. An early behavioral study tested aged and young adult rats on the Barnes circular platform task, which takes advantage of the rat's natural desire to avoid large open areas and to escape to a single dark hole out of 18 total all located around the perimeter. Aged rats failed to learn the spatial location of this escape tunnel on the Barnes circular platform task (Barnes, 1979; Barnes et al., 1980).

Further evidence that aged rats are impaired in learning and using the spatial relationships of landmarks to navigate but quite capable of using non-spatial strategies comes from the T-maze, the eight–arm radial maze, and the water maze. Aged and young adult rats were tested on a T-maze test in which one arm (the right / east) always yielded a liquid reward and the other none (Barnes et al., 1980). Rats from both groups learned the task, but the groups used different strategies. This became evident when the start arm was moved 180 degrees, the aged rats continued to turn right (now the west arm), whereas the young adult rats chose the spatially east arm. Thus, as seen in the monkeys, the aged rats navigated with an egocentric response strategy, whereas the young rats navigated with an allocentric spatial strategy.

In a spatially-demanding version of the radial arm maze, rats initially collect rewards from four arms and then are removed from the maze for a delay before they can collect the final four rewards. Aged rats require many more trials than young rats to learn to use the distal spatial landmarks (Barnes et al., 1980; Beatty et al., 1985; De Toledo-Morrell and Morrell, 1985; Gallagher et al., 1985; Mizumori et al., 1996). In contrast, aged rats performed equally well as young rats when the reward arms are predicted by local tactile cues, even with a delay and a cue-scramble before collection of the final four arms (Barnes et al., 1987). With local tactile cues the task is no longer a spatial one, but rather an associative response task not dependent upon episodic memory.

Probably the most-widely used test of episodic memory in rodents is the Morris water maze. The water maze takes advantage of the capable but reluctant swimming abilities of the rat. The rat is placed in a pool and learns to swim to an escape platform hidden below the water's surface. In a spatial version of the task the platform is located at the same position with respect to the landmarks on the room's walls; by shifting the rat's starting location, the rat is forced to navigate to the platform by using the spatial cues. Aged rats are consistently impaired on the spatial water maze in comparison to young rats (Burwell and Gallagher, 1993; Foster et al., 1991; Gage et al., 1984; Rapp et al., 1987). In a non-spatial version of the task, a flag hangs above the platform to serve as a cue; the platform and cue shift in training while the rat's start location remains the same from trial to trial. Aged rats are quite capable of learning the cued version of the water maze (Gallagher et al., 1993; Rapp et al., 1987; Rosenzweig et al., 1997; Shen and Barnes, 1996).

Gallagher and colleagues (Gallagher et al., 1993) have found that considerable individual differences exist among aged Long-Evans rats in learning the spatial version of the water maze. Some aged rats learned the task as well as young rats, whereas other aged rats were severely impaired (Gallagher et al., 1993). This makes the Long-Evans rat strain a particularly attractive animal model of human cognitive aging because it allows the investigation of the mechanisms underlying why some aged rats, like some aged humans, are impaired on spatial tasks but unimpaired on non-spatial ones, while others perform well on both types of tasks.

The hippocampal formation is critical for declarative memory

The declarative memory impairments associated with aging are similar to those that occur in patients who have suffered brain injuries to the medial temporal lobe and in animals whose medial temporal lobe has been lesioned. The medial temporal lobe belongs to the association cortices and comprises of the hippocampus, amygdala, and surrounding cortical areas (entorhinal, perirhinal, and parahippocampal (primate) / postrhinal (rodent) cortices). The first indication that the medial temporal lobe is involved in declarative memory came from a case study of the patient H.M. H.M. suffered from severe epilepsy and underwent a surgery in which the anterior 2/3 of the hippocampus, the amygdala, and a large section of the rhinal cortex were removed

bilaterally. Indeed the operation successfully alleviated the worst of the epilepsy seizures. Unfortunately, since the surgery, H.M. has been unable to make new episodic memories including spatial ones (Scoville and Milner, 1957). Doctors who visit H.M. and leave the room for five minutes, return as complete strangers to H.M. In contrast, H.M. can learn tasks dependent upon procedural memory, although he would declare that he had never seen the task before (Milner, 1962). From this dreadful amnesia, neuroscientists were pointed towards two critical discoveries: first, there are multiple memory systems in the brain accomplished by different brain regions; and second, the medial temporal lobe and the hippocampus play a critical role in the formation of new declarative memories.

Subsequent animal experiments with monkeys given medial temporal lobe lesions have confirmed the role of this region in declarative memory (Mishkin, 1978; Zola-Morgan and Squire, 1993). The contributions of individual regions within the medial temporal lobe, however, have proved surprisingly difficult to isolate in the monkey (although see Squire and Zola-Morgan (1991)).

Instead, studies in rats have proved most revealing, and they point to the hippocampus for its role in the same kinds of memories which are impaired in aging. The other regions of the medial temporal lobe have proved critical for memory systems which are less susceptible to aging. For example, the amygdala is involved in emotional memories (LeDoux, 2000), and the perirhinal cortex is involved in recognition memory (Brown and Aggleton, 2001). The most widely-studied behavioral deficits caused by hippocampal lesions are in spatial learning. Rats without a hippocampus do not learn the spatial water maze task (Morris et al., 1982), and just as in aging rats, hippocampal-lesioned rats are impaired on spatial versions of tasks but quite capable on response-based, non-spatial tasks (Eichenbaum et al., 1990; White and McDonald, 2002). Lesions of the hippocampus do not uniquely impair spatial tasks, however. Transitive inference (Dusek and Eichenbaum, 1997), social transmission of food preference (Bunsey and Eichenbaum, 1995), and sequence learning (Fortin et al., 2002) all are impaired by lesions of the hippocampus (although see Burton et al. (2000) for conflicting social transmission results).

In sum, behavioral evidence from lesion-studies suggests that the hippocampus plays a critical role in tasks which require the flexible representation of information, i.e.

tasks of declarative memory. Age-related memory deficits occur on these hippocampal-dependent declarative memory tasks. The following sections will therefore focus on the hippocampus: its anatomy, how its neurobiology changes with aging, and how it processes spatial information.

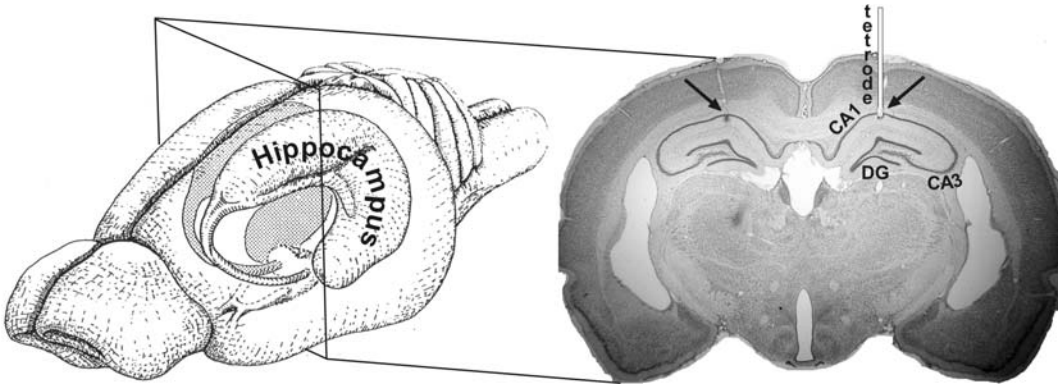

Figure 2
The hippocampus of the rat brain (adapted from Amaral and Witter, 1995).
Note the interlocking C's of the dentate gyrus (DG) and the CA1 and CA3 subregions. For electrophysiological recordings, the tetrodes (as drawn in the right hemisphere) passed through the overlying cortex to the CA1 pyramidal cell layer where recording sites are indicated with arrows.

Anatomy of the hippocampal formation

The hippocampal formation is comprised of four cytoarchitectonically distinct regions which are grouped together because of they form a predominantly unidirectional circuit of information flow. These regions are the entorhinal cortex, the dentate gyrus, the hippocampus proper (composed of the CA1, CA2 and CA3 subregions), and the subicular complex (Amaral and Witter, 1995). In this thesis I refer to the hippocampus as the dentate gyrus and CA1-3 subregions. The structure of the hippocampal formation is in principal similar in humans, monkeys and rats. The anatomical figures in this report will come from the rat to illustrate the principles used in the current study. Figure 2 shows the location of the hippocampus inside the rat brain. The hippocampus receives highly processed multi-modal information from the association cortices (Amaral and Witter, 1995). That is, inputs from all the sensory modalities, vision, hearing, touch, etc., have already converged and been preliminarily associated with one another by the time they reach the hippocampus.

The hippocampus forms a circuit which receives information from the association cortices, processes the relationships and sequences among the information, and then returns it to the association cortices. The gateway to the hippocampal circuit is the entorhinal cortex, which sends information to and receives the same, now-processed information back from the hippocampus. The three major subregions which do this processing are the dentate gyrus, the Cornu Ammonis 3 (Ammon's Horn 3 abbreviated CA3), and the Cornu Ammonis 1 (CA1). The CA2 subregion has been a matter of considerable controversy due to its less distinct anatomy (Amaral and Witter, 1995), and hence it will not be examined here. As shown in Figure 2, the three major subregions form interlocking C-shaped structures. The dentate gyrus composes one C, while the CA1 and CA3 subregions compose the other. Finally, the subiculum receives the output of the hippocampus and sends it back to the entorhinal cortex.

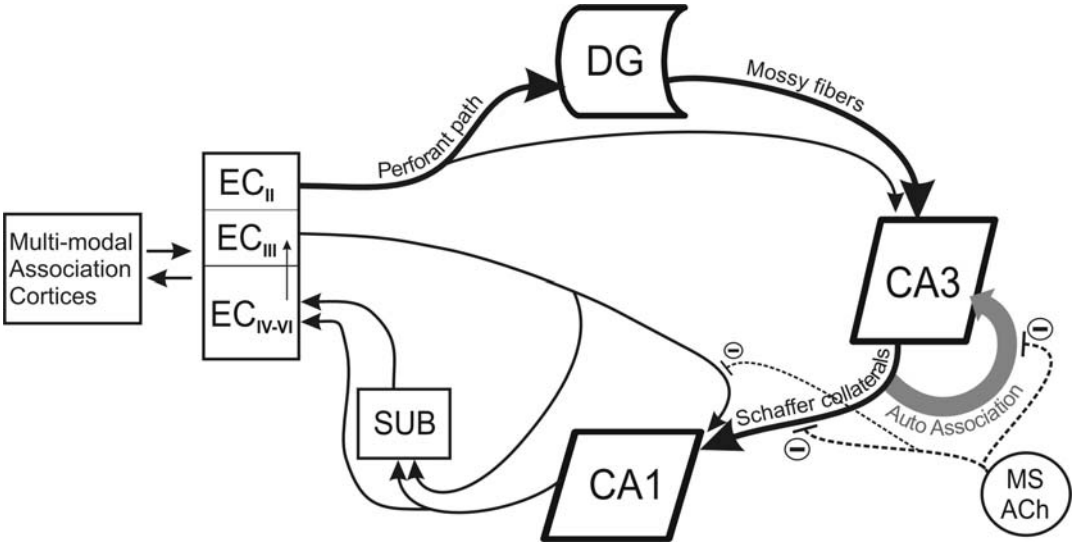

Figure 3
Information flow through the young hippocampus.
Information enters through the entorhinal cortex (EC) layers II and III in sequence to the dentate gyrus (DG), the CA3, the CA1, the subiculum (SUB) and returning to the entorhinal cortex layers IV-VI. The medial septum's (MS) cholinergic (ACh) modulation is also shown.

Information flow within the hippocampus formation is classically described as a trisynaptic circuit, signifying a cascade of processing (Amaral, 1993; Amaral and Witter, 1995), although there is also evidence of some feedback processing within the hippocampus (Penttonen et al., 1997). The information flow through the hippocampus

is depicted schematically in Figure 3, with the trisynaptic circuit shown in heavy arrows, and with an anatomical perspective in Figure 4. The first synaptic connections to enter the hippocampus arise from layer II of the entorhinal cortex, which sends highly processed sensory information through the perforant path to the dentate gyrus. These axons also branch off collaterals to the CA3 region. The second synaptic connections come from the dentate gyrus via the mossy fibers to the CA3. Thus the information from the entorhinal cortex arrives to CA3 both monosynaptically and disynaptically. This information is further processed within CA3 through auto-association fibers, which connect the CA3 pyramidal cells with one another. The third connection in the trisynaptic circuit brings the information from the CA3 cells via the Schaffer collaterals to the CA1 cells. Interestingly, CA1 also receives the information from the entorhinal cortex twice, trisynaptically from CA3 and monosynaptically through a direct connection from layer III of the entorhinal cortex (Amaral, 1993; Amaral and Witter, 1995).

CA1 projects its processed information to subiculum, where once again the entorhinal cortex (layer III) has also sent its information. Finally, the information is returned from CA1 to the entorhinal cortex (in this case the deep layers IV-VI) both monosynaptically through direct projections from CA1 and disynaptically through the subiculum. These simultaneous projections appear to be a guiding principle of the hippocampal circuit, allowing the processed information to be compared with a form of the original information at every step through parallel processing in addition to the classical serial cascade of processing (Amaral, 1993).

These synaptic connections of the hippocampus occur within a strict lamellar organization, which are worth noting in order to appreciate the fine changes in synaptic connectivity which occur with aging. Figure 5 labels the lamellae of the hippocampus, and Figure 4 places them in anatomical perspective. The lamellae are called strata and will be listed here from the apical receiving dendrites to the principal cell body layers and to the outgoing axonal fibers. The dentate gyrus is composed of three main lamellae. The stratum moleculare contains synaptic contacts between the entorhinal cortex layer II axons and the dentate dendrites (Freund and Buzsaki, 1996). Specifically the medial entorhinal cortex innervates the middle molecular dentate layer, whereas the lateral entorhinal cortex innervates the outer molecular dentate layer.

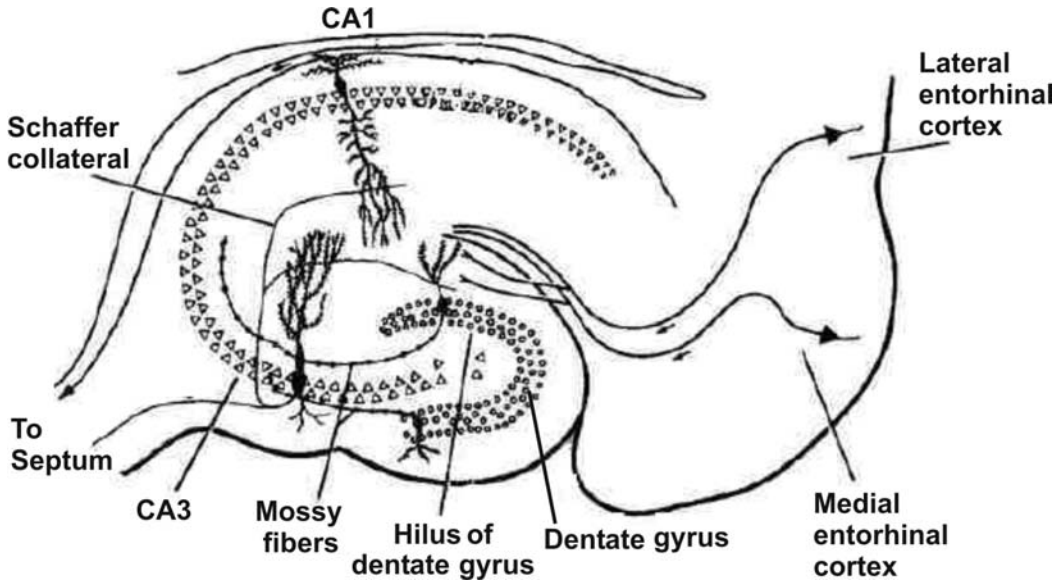

Figure 4
An anatomical perspective of the hippocampal lamellae and connectivity.
Adapted from O'Keefe and Nadel (1978).

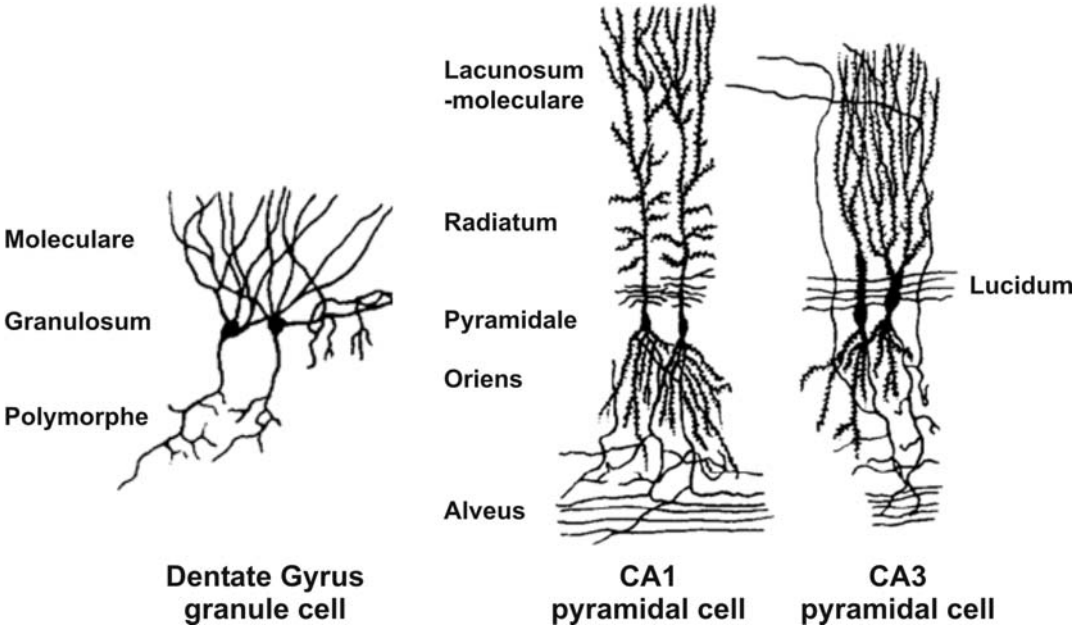

Figure 5
The hippocampal lamellae (adapted from O'Keefe and Nadel (1978).

The inner molecular dentate layer receives inhibitory feedback connection from hilar interneurons of the dentate gyrus. The stratum granulosum contains the granule cell bodies of the dentate gyrus which are small and numerous. The stratum polymorphe, located in the hilus of the dentate gyrus, contains the axonal fibers of the dentate gyrus granule cells and a variety of polymorphic interneurons which feed back to the inner molecular layer (Amaral, 1987).

The CA3 and CA1 subregions are organized in similar manners (Amaral and Witter, 1995). The stratum lacunosum-moleculare contains the entorhinal cortex inputs, from layer II for CA3 and from layer III for CA1. The stratum radiatum contains inputs from CA3 cells in both subregions. CA3 stratum radiatum is the location of CA3 auto-associative synapses, and CA1 stratum radiatum contains the synaptic inputs from the CA3 Schaffer collaterals. The dendrites of CA3 also possess an additional layer (not found in CA1) called the stratum lucidum which contains the synapses from the dentate gyrus mossy fibers. The stratum pyramidale of both CA3 and CA1 houses the excitatory pyramidal cell bodies. The stratum oriens and the stratum alveus contain the axonal fibers of each subregion, destined for innervation of CA1 and subiculum respectively.

Information processing in the hippocampus is modulated by many systems; one of particular significance in aging is cholinergic modulation which emerges from the medial septum through reciprocal pathways of the fimbria / fornix to the hippocampus. The cholinergic innervation, enacted through release of its neurotransmitter acetylcholine, has many effects upon the balance of how information is processed in the hippocampus. For the current purposes, these effects can be summarized by noting that increased cholinergic activity promotes synaptic plasticity in the hippocampus (Huerta and Lisman, 1993; Mizumori et al., 1989).

One way in which the cholinergic system may regulate synaptic plasticity of the hippocampus is through the generation of neuronal synchrony and rhythms. The medial septum has been called the pacemaker of the hippocampus because it coordinates the 4-8 hz theta rhythm through its cholinergic and GABAergic cells (Petsche et al., 1962). The cholinergic input modulates both hippocampal pyramidal cells and GABAergic interneurons (Frotscher and Leranth, 1985; Wainer et al., 1984). The inhibitory GABAergic projections of the medial septum terminate at the hippocampal GABAergic

interneurons, thus positioned to disinhibit the pyramidal cells (Freund and Antal, 1988). In this way the cholinergic and GABAergic projections from the medial septum are able to provide a synchronous orchestration of the entire hippocampal formation, known as the theta rhythm (Chrobak, 2000).

According to some theories of hippocampal learning, the theta rhythm is thought to set the stage for processing of new information in the hippocampus through regionally selective cholinergic inhibition (Buzsaki, 1989; Hasselmo and Schnell, 1994). The cholinergic system inhibits the CA3 auto-association fibers, the CA3-CA1 Schaffer collaterals, and to a lesser extent the entorhinal cortex layer III connection to CA1 (Hasselmo and Schnell, 1994). When the cholinergic inhibition is active, and therewith the theta rhythm, the hippocampus is primed to take in information from the entorhinal cortex in a controlled manner; when the cholinergic inhibition is inactive, the CA3 auto-associations generate sharp waves with strong synchronous excitatory input to the CA1 cells, possibly re-processing information within the hippocampus itself (Buzsaki, 1989; Hasselmo and Bower, 1993; Hasselmo et al., 1995).

Neurobiology of Aging

Due to behavioral impairments associated with aging on hippocampal-dependent tasks, researchers have focused on the hippocampal formation to identify neurobiological changes which would account for the behavioral deficits. The hippocampal circuit (described above), and indeed the entire brain, processes information with a delicate balance. For example, small changes in emphasis can lead to greater influence in CA3 of the direct input from the entorhinal cortex rather than the CA3 auto-association fibers (Hasselmo and Schnell, 1994), or small changes in emphasis could translate into behavioral differences, such as response-based or spatially-based navigation (White and McDonald, 2002). It is thus important to realize that even in aged animals with behavioral deficits on hippocampal-dependent tasks, the hippocampus is still active and processing information (Barnes et al., 1983; Maguire and Frith, 2003). Indeed, contrary to a common misconception, there is not widespread neuronal loss with aging (Erickson and Barnes, 2003). Instead, the memory deficits likely arise from relatively small age-related changes to the hippocampal circuit which cause shifts in how information is processed. These age-related changes to the

hippocampal neurobiology are regionally specific and include 1) loss of connectivity between the entorhinal cortex and the dentate gyrus and CA3; 2) loss of cholinergic modulation; 3) less inhibitory interneuron activity; and 4) less synaptic plasticity. The following sections detail these changes.

No neuronal loss with aging

The brains of elderly humans are smaller than those of younger counterparts (Resnick et al., 2003). The prefrontal cortex and the hippocampus experience particularly clear losses of volume measured with MRI even in cognitively normal aged subjects (Raz et al., 2004), and this rate of regional shrinkage is currently being analyzed as a possible predictor of which subjects will sink to Alzheimer's Disease and which will remain cognitively normal (Rodrigue and Raz, 2004).

Contrary to intuition, this shrinkage in normal aging does not result from a loss in the quantity of neurons. Unbiased stereological counting techniques have shown equal numbers of neurons from young and aged brains in the human neocortex (Pakkenberg and Gundersen, 1997) and in the hippocampal structures of humans (West, 1993), monkeys (Peters et al., 1996), rats (Rapp and Gallagher, 1996; Rasmussen et al., 1996), and mice (Calhoun et al., 1998). If neuronal death with aging cannot account for the decreased volume and more importantly for the memory impairments, what could? Instead of fewer neurons, it now seems likely that fewer synaptic connections between the neurons in older adults account for the results (Hedden and Gabrieli, 2004; Terry, 2000).

Reductions in entorhinal cortex connections to the hippocampus

In the aged hippocampus as a whole there are not fewer synapses. Nicolle and colleagues (Nicolle et al., 1999) crushed the entire hippocampi of behaviorally-characterized young and aged rats and then with Western blot analysis quantified the amount of three synaptic proteins. They found no differences between the groups even when the memory abilities were taken into account. On the other hand, quantification of synapses in a circuit-specific manner does reveal a loss of connectivity between the entorhinal cortex and the dentate gyrus and CA3 cells.

Geinisman and colleagues (Geinisman et al., 1992) counted the number of synapses in two molecular layers of the dorsal dentate gyrus in young and aged memory-impaired rats, and found a reduction in number of synaptic connections at the middle molecular layer, where the entorhinal cortex projections meet the dendrites of the dentate gyrus granule cells. Recently the same laboratory has counted the synapses at the CA1 stratum radiatum where the CA3 Schaffer collaterals connect with CA1 axons; here there is no loss with aging (Geinisman et al., 2004).

Smith and colleagues (Smith et al., 2000) performed an extensive and unbiased analysis of synaptophysin immunolabelling, a pre-synaptic marker, for all three areas of the trisynaptic circuit in behaviorally-characterized young and aged rats. They found no differences purely due to age in the dentate gyrus outer, middle, or inner molecular layers, in the CA3 stratum lucidum or stratum lacunosum-moleculare, or in the CA1 stratum radiatum or stratum lacunosum-moleculare. However, when the aged rats were divided between unimpaired and impaired, a single subfield stood out with about one-third fewer synaptic connections in the aged impaired rats than the aged unimpaired rats, namely the CA3 stratum lacunosum-moleculare. Moreover, a clear correlation between the individual spatial learning abilities and the amount of synapse labelling was seen, quite remarkably in all the subfields innervated by layer II of the entorhinal cortex, namely the dentate gyrus outer and middle molecular layers and the stratum lacunosum-moleculare of CA3. No other hippocampal subfields correlated with spatial learning, including the CA1 stratum lacunosum-moleculare which receives input from entorhinal cortex layer III. These findings suggest that aging results in synaptic loss to exquisitely specific circuits.

Further evidence for a loss of entorhinal inputs to the dentate gyrus comes from volumetric and electrophysiological analyses. The middle molecular layer of the dentate gyrus experiences a loss of volume with aging, whereas the inner molecular layer sees a relative increase in volume (Rapp et al., 1999). This suggests a re-organization within the aging hippocampus which emphasizes intrinsic connections while de-emphasizing entorhinal input. As would be expected with synapse loss, electrical stimulation of the perforant path elicits less of a response in aged rats than in adult rats (Barnes, 1979; Barnes and McNaughton, 1980; Foster et al., 1991). To some extent, there appears to be some compensation for the synaptic loss because the

response of dentate granule cells per remaining fiber is increased (Barnes, 1994; Rosenzweig and Barnes, 2003). That is, the remaining perforant path – dentate gyrus synapses seem to become stronger in aging. It is possible that these stronger synapses may result from a shift in the ratio of two classes of excitatory glutamate receptors within the aged dentate gyrus. The portion of excitatory electric potentials mediated by AMPA receptors, believed responsible for fast synaptic transmission in increased in the dentate gyrus of aged rats, while the portion mediated by NMDA receptors, critical for synaptic plasticity, is decreased in aged rats (Barnes et al., 1994). Thus, the aged dentate gyrus likely posesses fewer synapses and less learning capability (through NMDA-receptor mediated synaptic plasticity), but nevertheless it compensates somewhat by greater fast-synaptic transmission responses per fiber.

The story in the aged CA1 subregion is different. Firstly, in aged rats activity in CA3 consistently produces less excitation of CA1 than it does in young rats, even in the response per fiber (Rosenzweig et al., 1997; Wu et al., 2002). Secondly, the excitatory response per fiber is reduced for transmission through both AMPA receptors and NMDA receptors (Barnes et al., 1994; Barnes et al., 1997). Drawing from these data Barnes (1994) has proposed that the aged CA1 subregion contains fewer synapses. Recent evidence, however, indicates that the number of CA1 synapses is preserved with aging (Geinisman et al., 2004; Smith et al., 2000). Instead, a recent report examining the CA1 post-synaptic density with even greater specificity shows that the size of the perforated synapses is reduced with aging (Nicholson et al., 2004). Perforated synapses contain large numbers of both AMPA and NMDA receptors, and a reduction in the synapse size implies fewer excitatory glutamate receptors (Ganeshina et al., 2004). An interesting test of these ideas would be to measure the size of perforated synapses in the aged dentate gyrus because the data discussed above would predict an increase in synapse size with aging. An actual count of the numbers of excitatory receptors in a hippocampal subregion has thus far proved too daunting a task for researchers, and measurements in aged individuals of the messenger RNA level of NMDA subunits have not yielded a consistent conclusion (Adams et al., 2001; Bai et al., 2004; Clayton and Browning, 2001).

In total, the loss of synapses from the entorhinal cortex reduces the input the hippocampus receives from its primary source of multi-modal sensory information.

Furthermore, the intrinsic hippocampal synaptic connections in the dentate gyrus and CA3 are left intact or are increased in emphasis. These changes may alter the ability of the aged hippocampus to process new cortical information.

Reduced cholinergic modulation

Another circuit which is critical for the processing of new information in the hippocampus and which suffers age-related degeneration is the basal forebrain's cholinergic system. As discussed earlier, the medial septum of the basal forebrain governs the theta rhythm and therewith can set the stage for hippocampal processing of new information from the entorhinal cortex (Hasselmo, 2000). Early studies in the demented aged population noted a clear loss of cholinergic cells in Alzheimer's Disease patients in comparison to elderly controls, and this led to the influential "cholinergic hypothesis of geriatric memory dysfunction" (Bartus et al., 1982). However, early studies comparing non-demented subjects with young adults gave conflicting results (Bartus et al., 1982), and the cholinergic hypothesis has since proved an over-simplification of even Alzheimer's Disease. Recent studies, taking advantage of greater specificity in anatomical techniques and animal models which can take individual differences into account, have found that with normal aging the cholinergic system which modulates the hippocampus is indeed compromised in several different ways, including cell loss in the medial septum and a blunted response in the hippocampus to cholinergic stimulation.

It is now clear that with normal aging there is a considerable decrease in the number of cholinergic cells in the medial septum. To count a particular type of cell in the brain, one must identify the cells by attaching an immunohistochemical marker which is specific for that cell type. The two most common labels of cholinergic cells neurons are the enzymes choline acetyltransferase (ChAT) and acetylcholinesterase (AChE). ChAT is the catalyst for the creation of the neurotransmitter acetylcholine. AChE inactivates acetylcholine to remove it from the synapse. In aged rats and aged monkeys the number of neurons in the medial septum which are positive for either ChAT or AChE, and therefore termed cholinergic, is considerably less than in young controls (Fischer et al., 1989; Fischer et al., 1991; Gallagher et al., 1990; Luine and Hearns, 1990; Stroessner-Johnson et al., 1992). As Stroessner-Johnson and colleagues

(1992) pointed out, this age-related change may reflect frank cell death of the cholinergic cells, or it may reflect cells which no longer produce detectable levels of the enzymes. In either case the results clearly suggest that the functional integrity of the cholinergic system is compromised with normal aging. Furthermore, the status of the cholinergic input to the hippocampus is correlated with cognitive function. Aged animals which perform as well as young animals on tests of spatial learning have better preserved basal forebrain cholinergic systems in comparison to cognitively impaired aged animals (Fischer et al., 1989; Fischer et al., 1991; Gallagher et al., 1990; Luine and Hearns, 1990).

In the hippocampal cells which receive the cholinergic projections, there is no loss of muscarinic (acetylcholine) receptors in aged animals (Chouinard et al., 1995). However, there is evidence from both electrophysiological and chemical stimulation that the response of hippocampal neurons to the cholinergic system is blunted. Shen and Barnes (Shen and Barnes, 1996) electrically stimulated the stratum oriens of CA1 and CA3 and the granular layer of the dentate gyrus, where the incoming cholinergic fibers are dense, and they recorded the excitatory post-synaptic potential (EPSP) with intracellular electrodes in individual principal cells from all subregions. Cells of aged rats had much smaller responses to the stimulation, and this was due to compromised cholinergic transmission since the response was blunted even in the presence of glutamatergic and GABAergic antagonists. Chemical stimulation through muscarinic receptor agonists also results in a blunted response in the hippocampus of aged memory-impaired rats (Chouinard et al., 1995; Nicolle et al., 1999; Nicolle et al., 2001). The decreased response to opening of the acetylcholine receptor arises from poor coupling of the receptor to its second messenger system, the phosphoinositide signal transduction pathway. Furthermore, the blunted phophoinositide turnover is correlated with spatial learning abilities in young and aged rats (Nicolle et al., 1999).

These results from the medial septum and the hippocampus suggest that dysfunction of the cholinergic system may play a critical role in age-related memory impairments. To test this, the basal forebrain cholinergic neurons of young rats have been selectively killed with the immunotoxin 192 IgG-saporin. These lesions did not produce spatial learning deficits in young rats (Baxter et al., 1995; Baxter et al., 1996; Berger-Sweeney et al., 1994; McMahan et al., 1997), and the lesions did not cause

previously unimpaired aged rats to become cognitively impaired (Baxter and Gallagher, 1996). In sum, these studies indicate that disruption of the cholinergic modulation alone does not produce the memory impairment associated with aging. Instead it is the combination of cholinergic dysfunction and other age-related changes to the hippocampal circuitry which produces cognitive memory impairments.

Reduction in inhibitory interneuron activity

Compounding the dysfunction of the cholinergic system is a loss of modulation from the inhibitory interneurons of the hippocampus. Markers for GABA-containing cells are reduced in aging rats for a wide-variety of interneuron types and across the three hippocampal subregions (Cadacio et al., 2003; Stanley and Shetty, 2004; Vela et al., 2003), as well as the entorhinal cortex (Miettinen et al., 1993). Interestingly, as with the principal cells, the actual interneuron number is not decreased, but instead the enzymes which produce the neurotransmitter GABA are reduced (Stanley and Shetty, 2004). This could arise from the loss of synapses seen in the hippocampus of aging animals but it is not yet clear what precise roles interneurons with reduced GABA levels would play in the hippocampal circuit. It is clear, though, that significant decreases in inhibitory activity would upset the balance of information processing in the aged hippocampus.

Weakened synaptic plasticity

In order for a network of neurons to learn new material, the connections between neurons must be strengthened. These fast changes in connectivity take place at the synapses, with new spines able to grow in 30 minutes or less (Maletic-Savatic et al., 1999). In prescient theoretical work, Donald Hebb proposed that a pre-synaptic and a post-synaptic neuron which become active simultaneously would increase the strength of the synaptic connections between them and accomplish learning (Hebb, 1949). Twenty-five years later a process was discovered which seemed to reflect Hebb's ideas in an experiment. By stimulating the perforant pathway with a high frequency and strength, the neurons in the dentate gyrus exhibited a long-lasting increase of synaptic strength (Bliss and Lømo, 1973). Succeeding, weaker stimulations of the same pathway elicited strong responses even days afterwards. The activity-dependent increase in the

strength of the synaptic connections (known now as long-term potentiation) has served as an attractive model for how synapses can accomplish learning.

In aged rats long-term synaptic potentiation (LTP) has been extensively studied in the entorhinal cortex – dentate gyrus connection and in the CA3 – CA1 connection. To summarize findings across numerous stimulation paradigms, LTP in aged memory-impaired rats requires greater stimulation to be induced but it can reach the same magnitude as young rats, although it then decays faster (for excellent reviews, see (Foster, 1999; Rosenzweig and Barnes, 2003). When the stimulation is at low levels, aged rats consistently show LTP-induction deficits in CA1 (Deupree et al., 1993; Moore et al., 1993; Rosenzweig et al., 1997) and in the dentate gyrus (Barnes et al., 2000), but at more intense stimulation levels, induction of LTP is normal in aged rats (Barnes, 1979; Landfield et al., 1978). The fact that enhanced levels of synaptic plasticity can be reached in aged memory-impaired animals suggests that the fundamental mechanisms for induction remain, but the threshold for changes is shifted.

Although the LTP eventually reaches the same magnitude in aged and young rodents, the enhancement declines faster in aged rodents (Bach et al., 1999; Barnes, 1979; Barnes and McNaughton, 1985). Moreover, in these experiments the rates of decay correlated with the rates of spatial learning. The rapid decay of long-term potentiation in aged animals may relate to a reduced threshold for its counterpart, the reduction of synaptic strength called long-term depression (LTD). LTD is induced with low-frequency stimulation (in contrast to the high-frequency used for LTP), and aged rats are more prone to a weakening of synaptic strength than young animals(Foster and Norris, 1997; Norris et al., 1996). In sum, the increased threshold for synaptic enhancement and the decreased threshold for synaptic weakening provide striking parallels to behavioral learning and forgetting in aged animals.

The mechanisms underlying these changes in synaptic plasticity are once again likely due to small shifts in the balance of information processing. In this case of less synaptic plasticity in aging, the focus has been on the balance between depolarization and hyperpolarization of the cell and on the balance of calcium ions inside the cell. At the neuron's steady-state, the charged ions are balanced inside and outside the cell membrane. With inhibitory input the cell hyperpolarizes, and it becomes less likely to produce action potentials (neuronal communication through large self-propagating

depolarization). With excitatory input the cell depolarizes from its steady-state, and with enough excitation the threshold for an action potential will be reached. During action potentials calcium channels in the neuron membrane open, Ca^{2+} enters, and its depolarizing influence initiates cascades within the cell which can change the state of proteins. After the depolarization of the action potential, there is a period of hyperpolarization caused by an outflow of potassium through open potassium channels. This after-hyperpolarization (AHP) prevents further action potentials for the duration of the AHP. The potassium currents are readily modulated by a number of mechanisms; in the context of aging, it is important to note that activation of muscarinic cholinergic receptors decreases the potassium currents and therewith the after-hyperpolarization (Weiss et al., 2000).

During learning the after-hyperpolarization is reduced in CA1 and CA3, presumably allowing greater excitation and more action potentials (Moyer et al., 1996; Moyer et al., 2000). In aged rats and aged rabbits the after-hyperpolarization is in general extended (Landfield and Pitler, 1984; Moyer et al., 1992; Moyer et al., 2000; Oh et al., 1999). Furthermore, those aged rats which do learn a hippocampal-dependent task do show the reduction in after-hyperpolarization, whereas those aged rats which do not learn the task do not show any reduction (Moyer et al., 2000). Certainly one mechanism for these age-related reductions may be their blunted cholinergic system, which would reduce their ability to decrease the after-hyperpolarization. Thus, the reduced excitation and Ca^{2+} influx due to the increased after-hyperpolarization may discourage synaptic plasticity and new learning in aged memory-impaired animals. A small shift, therefore, in the balance of hyperpolarization and depolarization likely contributes to age-related memory impairments.

Summary of the Neurobiology of Aging

To summarize the neurobiological changes with aging, the key point is that it is small changes to the hippocampal system which likely lead to impaired memory. Unlike patients of Alzheimer's Disease, whose hippocampus is ravaged by neuronal death and up to 95% loss of cholinergic input (Davies and Maloney, 1976), the normal aging population experiences far more subtle deterioration. With normal aging comes a loss of connectivity from the entorhinal cortex, a decrease in cholinergic and

interneuron modulation, and reduced capabilities for synaptic plasticity. These losses are more pronounced in aged memory-impaired animals than in aged memory-intact animals. It, therefore, seems likely that the changes cause small shifts in the balance of information processing within the aged memory-impaired hippocampus which make it more difficult to learn new material.

Single-cell recordings

In order to draw a closer association between the behavioral performance and the neuronal activity of aged animals, investigations have examined the cells as they are actively processing information. By placing extracellular electrodes near to the soma of cells inside the brain, it is possible to record the action potentials of neurons as they communicate with one another through changes in electric potentials. The great advantage of this technique is that we can monitor cell activity while the animal is awake and even performing a task. By providing a window to when neurons are active, single cell recordings have provided unique insight into what information is processed within particular brain regions. In other words, by knowing when action potentials occur (to the nearest tenths of a millisecond), we can ask how this activity relates to what the animal was doing at that particular time.

In the hipppocampus of the rat (O'Keefe and Dostrovsky, 1971), the monkey (Rolls, 1999), and the human (Ekstrom et al., 2003) neurons actively process spatial information. This is most obvious from single-cell recordings of rats which are freely moving around an environment. The pyramidal cells of the CA1 and CA3 hippocampus fire action potentials when the rat occupies particular places (for review, see (Muller, 1996)). "Place cells" have high firing rates in a particular area of an environment, referred to as the place field; outside this place field the cells are nearly silent. For examples of place cells and their fields, please see Figure 14. With thousands of cells active within an environment and each cell with its own place field, these neurons could compute the rat's spatial location. Wilson and McNaughton (Wilson and McNaughton, 1993), recording simultaneously from as many as 141 pyramidal cells, were able to estimate the rat's position to 1cm accuracy even with only this number of cells.

While it is clear that these cells participate in a broad system of spatial processing within the hippocampal formation and beyond which is important for

navigation (Redish and Touretzky, 1997), it is not so clear just what the activity of place cells implies about the function of the hippocampus. The question "what is the role of the hippocampus in learning and memory" has entertained researchers for many years. In 1948, Edward Tolman published a summary of his ideas on how rats learn to navigate (Tolman, 1948). He provided evidence that the brain of rats contains a "cognitive map" of their world, which for navigation is formed by working the environment's sensory stimuli into a map-like representation. His experiments showed that rats could flexibly approach goals, taking new paths without resorting to prior routines. Tolman did not attribute the cognitive map to any particular brain region. Thirty years later John O'Keefe and Lynn Nadel applied Tolman's theory to brain physiology and asserted that place cells of the hippocampus reflect elements of the "cognitive map" (O'Keefe and Nadel, 1978). Thus, the cognitive map theory states that it is the hippocampus which allows the rat to know where it is and where it is going. According to this view, the rat hippocampus has especially evolved to process space and place cells form the brain's representation of space. This view has proven attractive over the years because it makes clear predictions of how each hippocampal cell processes its information: it uses information from all sensory modalities to create a place code and together the cells compose the cognitive map. The cognitive map theory has been well-supported by the rodent literature on place cells and experiments which show that the monkey and human hippocampi are also very much involved in spatial processing. However, it is also clear that the human hippocampus is involved in more than just space and supports episodic memory (Burgess et al., 2002).

If the human hippocampus broadly supports episodic memory, should the role of the hippocampus of animals be specific to spatial functions or can a broad theory of the hippocampus encompass both the human and animal literature? The majority of single-cell recordings of hippocampal cells have come from tasks which have some spatial components to them, often making it possible to construe the results in either a non-spatial or a spatial light. Three early place cell results illustrate this point as researchers tried to answer if place cells of the rodent hippocampus encode more than just space.

Firstly, in their groundbreaking book The Hippocampus as a Cognitive Map, that ascribed spatial processing to the hippocampus, O'Keefe and Nadel (1978) made note of "misplace" cells which are highly active only when an expected stimulus is

missing from a place. For example a misplace cell is active when the rat approaches its feeding bowl, but finds it empty rather than full of the usual food (O'Keefe, 1999). At the same location with food present, the misplace cell of the hippocampus is silent. Secondly, place cells are modulated not just by place but by the velocity of the rat. Cells fire at higher firing rates as the rat moves faster (McNaughton et al., 1983). Thirdly, changes in the environment cause drastic changes in the firing patterns of place cells, termed remapping or creating new spatial representations (Hill, 1978). This suggests the existence of multiple maps in the hippocampus (for more discussion of new spatial representations, see the subsequent section on "Place cells as a memory model"). These three characteristics of place cells can be interpreted in two ways. John O'Keefe has argued that they are all components of a broad definition of spatial processing (O'Keefe, 1993; O'Keefe, 1999): "misplace" cells do encode an event at a particular location; velocity is an essential component to how the rat moves through its spatial environment; and different spatial environments could be represented by different cognitive maps in the hippocampus. Other researchers, on the other hand, view these attributes as support for theories that the hippocampus encodes broad contexts (both spatial and non-spatial) (for example, Redish (2001)).

Further research has shown more convincing evidence that hippocampal cells encode non-spatial information also. First, changes in task within a single environment can generate new spatial representations (Markus et al., 1995). Second, hippocampal cells have elevated firing rates for particular events during a delayed non-match-to-sample task (Deadwyler and Hampson, 2004; Hampson et al., 1993; Hampson et al., 1999). Third, hippocampal cells fire preferentially for particular cues, goals, or contexts during non-spatial tasks (Eichenbaum et al., 1987; Wible et al., 1986; Wood et al., 2000; Young et al., 1994). These results provide strong evidence that even the rodent hippocampus does encode non-spatial information (although some researchers have capably argued that space is always involved (O'Keefe, 1993; O'Keefe, 1999)).

Numerous theories have attempted to encompass both spatial and non-spatial aspects of hippocampal activity. In this thesis I will examine four attractive alternative theories to the cognitive map theory. The multiple map hypotheses concluded that the hippocampus contains many "maps", in which external cues and internal cues (self-motion information) are bound together (McNaughton et al., 1996; Redish and

Touretzky, 1997; Redish, 1999; Redish, 2001). These hypotheses assert that non-spatial correlates of hippocampal activity arise from changes between different internal cognitive maps. According to their view, changes in goals or tasks require different maps. It is worth noting that Tolman's original ideas did not limit the cognitive map to spatial inputs; all aspects of cognition could be involved (Tolman, 1948). Recently, David Redish elegantly used the multiple map theory to draw together data on hippocampal function: the hippocampus encodes contexts, and it serves to bridge contextual gaps by recalling the correct contextual representation (Redish, 2001). This theory reconciles spatial and non-spatial information coding by hippocampal cells, as well as providing an explanation of why the hippocampus is required for performance across delays but not needed when no delay is forced.

The episodic memory theory states that the hippocampus is responsible for the rapid formation of memories of events, in a manner that can be flexibly accessed later (Cohen and Eichenbaum, 1993). In support of this, hippocampal place cells fire differentially on the start arm of a T-maze depending on whether the rat will turn left or right (Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000), suggesting the cells may be active based on individual episodes. According to this episodic memory view, the activity of place cells reflects representations of places where significant events occur within episodic memories (Eichenbaum et al., 1999). This theory fits well with literature from rodent, monkey, and human when place memory is considered a part of episodic memory which is encoded by the hippocampus using relationships between cues and contexts (see Cohen and Eichenbaum (1993)). It remains to be explained, however, exactly what determines how individual cells encode episodic memory (for some interesting possibilities, see Eichenbaum (2004); Huxter et al. (2003)).

The hippocampal memory indexing theory states that the hippocampus links together many different cortical associations (Teyler and DiScenna, 1986). The hippocampus thereby forms an index of which neocortical areas were active during an event. Recall of the hippocampal index leads to recall of the cortical associations also. In this hypothesis the associative memories are stored elsewhere, and the hippocampus simply allows access to the connections between them, at least until the cortices form a connection themselves over time.

The theory of conjunctive representations attributes rapid encoding of one-time experiences to the hippocampus (O'Reilly and Rudy, 2001). For any event a conjunction is formed to bind together many stimuli within the hippocampus. The neocortex, in contrast, can only learn conjunctions slowly over may trial repetitions.

The episodic memory theory, the indexing theory, and the conjunctive representation theory all assert that the hippocampus processes complex associations, of which spatial relationships are only one. Key differences between these theories lie in what kind of memory is formed and where it is stored. In the episodic memory and the indexing hypotheses, associations of memories are bound together by the hippocampus, and it is required for access to them. In episodic theory the associations are located in the hippocampus, whereas in the indexing theory the associations remain in the cortices. In the conjunctive representations theory an actual new association is formed out of the event stimuli and lies within the hippocampus.

All five of the hippocampal theories discussed here do share similarities. All agree that the hippocampus rapidly associates multi-modal complex stimuli in a manner which allows flexible retrieval and that the most prominent reflections of these memories are the hippocampal place cells.

Place cells as a memory model

Regardless of what they imply for hippocampal theory, place cells possess four characteristics which make them a useful window into how the hippocampus stores information. The first property of place cells which attracts memory researchers is that they are not simply sensory neurons. The hippocampus receives multi-modal information so it is not a surprise that place fields are controlled by contributions from all the sensory modalities. Rats navigate by vision, smell, touch, hearing, and self-motion information; all of these have influence over place cell firing patterns (Muller, 1996). The clearest of these controls are vision and self-motion, although part of this may arise from the fact that these are the easiest for the human experimenter to control and manipulate. Place cells are often recorded in an open arena or arm maze with prominent visual cues on the walls, making it easy for the experimenter to manipulate these cues. This characteristic means that researchers can study how the sensory modalities interact to control stored patterns of activity.

The second property of place cells which makes them attractive as a model of memory is their remarkable stability across time. In young adult rats place cells are active in the same location with respect to each other and to the environment both within a continuous session in an environment and when the rat re-enters the same environment after an absence (illustrated by cell Y1 of Figure 14; Barnes et al., 1987; Muller and Kubie, 1987; Thompson and Best, 1990). This appears to be a function of memory recall because different environments are represented by different sets of place cells (discussed later) and re-entering the original environment primes the retrieval of the original spatial representation. This characteristic of place cells indicates that a representation of the environment is stored in the hippocampus, and place cells allow researchers an easy access to explore the mechanisms of these memories.

The third important feature of place cells is that they are largely controlled by visual landmarks, and therefore the place cells rotate with rotations of the visual landmarks. When the rat is removed from an arena and the visual landmarks of an arena are then rotated by 90°, upon re-entry the place cells follow the landmark rotation by almost exactly 90° (illustrated by cell Y2 of Figure 14; Muller and Kubie, 1987; O'Keefe and Conway, 1978). Because tests of spatial memory, such as the water maze, rely upon use of the visual landmarks, the fact that place cells rotate with visual cues strengthens the link between hippocampal cells and spatial navigation. The rotations provide a simple way to test how well the cues of a particular environment control the hippocampal spatial representations.

The fourth attraction of place cells for memory researchers is the creation of new spatial representations in new environments. When a rat enters a visually new arena from familiar one, the place cells drastically alter their place field firing patterns, quickly forming completely new firing patterns in relation of the cells to each other and to the visual landmarks (Frank et al., 2004; Hill, 1978; Wilson and McNaughton, 1993). For the two environments two different sets of hippocampal neurons are active. Thus, a given place cell may be silent in one environment and active in the other (as shown by cell Y1 of Figure 14). Alternatively, the cell may use two different place field locations to represent the two environments. Each environment recalls into activity the set of neurons bound to it, much resembling a stored memory. The learning of a new environment and creation of new spatial representation is not always rapid. When two

environments are similar, place cells may initially use the same representation but with additional exposure may slowly (over as many as twenty days) develop distinct representations for each (Lever et al., 2002). Place cells, therefore, provide researchers the perfect opportunity to study the storage of a memory from its creation in the hippocampus.

Cues which control place cells

As mentioned earlier, place cells are controlled by many different kinds of cues. For example, the hippocampus may create new spatial representations when the visual information has remained the same but other information indicates change. Markus and colleagues (Markus et al., 1995) found that the hippocampus used two different spatial representations for two different tasks within a single environment. Furthermore, rats which walk between two visually identical arenas can develop two different spatial representations for the two arenas since the self-motion information indicates change (Skaggs and McNaughton, 1998; Tanila, 1999). Self-motion refers to the ability of animals to keep track of how far and in what direction they have walked. Rats are adept at using this idiothetic information to navigate (Gallistel, 1990; Redish, 1999; Alyan and McNaughton, 1999; Mittelstaedt and Mittelstaedt, 1980), and place cells can be maintained by idiothetic information with considerable accuracy in the dark (Quirk et al., 1990), in blind rats (Save et al., 1998), when the visual landmarks are removed (Muller and Kubie, 1987), and when self-motion is simply the most salient cue (Gothard et al., 1996; Skaggs and McNaughton, 1998; Tanila, 1999)).

Several theories hold that an important role of the hippocampus is to bind these different sources of information with each other, idiothetic cues, external environment cues, as well as contextual information (McNaughton et al., 1996; Redish, 2001; Redish, 1999; Touretzky and Redish, 1996). These theories are supported by experiments which show when the conflict between sources of information is too great, the place cells form new representations. Small rotations (45°) of the landmarks seen by the rat result in similar rotations of the place fields, whereas large rotations (180°) of the landmarks result in new spatial representations (Knierim et al., 1998), as if the large conflict could only be resolved by binding the relationships anew. Additionally, changes in context, such as task, result in changed spatial representations despite

unchanged visual landmarks (Markus et al., 1995). New spatial representations may be created when a certain threshold of changes has been reached, and the impetus for new place fields may come from above-threshold changes in any of the sources of information. The binding of the pieces of information to the spatial representation likely occurs through synaptic plasticity in the hippocampus (McNaughton et al., 1996; Redish and Touretzky, 1997), and thus place cells provide attractive possibilities to study the cellular mechanisms of memory (Milner et al., 1998).

Place cells and spatial learning

Place cells appear to be a prominent reflection of hippocampal spatial processing, and it is important to address what is the functional significance of place cells in the performance of spatial tasks. Several studies indicate that the relationship between place cell firing and the spatial location of a goal is critical for finding that goal. O'Keefe and Speakman (O'Keefe and Speakman, 1987) recorded cells as the rat collected a reward at the end of one arm in a four-armed plus maze. On some trials the visual cues were removed, and performance deteriorated. Interestingly, the arm which the rats selected in error as the goal could be predicted by the pattern of place fields. The spatial representation remained in register with the anticipated goal arm, suggesting this relationship is important for navigation.

Subsequent studies have established that a disturbance in the relationship between the place cells and spatial location of a goal causes a deterioration of performance in finding that goal. In a study by Lenck-Santini and colleagues (Lenck-Santini et al., 2001), rats performed a continuous spatial alternation task in a 3-armed Y-maze. Through a series of rotations, some place fields became out-of-register with the goal arm, and for these rats, performance on the task was poor. Place fields of other rats maintained their correct relationship with the goal arm, and for these rats, performance on the task remained accurate. Lenck-Santini and colleagues (Lenck-Santini et al., 2002) tightened the link between place fields and navigation goals by recording from place cells while the rats performed one of three goal-directed tasks. The rats learned to find a goal area by spatial relationships, by a wall cue, and by a floor cue. Then a visible rotation of the landmarks caused the place fields to become out-of-register with the goal location, because the conflict between self-motion and landmarks meant that a

majority of the place fields did not rotate while the goal did. Performance on the two cue-guided tasks was unaffected by the inconsistent relationship between goal and spatial representation. Performance on the spatial navigation task, on the other hand, was severely disrupted by a disruption in the relationship between goal and spatial representation.

Recently, Rosenzweig and colleagues (Rosenzweig et al., 2003) took advantage of the age-related impairments in spatial memory to strengthen this connection between performance on a spatial task and place field adjustments. They tested this relationship in a cleverly designed experiment which manipulated the relationship between external cues and self-motion cues. The goal location on a linear track remained constant with respect to the external visual cues, but the start box was shifted for each trial causing the walking distance to the goal location to vary. On each trial place fields were initially determined by the self-motion cues, and successful performance entailed a switch in the control of place fields from self-motion cues to external landmark cues well before the goal area was reached (Gothard et al., 1996). In a collection of both young and aged rats the learning of a goal location was correlated with how readily the place fields of the rats switched into control by spatial cues. Taken together, the three results of Lenck-Santini and colleagues (2001 and 2002) and Rosenzweig and colleagues (2003) strongly suggest that a consistent place field-goal relationship is essential for finding goals which require spatial navigation.

How is it then that the hippocampal cells participate in spatial navigation? Almost since their discovery, place cells have provided an attractive theoretical contribution to theories on how spatial navigation occurs (O'Keefe and Nadel, 1978). Place cells provide a representation of the current environment and help to relate the current spatial view with past views and with the internal representation of a goal. Recently, experimental support for the role of place cells has gotten stronger. Early evidence suggested that the reward locations may have a particularly high density of place fields (Eichenbaum et al., 1987; Kobayashi et al., 1997), but it was not clear whether the firing was due to sensory associations or emotional guidance. To resolve this, Hollup and colleagues (Hollup et al., 2001) cleverly adapted the water maze to place cell recordings by making it an annulus (a ring) with a removable rescue platform. This enabled place cells to be sampled over the entire arena and to be recorded also in

the absence of the goal's sensory cues. In this setup the place fields over-accumulated at the goal platform area. Twice as many cells had peak firing rates around the goal (even in its absence) as over the rest of the water maze. This suggests that some hippocampal neurons may contribute to spatial navigation by signaling regions of particular behavioral significance.

Another way that place cells may participate in navigation and memory is by signaling particular sequences of events. Wood and colleagues (Wood et al., 2000) and Frank and colleagues (Frank et al., 2000) showed that place cells could be differentially activated on the start of a T-maze depending on whether a right or left turn had been or would be made. In a comprehensive work Ferbinteanu and Shapiro (Ferbinteanu and Shapiro, 2003) advanced these findings by recording cells as the rats performed a hippocampal-dependent T-maze task. They found three classes of place cells: 1) some showed retrospective memory encoding, that is the location-specific firing depended on where the rats had come from; 2) some showed prospective memory coding, that is the location-specific firing depended on where the rats were going to; and 3) some cells fired only for the spatial location regardless of history or future. Interestingly, when the rats made a mistake behaviorally, the journey-dependent firing of cells was decreased, particularly for future encoding, which suggests that disrupted coding correlates with memory performance. The hippocampal neurons, thus encoded information about the recent past, the present, and the imminent future, consistent with a possible mechanism for episodic memory.

Aging place cells

CA1 and CA3 pyramidal cells reflect how spatial information is processed and stored in the hippocampus. Because place cells are not simply sensory neurons, because place cells are stable over time and repetition, because place cells clearly use the visual landmarks, and because place cells form new spatial representations of new environments, they provide an intriguing window into the formation of memories by the brain. Furthermore, consistent spatial representations are necessary for correct performance on spatial tasks and serve as a likely step in the mechanisms of spatial navigation and episodic memory. Because these are precisely the behavioral impairments from which some aged individuals suffer, researchers have turned to place

cells in order to tighten the connection between the behavioral deficits of aged animals and the neurobiological deteriorations in the hippocampus region. Hence, comparing the information processing of hippocampal place cells of young and aged rats represents a powerful means of uncovering what goes wrong in the hippocampus of aged memory-impaired individuals.

Consistent with the relatively small neurobiological changes to the hippocampus of aged individuals, the basic properties of place cells do not differ between aged rats and young rats (Barnes et al., 1983; Markus et al., 1994; Mizumori et al., 1996; Mizumori and Kalyani, 1997; Shen et al., 1997). Most importantly, the hippocampal cells of aged rats have place fields which are equally crisp as those of young rats (for examples, see Figure 14). The average and peak firing rates, the inter-spike intervals, the spike amplitude, and the spike width are all similar in young and aged rats. On the other hand, the results are inconsistent on whether the spatial selectivity of place cells from aged animals is decreased (Barnes et al., 1983; Mizumori et al., 1996; Tanila et al., 1997a; Tanila et al., 1997b), similar (Barnes et al., 1997; Markus et al., 1994; Mizumori et al., 1996; Shen et al., 1997; Tanila et al., 1997a), or increased (Mizumori et al., 1996; Shen et al., 1997) in comparison to those of young animals (for a detailed discussion see Barnes (1998)). These differences are likely due to differences in tasks between the experiments and to differences in how spatial selectivity is calculated. Suffice it to say, that in general the place cells of aged and young rats have equally robust firing properties and fields.

Differences between the spatial representations of young and aged rats are brought out when the hippocampus is challenged to encode changes in the environment. In an elegantly simple experiment, Barnes and colleagues (Barnes et al., 1997) recorded place cells in a familiar environment, and then took the rats on a tour of several new environments. When the rats were placed back into the familiar environment, the authors performed a correlation analysis between the place fields used initially in the familiar environment and those used after the new exposures. The young rats used the same place fields to represent both sessions in the familiar environment. The aged rats, on the other hand, recalled the former place fields correctly only about 70% of the time. On thirty percent of the occasions the aged rats used a completely novel arrangement of place fields to represent the familiar environment. As a control, the authors compared

the place cell activity within uninterrupted sessions; in this case the aged rats, as well as the young, maintained the same spatial representations throughout the sessions. These results suggest that the aged rats had no difficulties maintaining a consistent representation during uninterrupted exposure to an environment, but that new experiences could interfere with successful recall of even a highly familiar environment. The aged rats, thus, had multi-stable representations.

To test how this place cell encoding deficit related to spatial navigation abilities, the authors (Barnes et al., 1997) compared water maze data from ninety-eight young and ninety-three aged rats (these rats were different individuals than those recorded). After four days of training the young rats quickly found the hidden platform almost every time, whereas the aged rats sometimes found it and sometimes had much longer search paths. The variability of the aged rats was not due to between-rat differences, but rather each aged rat had good performances and bad performances. Together these data suggest that the aged rats sometimes got lost; that is, their place cells failed to recall the correct spatial representation, and this caused them to search incorrectly for the water maze platform.

In the same year a second set of experiments on the place cells of aged rats found results seemingly contradictory to those of Barnes and colleagues. Tanila and colleagues (Tanila et al., 1997a; Tanila et al., 1997b) recorded place cells as rats collected rewards at the ends of a four-armed plus maze. The rats were pre-tested on the spatial water maze, and accordingly grouped into young memory-intact rats, aged memory-intact rats, and aged memory-impaired rats. The maze environment was rich with sensory cues: distal cues included visual landmarks on the walls, and local cues unique for each arm included assorted textures and assorted smells. After the place cell firing patterns were established in this familiar environment, the rat was removed and the spatial arrangement of these cues was manipulated, either by changing the relationships of the existing visual and floor cues or by creating a novel environment with novel cues.

Under both manipulations the place cells of young rats and aged memory-intact rats formed new spatial representations, either with repeated exposures to the altered cues or rapidly upon exposure to the novel cues. In contrast, the place cells of aged memory-impaired rats did not develop new spatial representations; instead the place

cells retained their original shape and location with respect to the maze center often following the rotation of a particular cue to another arm. These data suggest that the hippocampus of aged memory-impaired rats incorporates a limited scope of environmental information into their spatial representations and incorporates environmental changes with less plasticity of their spatial representations. Recently, Oler and Markus (Oler and Markus, 2000) showed that the rigidity of place fields in aged rats extends to changes in task demands which are sufficient to induce changes in place fields of middle-aged rats, even within the same environment. Taken together, these results suggest that the spatial memory impairment of aged rats may arise from hippocampal place cells less able to encode new information by creation of appropriate new spatial representations.

Further evidence that aged place cells are less capable of change in place fields comes from a study of place field size on a linear track. When young rats run repeated laps on a circular track, the place fields expand backwards (Mehta et al 1995). In contrast, the place fields of aged rats do not increase their size (Shen and Barnes 1996). According to theoretical models of synaptic plasticity which had predicted the backwards place field expansion even prior to the experiments (Blum and Abbot 1996), if two cells are active repeatedly in the same sequence, then the first cell will begin to predict and encourage the firing of the second cell. As the synaptic connections between the first and second place cells get stronger and stronger, the second place cell begins to fire earlier and earlier on the circular track. The fact that the place cells of aged rats do not undergo this expansion provides evidence that the spatial representations of aged rats are affected by weakened synaptic plasticity and therefore are less likely to change.

As discussed earlier (in the section on Place cells and spatial learning), Rosenzweig and colleagues (2003) evaluated the place cells of aged rats as the animals were performing a navigation task. They found that the aged rats were impaired at finding a spatial goal, and the place cells of aged rats failed to realign as readily as young rats to the spatial cues and away from the self-motion cues. This strongly suggests that the delays of the place fields of aged rats in adjusting to external landmark control contribute to spatial learning deficits.

Concluding remarks to the introduction

This literature review has strived to illustrate the contribution hippocampal place cells can make to the understanding of the aging brain. Many aged individuals are impaired on hippocampal-dependent spatial tasks. The neurobiology of the aging hippocampus indicates that it is relatively small but specific deteriorations which compound each other to render the aged individual memory-impaired. The connection between the behavioral impairments and the neurobiological changes has been drawn closer by recent place cell studies of information processing in the awake behaving rat. These studies suggested that alterations in place cell properties, from multi-stability to rigidity, may underlie the impairment in spatial learning associated with aging. These studies, on the other hand, raise the question how the spatial representations of aged rats can be both unstable and overly stable. Furthermore, the study by Rosenzweig and colleagues (2003) makes it clear that individual differences can play an important role in the behaviors and spatial representations of aged rats. The current studies will therefore examine place cell encoding of rats individually characterized on the most common test of spatial learning ability, in contrast to many past studies which have only looked at the aged rats as groups classified by behavior. The current set of experiments aimed to clarify the conflicting reports of unstable and inflexible aged hippocampal representations by testing the hypothesis that the individual spatial learning abilities of aged rats could arise from the abilities of their hippocampi to encode spatial information.

3. Aims of these Studies

The aim of this thesis was to investigate the mechanisms in the hippocampus behind memory impairment of aged individuals. Young and aged rats were first tested for their spatial learning abilities, and then the activity of single neurons (place cells) of these rats was used as a window into the information processing system of the hippocampus.

These studies evaluate how individual spatial learning abilities of young and aged rats may arise from the abilities of hippocampal pyramidal cells to encode spatial information. In these experiments the hippocampal place cells of these rats were challenged to process changes in their spatial context. Specifically, the studies addressed the following questions:

1. Does age alter the basic firing characteristics of hippocampal place cells (I, II, and IV)?

2. Does age compromise the ability of the hippocampus to process changes in the external environment (I and II)?

3. Does age compromise the ability of the hippocampus to encode new visual landmarks even after multiple exposures (II)?

4. Is the failure to encode new external environments specific to one of the hippocampal subregions (IV)?

5. Does age compromise the ability of the hippocampus to process changes indicated by self-motion information when the visual landmarks remain the same (III)?

6. Of these properties of place cells mentioned above, which ones relate best to age-associated memory impairments (I, II, III, and IV)?

4. Materials and Methods

Subjects

The subjects were young (6-8 months old) and aged (24-28 months old) male Long-Evans rats. All of the studies used the same core of 6 young and 9 aged rats which were used in Study I; studies II-IV used a few additional rats to the core of Study I. Study II used an additional 2 young and 2 aged rats (total 8 young rats and 11 aged rats). Study III included 5 young rats matched with 10 aged rats (the same as those in Study II) and an additional 6 young cholinergic-lesioned rats matched with 6 young control rats. Study IV included 18 young and 17 aged rats, bringing together recordings from the same series of Kuopio rats and recordings from the State University of New York at Stony Brook, USA.

Electrode implantation and recordings were done in Kuopio, Finland, with the exception of 4 young and 5 aged rats of Study IV which were recorded in Stony Brook, New York, USA. All experiments were conducted in accordance with the guidelines of the Council of Europe and the U.S. National Institutes of Health.

Behavioral pre-Screening

To determine the spatial learning abilities of each, the young and aged rats were pre-screened on the spatial water maze task at Johns Hopkins University before they were shipped to Kuopio, Finland for the electrophysiological experiments. Those young rats which received the cholinergic-lesion and their controls (part of Study III) were not tested on the water maze since it has been shown that medial septum cholinergic lesions do not impair spatial water maze learning (Baxter et al., 1995; Berger-Sweeney et al., 1994).

Prior to electrophysiological experiments, the rats were trained on a spatial navigation task in the Morris water maze using training trials to assess acquisition and probe trials to assess search strategy in locating a submerged escape platform. Rats received three trials per day for eight consecutive days using a 60 s inter-trial interval. The location of the platform remained constant in one quadrant of the maze, and the starting position for each trial was varied among four equally spaced positions around the perimeter of the maze.

Every sixth trial was a probe trial during which the platform was retracted and unavailable for escape for the first 30 s of the trial; after this time it was raised and made available for escape. The probe trials assessed if there was development of a spatial bias in searching for the escape platform. The primary measure, referred to as the learning index, is derived from the probe trials that were interpolated after each set of five training trials. The learning index is computed as the average proximity of the rat (in cm) to the target platform location on probe trials 2-4; thus low index values represent more accurate search patterns acquired more rapidly during learning, while high index values indicate poor performance (Gallagher et al., 1993).

In order to test visual acuity and swimming ability independent of the ability to process spatial information, each rat was given six "cued" training trials on the day after completion of the training trials. During these trials the submerged platform was replaced with a visible platform at 2 cm above the surface of the water, and the location of the escape platform was varied randomly among the quadrants of the pool from trial to trial. Each rat was allowed 30 s to reach the platform, and was allowed to remain there briefly before being returned to a holding cage for 5 s before the next trial.

Surgeries

Lesions

In Study III, 12 young rats underwent immunotoxic cholinergic lesions (or sham lesions) of the medial septum at Johns Hopkins University prior to electrophyisological experiments. Under Nembutal anesthesia (50mg/kg), 192 IgG saporin (0.5μg/μl, Chemican, Temecula, CA) or phosphate-buffered saline was injected at two depths bilaterally at AP = +0.45 mm and ML = +0.6 mm referenced to Bregma. A total volume of 0.3 μl was infused to the sites at DV = -7.8 mm, and a total volume of 0.2 μl was infused into the sites at DV = -6.2 mm.

Implantation of Electrodes

Under general anesthesia (pentobarbital and chloral hydrate each 40 mg/kg i.p., supplemented with ketamine 20 mg/kg i.m.) each rat was implanted with two or four movable tetrodes (a bundle of four twisted 30 μm Nichrome wires containing 10% iron) aimed at CA1 of each hemisphere (anteroposterior –3.3 mm, mediolateral ±1.8 mm,

dorsoventral –2.2 mm from the dura). Each tetrode was attached to a custom-built microdrive which allowed controlled advances of the tetrodes toward the hippocampus once the rat had recovered from surgery. In addition, bipolar stimulation electrodes were implanted in the lateral hypothalamus to deliver rewarding brain stimulation (AP -0.5 mm, ML ±1.6 mm, DV -7.8 mm from the dura). Rats were given one week of monitored rest to recover from the surgery.

Behavioral training and Screening for cells

After the rats were fully recovered from the implantation surgery (one week in the home cage), they were brought into the electrophysiology recording room and the cable was attached which brought the electrodes' signal from the rat's head via pre-amplifier to the amplifiers, oscilloscope, and computer. To monitor the neural activity, it was amplified 5000-10 000 times, bandpass filtered (0.3 – 5 kHz), and digitized at 25 kHz using Enhanced Discovery software (DataWave, Longmont, Colorado, USA). The position of the rat, which had two small light bulbs mounted on the headstage, was tracked through a video camera on the ceiling above the arena and digitized at 60 Hz.

The rat was placed into a brown pasteboard cylinder (diameter 70 cm, height 50 cm) with three distinct two dimensional patterns attached to the cylinder wall serving as landmark cues (see Figure 7, left column, first three rows). This arena and all other experimental arenas were frames placed on a black plastic table (diameter 1.1 m) that was cleaned thoroughly between trials to minimize olfactory cues. The table and frames were surrounded by a floor-to-ceiling circle of black curtain (diameter 2.2 m); the rat remained inside this curtained area for the entirety of each training and recording session. The arena itself was lit by four incandescent bulbs arranged symmetrically 1.5 m above the center of the arena floor. The room was almost soundproof with the ventilation creating a diffuse background white-noise. These factors all served to limit the orienting cues outside the arena frame.

Within the cylindrical environment the animals were trained to search for randomly distributed loci where rewarding brain stimulation leading to the release of dopamine within the lateral hypothalamus was delivered. This stimulation, thus, encouraged continuous exploration without causing any area to be more rewarded than others. For further details of the stimulation, see Hetherington and Shapiro (1997).

Once learned, the stimulus current was adjusted to the minimum level which kept the rat constantly moving and assured equal exploration of the entire arena. For rest, the rat was placed in a bucket (32 cm diameter and 33 cm height) next to or above the arena. The rats became highly familiar with the cylindrical environment during the 2-3 weeks of daily screening for cells.

The electrodes were advanced slowly (maximum 80 μm per day) to the CA1 hippocampus. The hippocampus was identified by four characteristic features: sharp wave-associated rhythmic 200 Hz "ripples" during rest, theta wave rhythms during exploration, and single unit pyramidal cell activity which showed complex spike bursts and duration of the negative spike >300 μsec, and location-specific activity. Once action potentials were found from hippocampal cells with clear place fields and clear amplitude differences between the four tetrode channels (indicating good isolation), the electrodes were not advanced any further and recordings could be made. Experiments were done each day while the cells were well-isolated. When isolation was lost before all experiments were complete, the electrodes were advanced until new cells could be found (perhaps advancing all the way to CA3 hippocampus).

Experimental procedures

Study I

Study I challenged the place cells of young and aged rats to process an alteration of the external environment. During the recording sessions, the familiar cylindrical environment (Fam) was alternated with a modified, square arena (Sq) composed of flat brown walls (63 cm x 63 cm) and the identical three landmarks transferred from the cylinder (see Figure 14, top row). These cues appeared rotated 90° with respect to the experimental room, in order to test the importance of the landmarks in determining the location of place fields. Each recording session consisted of five 7-min trials separated by 5-min pauses during which the rat was placed in a bucket (diameter 32 cm, height 33 cm) suspended above the arena while environment manipulations were made and floor was cleaned. The bucket was spun to mildly disorient the animals prior to reentry. Each session followed the sequence Fam1-Sq2-Fam2-Sq2-Fam3 (see Figure 14, top row). All but one rat underwent two such recording sessions separated by 3-4 days. This second session allowed us to sample an additional ensemble of cells from each rat.

Study II

Study II challenged the place cells of young and aged rats to process a completely novel external environment. It was composed of two experiments which involved a familiar and a new environment. During each recording session, the rats were initially exposed to the familiar cylindrical environment (Fam), then they were introduced to a novel environment (New) for multiple trials, and then returned to the familiar environment in a final trial (see Figure 15, top row). The novel environment was formed by black plastic walls composed into a hexagon (diameter 80 cm, height 40 cm), and a novel and distinct landmark cue was positioned on each of three walls. In Experiment 1 (young rats = 6, aged rats = 9), each initial recording session consisted of five 7-min trials (Fam1-New1-New2-New3-Fam2) separated by 5-min pauses during which the rat was placed in the bucket suspended above the arena while environment manipulations were made (see Figure 15 top row). The bucket was spun to mildly disorient the animals prior to reentry with the exception of between trial New1 and trial New2. The rats underwent two recording sessions separated by 3-4 days. The second session allowed us to test for delayed acquisition of spatial representations for the novel environment. In addition, in one of the exposures to the novel environment we tested whether the spatial representations were anchored to the landmark cues by rotating those cues and the arena frame by 90°. Hence, session 2 followed the sequence Fam1-New1-New2-New3R-Fam2 (see Figure 16, top row).

In Experiment 2, we recorded place cells from an additional two aged memory-impaired (28 months old) and two young rats (8 months old) given repeated exposures to the novel environment. Each rat explored the same familiar and novel arenas for as many as eight consecutive daily sessions with the sequence Fam1-New1-New2R-New3-Fam2 (see Figure 17, top row). Trial New2R was a 90° rotation of the entire arena including landmarks. For counterbalancing, the direction of rotation alternated each session, either 90° clockwise or anti-clockwise.

Study III

Study III challenged the place cells of young and aged rats to process a change indicated by changes in the self-motion information. Study III set visual and self-

motion inputs in conflict by recording place cells as the rat walked between two visually-identical compartments. The recording environment consisted of two rectangular compartments, each with three identical cue cards on the walls (see Figure 6, length 41cm, width 34 cm, and height 32 cm). The compartments were joined by a common wall that contained a hidden double-leaf door that could swing to open a 10-cm-wide walkway. The compartments are hereafter referred to as Box A and Box B. Special care was taken to make the compartments as visually identical as possible. Whenever the rat was lifted into one of the compartments, the other was covered with a cardboard sheet to prevent the rat from seeing the entire layout.

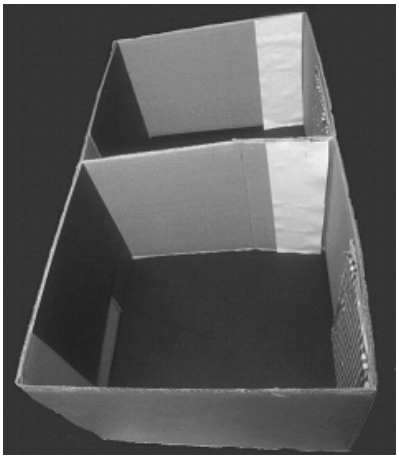

Figure 6
The two visually identical boxes of Study III. Notice the hidden double-leaf door on the right side of the dividing wall.

The place cell recording protocol followed the sequence of trials A1 – B2 – B3 – A4 (see Figure 21, top row). All recording trials lasted for 5 min. Initially the rat was placed in Box A and allowed 15-25 min to familiarize itself with the novel environment. Place cells were recorded for the last 5 min of this exploration period. The experimenter then quietly opened the hidden door by swinging open the two overlapping leaves. This allowed the rat to walk into Box B. Once the rat had entered, the door was closed and the trial B2 began. After 5 min, the rat was lifted out and placed into a bucket (diameter 32 cm, height 33 cm) suspended above the compartments. There the animal rested for 5 min while the compartment floors were cleaned. The bucket was not spun in order to avoid disorienting the rat. The rat was lifted back into compartment B for trial B3 and then, after the 5 min recording trial, the door was again opened and the rat walked back into Box A for trial A4.

Study IV

Study IV re-analyzed and amalgamated recordings from several earlier studies to provide sufficient data for comparison of the activity of young and aged cells from CA1 and CA3 hippcampus during exploration of familiar and novel environments. Data were taken from Studies I-III of consecutive recordings in the initial familiar cylinder environment and a second novel environment, either the altered square arena, the novel hexagon arena, or Box A of the two identical boxes. In addition, data was taken from a study published earlier (Tanila et al., 1997b) and carried out in the State University of New York at Stony Brook, USA. The procedure was similar to the Kuopio experiments, except the familiar environment was a plus maze with rewards given at the end of each arm. For the novel environment the surrounding black curtains were opened, new visual landmarks were hung, and the plus maze floor odors were removed. The familiar and novel environments used in Study IV are shown in Figure 7.

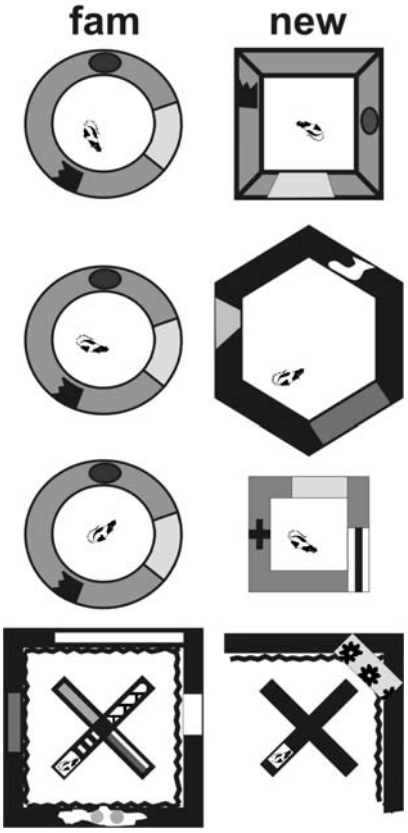

Figure 7
The familiar and new arenas of Study IV. Rats explored the familiar (fam) and new arenas successively. Each row represents a session; each fam-new session was recorded on a separate day. Rows 1-3 denote the environments of Studies I-III. Row 4 shows the familiar and novel arenas of the Stony Brook experiments (Tanila et al., 1997b).

Place cell analysis

The recordings were made with Enhanced Discovery software, and cells were isolated off-line by clusters defined with waveform parameters using Autocut software (both from DataWave Technologies, Longmont, CO, USA). Place cells were defined as pyramidal cells (based on the presence of complex spikes and duration of the negative spike more than 300 μs) with a place field in at least one of the recording environments. Place fields were defined as a set of at least six adjacent pixels (each pixel was 3.5 cm x 3.5 cm) with firing rates above 0.5 Hz and two times above the cell's overall mean firing rate. Cells were evaluated separately for each study and included into the study only if they had clear place fields in at least one recording environment and showed clear amplitude differences between the four tetrode channels (indicating good isolation).

To compare the properties of place cells between groups, analyses were based upon the overall firing rates, which were calculated for periods when the rat was moving at least 2 cm/s. This speed filter minimized contamination of the location-specific firing with spikes during sharp wave activity (see Chrobak and Buzsaki (1998)). We also compared spatial selectivity, a quantitative evaluation of how specific the place field is for a particular area in the environment. We calculated the spatial selectivity with the spatial information content (Markus et al., 1995; Skaggs et al., 1993), restricted to trials in which the mean firing rate of the cell was above 0.1 Hz. The formula used was:

$$\text{spatial information content} = \Sigma P_i(FR_i/FR)\log_2(FR_i/FR)$$

where i is the pixel number, P_i is the probability for occupancy of pixel i, FR_i is the mean firing rate for bin i, and FR is the overall firing rate.

In order to measure the extent to which a spatial representation changed between trials in the different compartments, we quantified the similarity between firing rate maps calculated as pixel-to-pixel cross-correlations. We calculated the cross-correlations only if a place field was evident in at least one of the trials. As a second measure of the neuronal response to the movement across compartments, we calculated the magnitude of firing rate change (independent of direction) between environments, computed as:

$$\text{firing rate change} = \text{ABS}\,[(fr_1-fr_2) / (fr_1+fr_2)]$$

such that ABS = absolute value, fr_1=firing rate in the first trial, and fr_2=firing rate in the second trial. This measure allowed us to account for cells which were silent in one of the environments, a situation in which spatial correlations cannot be used.

Histology

At the end of the study, the rats were deeply anesthetized (with pentobaritol and chloral hydrate each 50 mg/kg i.p.), and the recording sites were marked by passing anodal current (30 μA, 5 sec) through the electrodes. The animals were perfused with buffered 4% formalin, and the brains were cut into 50 μm sections. The locations of the electrodes tips were confirmed by Prussian blue reaction (Tanila et al., 1997a; see histological examples in Figure 2). The immunotoxin lesions were evaluated by AChE staining of sections taken through the hippocampus, as described by (Hedreen et al., 1985), and by loss of ChAT-positive neurons in the medial septum, as described in (Mikkonen et al., 1997).

Statistical Analysis

Since the number of cells recorded in each varied randomly, each rat was treated as an equal statistical unit by averaging all simultaneously recorded cells for each trial in Studies I-III. The young and aged groups of rats were then compared by one-way analysis of variance. Finally spatial memory index scores of each individual rat were correlated with place field correlation parameters. Study IV was an exception since the large number of cells minimized such bias and so the analysis was done on a cell by cell basis. Additionally, since only two trials (familiar and novel environments) were used, an analysis of variance with repeated measures was performed for the firing rate and place field parameters.

5. Results

Spatial learning and spatial cells

As found in previous studies (Gallagher et al., 1993), the aged (24-26 months) rats as a group had a significantly higher spatial learning index scores, indicating impaired spatial memory, compared to young adult (6-8 months) rats (253±11 versus 183±9 cm, mean±SEM; $t(17) = -4.6$, $p < 0.001$). As shown in Figure 8, some of the aged rats had scores within the range of young performance, whereas others performed outside the range of the young subject population. By contrast, during simple training to locate a visible platform, the aged rats had escape latencies equivalent to those of young rats (7.3±1.2 s vs. 8.1±1.4 s, respectively; $t(13) = 0.43$, $p > 0.60$).

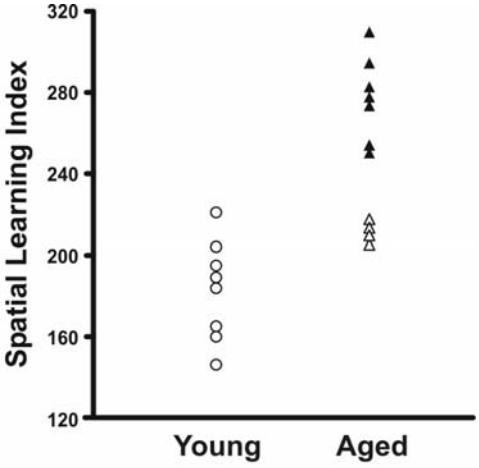

Figure 8
Learning index scores from the behavioral characterization of the individual rats used in the recording studies.
The learning index is derived from interpolated probe trials during place learning in the water maze (Gallagher et al., 1993). Lower values represent more rapid and accurate acquisition of a search for the escape platform.

We recorded simultaneously from an average of 4.2±0.5 (mean±SEM) place cells per experimental session for a grand total in Studies I-III of 146 young cells and 295 aged cells. These totals were composed of both CA1 and CA3 cells which were combined for Studies I-III to increase the statistical power for each experiment. In addition to the cells of the young and aged rats, Study III examined 13 place cells from cholinergic-lesioned animals.

Study IV combined all these experiments with a familiar and novel environment in order to analyze the effect of age upon the CA1 and CA3 subregions. Study IV analyzed 139 young CA1 and 221 aged CA1 place cells, and 36 young CA3 cells and 109 aged CA3 cells. Figure 9 depicts all the recording sites which yielded place cells for Study IV.

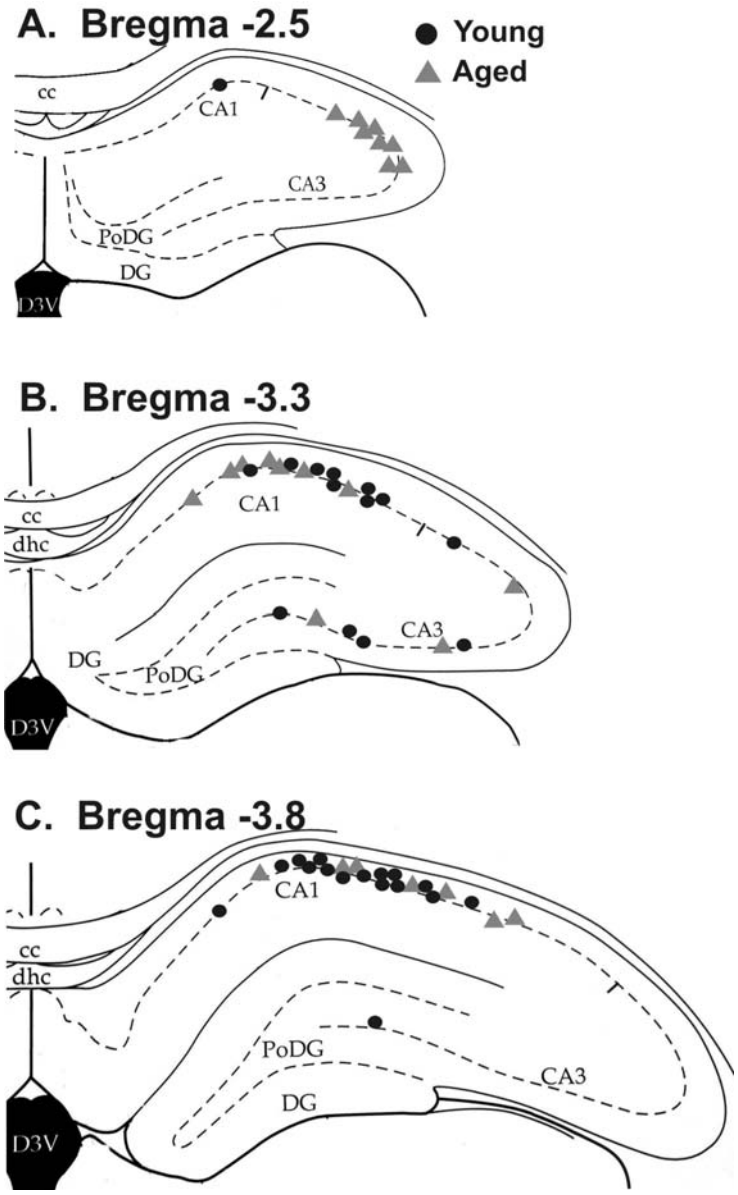

Figure 9
Recording sites of place cell recordings in Study IV from the CA1 and CA3 subregions (adapted from Paxinos and Watson (1998)).
Recordings took place in the dorsal hippocampus at different distances posterior to the Bregma skull coordinates (indicated by A, B, and C). The sites are derived from histological slices showing the electrode locations, such as the example in Figure 2. Sites are marked with a grey triangle (aged) or black circle (young). A marker indicates at least one (likely more) place cell was recorded from that site.

Because the numbers of recorded cells differed among electrodes, using individual cells as the unit for assessments could potentially skew the results towards those characteristic of tetrodes with a large number of recorded cells. In order to exclude this potential bias, we performed the same analyses based on ensembles of cells rather than individual cells. The ensemble values consisted of the average of all cells recorded simultaneously by one tetrode. The results using both methods were similar, indicating that any bias in the analyses based on individual cells would be minimal (see Appendix Study IV, Supplemental Table 2 for details).

Aged CA3 place cells have abnormally high firing rates.

We examined the overall firing rates and the spatial selectivity of the young and aged hippocampal cells in familiar and novel environments. In both the familiar and novel (or altered) environments (Studies I and II, but not Study III) the place cells of aged rats had significantly higher firing rates than those of the young rats (see Figure 10A and 10B; age effect I: $F(1, 27) = 5.8$, $p=0.02$); age effect II: $F(1, 13) = 4.3$, $p=0.05$). The pyramidal cells of both young and aged rats did, however, exhibit robust location-specific activity under almost all conditions (see Figure 11; age effect in Studies I and II on spatial selectivity: non-significant).

Analysis of the individual hippocampal layers permitted by Study IV revealed that the CA3 cells of aged rats were much more active than those of the young rats, but that CA1 cells had similar firing rates across ages (see Figure 10D; CA3 age effect: $F(1, 121) = 14.5$, $p<0.001$; CA1 age effect: $F(1, 274) = 1.1$, $p>0.3$). The overall spatial selectivity of aged CA3 cells was not compromised, although the cells of young rats had higher spatial selectivity in the novel environment than those of aged rats (see Figure 11D). In sum, in comparison to the cells of young rats, the place cells of aged rats were active at higher rates, which could be attributed to hyperactivity of the CA3 subregion, and this was not secondary to an overall loss in spatial selectivity.

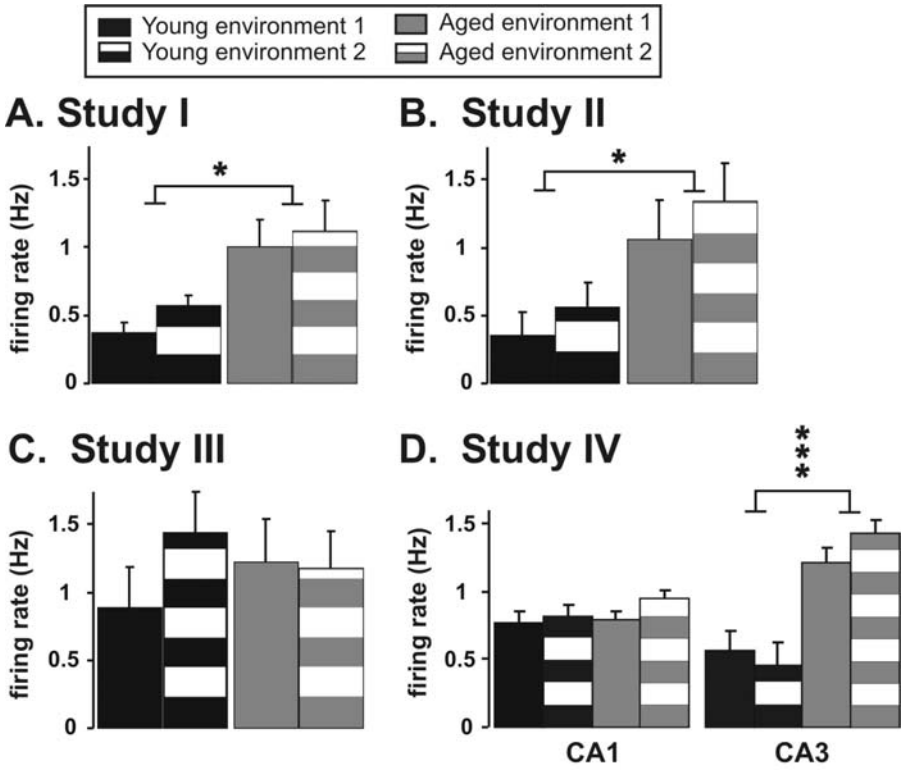

Figure 10
The mean firing rates of place cells for young and aged rats across familiar and changed environments.
A. Study I - familiar and altered environments.
B. Study II - familiar and novel environments.
C. Study III - visually identical Boxes A and B.
D. Study IV - familiar and novel environments.
* indicates statistical significance $p<0.05$.
*** indicates statistical significance $p<0.001$.

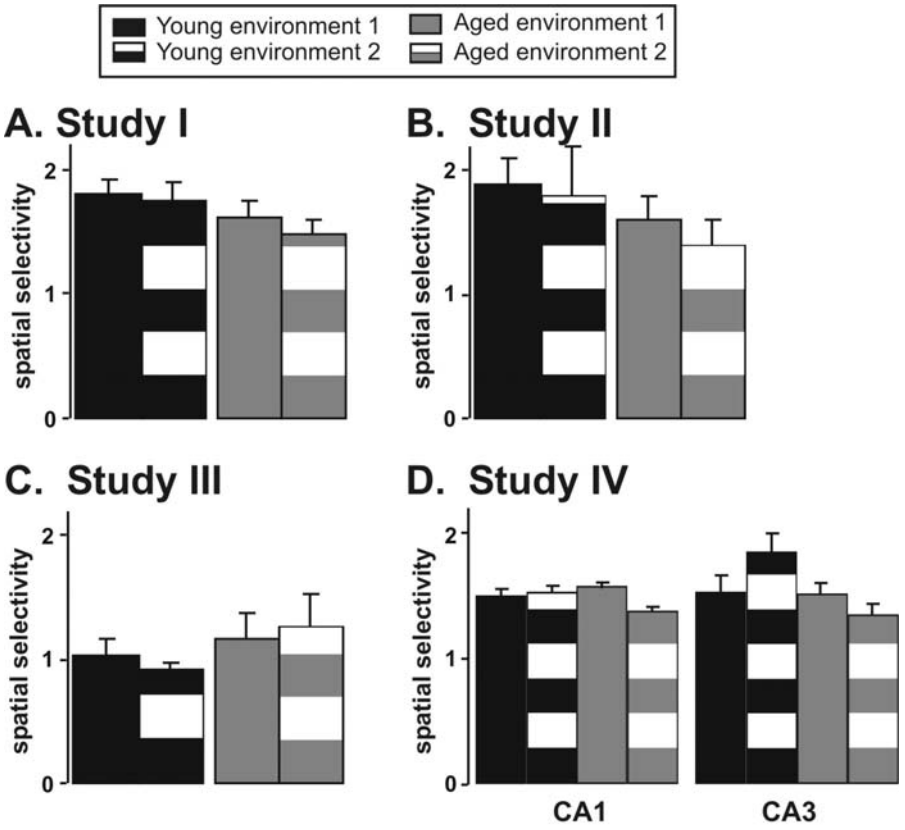

Figure 11
The spatial selectivity of place cells for young and aged rats across familiar and changed environments. Spatial selectivity was measured by the spatial information content.
A. Study I - familiar and altered environments.
B. Study II - familiar and novel environments.
C. Study III - visually identical Boxes A and B.
D. Study IV - familiar and novel environments.

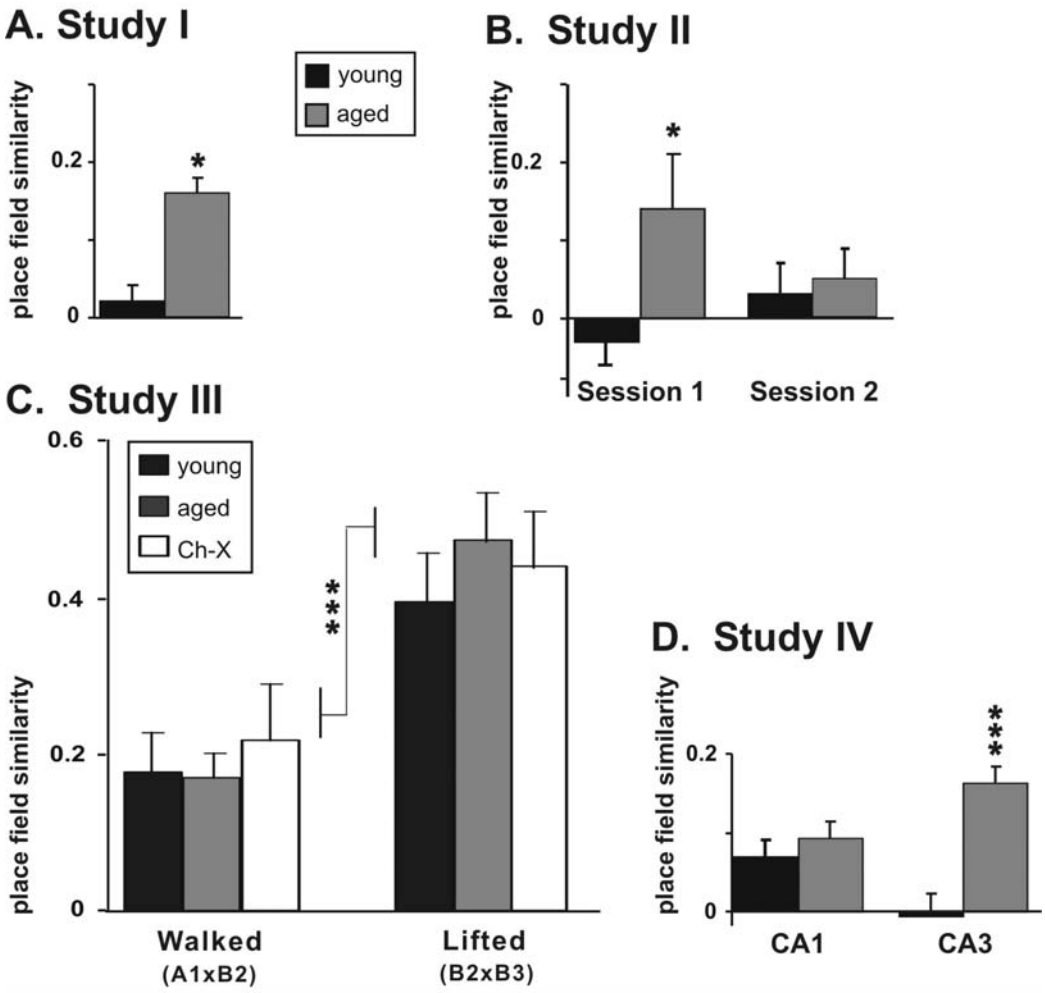

Figure 12
The place field similarities for young and aged rats across familiar and changed environments.
A. Study I - familiar and altered environments.
B. Study II - familiar and novel environments.
C. Study III - visually identical Boxes A and B.
This study includes young rats with cholinergic lesions (Ch-X).
D. Study IV - familiar and novel environments.
* indicates statistical significance $p<0.05$.
*** indicates statistical significance $p<0.001$.

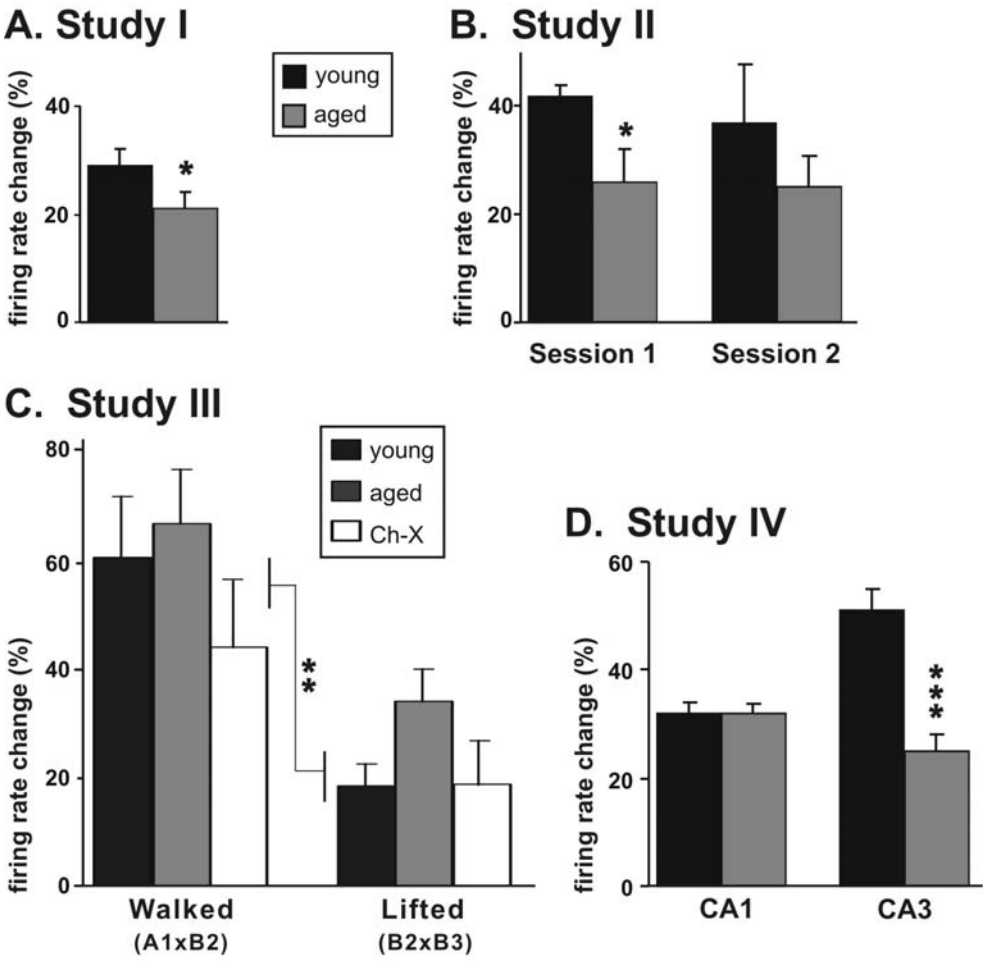

Figure 13
The firing rate change (independent of direction) of place cells for young and aged rats across familiar and changed environments.
A. Study I - familiar and altered environments.
B. Study II - familiar and novel environments.
C. Study III - visually identical Boxes A and B.
This study includes young rats with cholinergic lesions (Ch-X).
D. Study IV - familiar and novel environments.
* indicates statistical significance $p<0.05$.
** indicates statistical significance $p<0.01$.
*** indicates statistical significance $p<0.001$.

Age compromises the ability to process changes in the external environment.

When the external environment changes above a threshold level, hippocampal place cells can reflect this change by two mechanisms: 1) the location of intense firing can drastically change (termed place field remapping) or 2) the firing rate can change

although the place field remains the same. Alternatively, if the surrounding changes do not reach a threshold, the place fields may remain unchanged in location and firing rate. Studies I and II investigated place cell changes under conditions of an altered external visual environment (I) or a novel external environment (II).

Study I found that the place cell of aged rats were much less likely than those of young rats to change their firing patterns in the face of visual environment alterations (pixel-to-pixel place field similarity correlations in Figure 12A: young = 0.02 ± 0.02, aged = 0.16 ± 0.03, mean ± SEM; $t(27) = -4.0$, $p<0.001$; firing rate percent change in Figure 13A: young = 30 ± 3% Hz , aged = 23 ± 2%; $t(27)=2.0$, $p=0.05$). For distribution of the place field similarities across rats, see Figure 22. Cells of the young rats often created new spatial representations of the altered environment (see Figure 14, cell1 Y1), whereas cells of aged rats often carried over the spatial representations from the familiar environment to the altered one (see Figure 14, cells A1-A2).

Study II extended this finding to a novel environment. Upon first entry to the novel environment from a familiar one, some aged rats used similar spatial representations for both environments whereas all young rats quickly created separate representations for each arena (see Figure 15; place field similarity correlations Fam1-New1 in Figure 12B: young = -0.03±0.03, aged = 0.14±0.07; $t(13)=-2.3$, $p=0.04$; firing rate percent change in Figure 13B: young = 42±2%, aged = 26±6%; $t(13)=2.4$, $p=0.03$). On the second day of exposure to this novel environment, however, both the young and aged rats treated the hexagon environment as different from the familiar cylinder. Now the aged rats developed distinct representations and changed their firing rates, much as the young rats did during their first exposure to the novel arena (see Figure 16; place field similarity Fam1-New1 in Figure 12B: young = 0.03±0.04, aged = 0.05±0.04; $t(12) = -0.4$, $p>0.50$; firing rate percent change in Figure 13B : young = 37±11%, aged = 25±6%; $t(12) = 1.0$, $p>0.30$). For distribution of the place field similarities across rats, see Appendix, Study II, Table 2. Thus, when the visual world changes a little (I) or a lot (II), the hippocampus of aged rats does not readily change its place cell firing patterns. Study II indicates, however, that the creation of new representations in aged rats may simply require more exposure than in young rats.

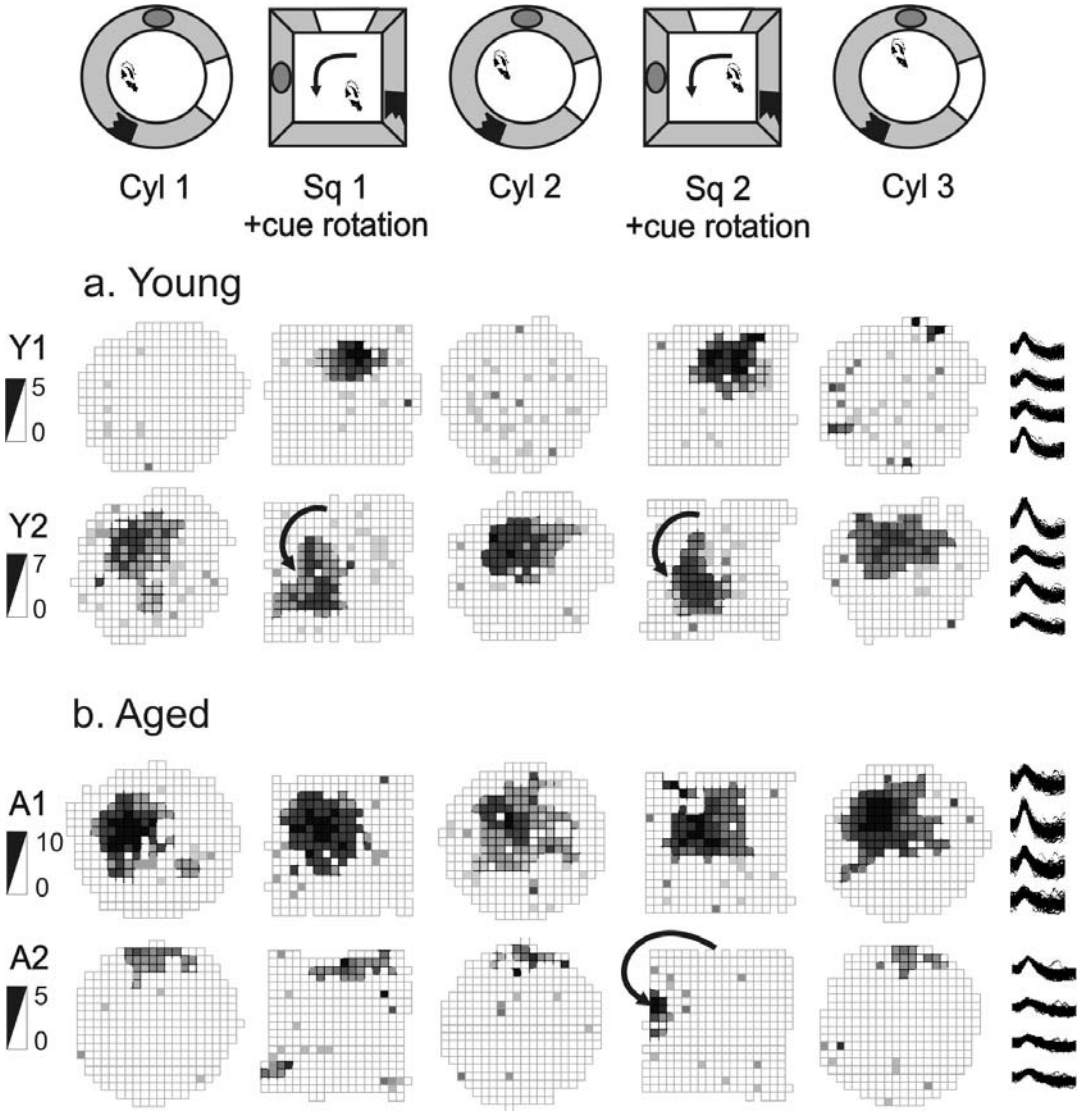

Figure 14
Place fields of hippocampal cells in young and aged memory-impaired rats in Study I. The top row depicts the experimental setup with the rats exploring a familiar cylindrical arena (Cyl) and a novel square arena (Sq). Each subsequent row represents the activity of one cell over the entire experiment; individual grids represent the floor space where the rat was moving. Firing rate scales are provided on the left of the figure, such that darker pixels indicate areas in which more action potentials occurred. White pixels indicate no action potentials, but that the rat did visit the area. Example tetrode waveforms of each cell are shown on the right side. Data are shown (a) for two place cells of two young rats and (b) for two cells of two aged, memory-impaired rats. Cell Y1 is an example of the generation of new spatial representations by cells of young rats. Cell Y2 shows a place cell whose field rotated, following the landmarks in the square. Cell A1 shows an example of rigid place fields of aged memory-impaired rats despite changes in the environment. Cell A2 is rigid in response to the first exposure to the novel-square, but then rotates with the square's landmarks in the second trial.

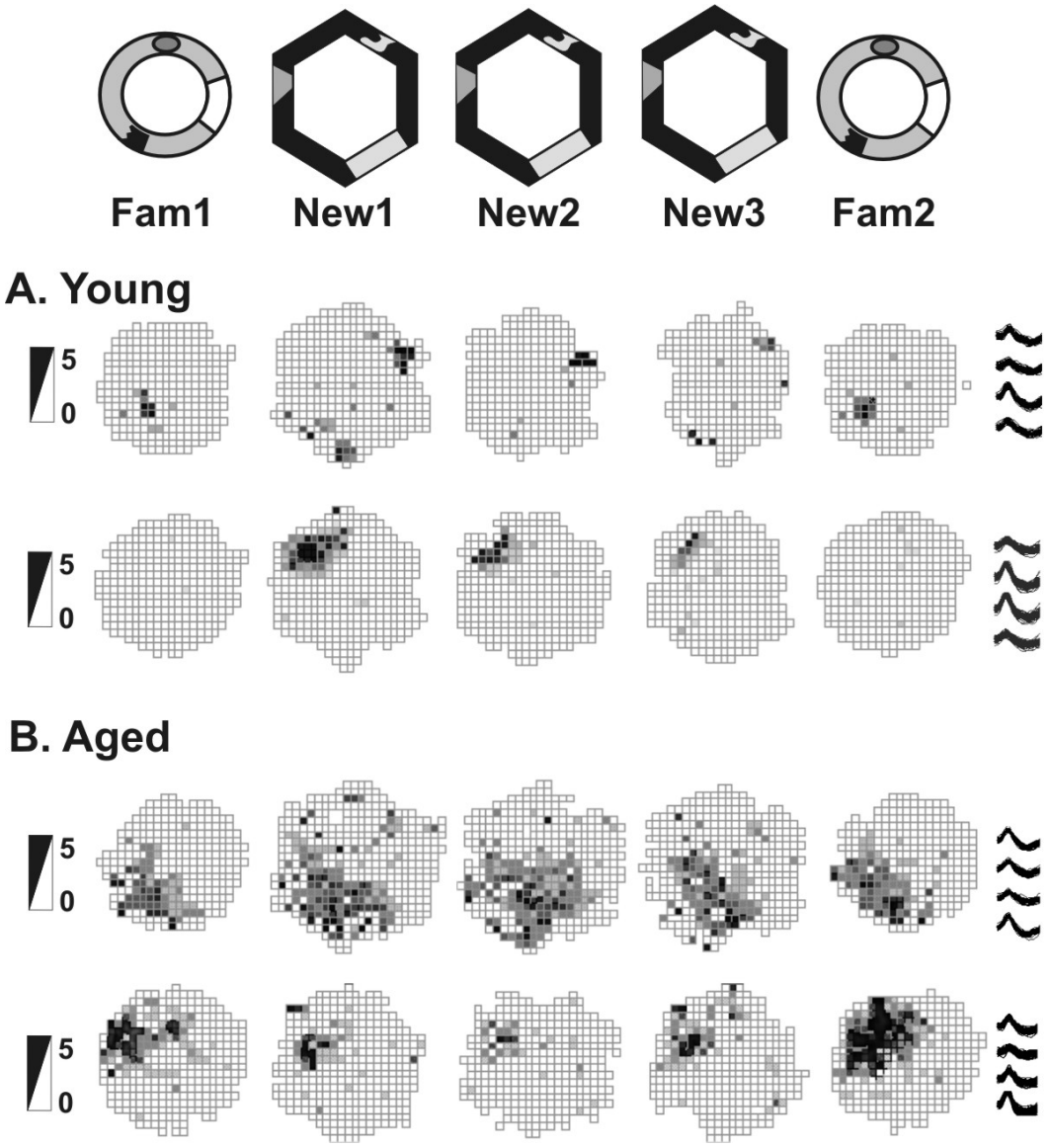

Figure 15
Initial spatial representations of a novel environment (from Session 1 of Study II).
Place fields of hippocampal cells in rats exploring the familiar cylinder (Fam) and the novel hexagon (New) arenas during Session 1 are shown. Data are shown (A) for two cells of two young rats (rat H5 cell 4 and rat G9 cell 12) and (B) for two cells of two aged rats (rat G6 cell 8 and rat H2 cell 3). In Session 1 the fields of young rats formed distinct spatial representations for each environment, whereas the fields of aged rats remained rigid despite the changes in the surrounding environments. Firing rate scales in spikes per second are provided on the left of the figure; example waveforms of each cell from all tetrode tips are shown on the right side.

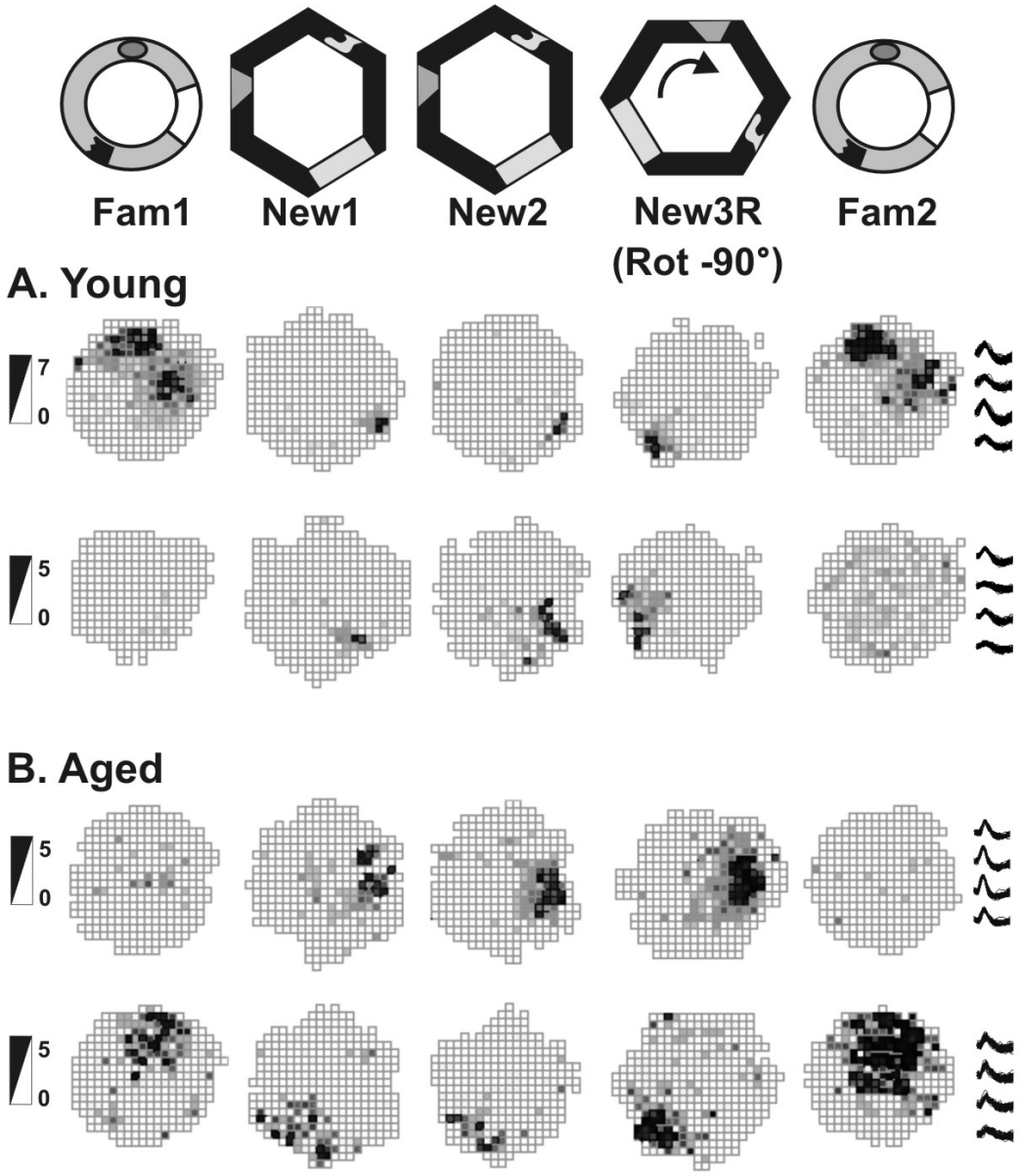

Figure 16
Spatial representations in the second exposure to the novel arena (from Session 2 of Study II).
Place fields of hippocampal cells in rats exploring the familiar cylinder (Fam) and the novel hexagon (New) arenas during Session 2 are shown. Data are shown (A) for two cells of two young rats (rat G9 cell 10 and rat H5 cell 3) and (B) for two cells of two aged rats (rat G5 cell 1 and rat G6 cell 7). In Session 2 the fields of young and aged rats formed distinct spatial representations for each environment. When the new hexagon arena was rotated 90º clockwise prior to the trial New3R, the fields of young rats followed the landmarks cues by rotating their spatial representations. The fields of aged, memory-impaired rats failed to rotate with the arena; instead they remained in the location of trial New2. Firing rate scales in spikes per second are provided on the left of the figure; example tetrode waveforms of each cell are shown on the right side.

Place cells of aged rats do not consistently use new visual landmarks even after multiple exposures.

Since the spatial water maze task is based on the ability to use visual landmarks to navigate, we tested if the place cells of young and aged rats would learn to incorporate rotations of novel visual landmarks after multiple exposures. In the second session of Study II, several aged rats which had now created new spatial representations for the new environment failed to rotate with a 90° rotation of the visual landmarks, whereas all of cells of young rats rotated with the landmarks (see Figure 16). Because cells of some of the aged animals followed the cues whereas others did not (as shown by Study II, Table 2 in the appendix), the means of the young and aged rats were not significantly different (New2-New3R-Aligned correlation: young = 0.29±0.08, aged = 0.22±0.09; $t(12) = 0.6$, $p>0.5$; New2-New3R not aligned correlation: young = 0.01±0.02; aged = 0.14±0.07; $t(12) = -1.4$, $p>0.15$). For distribution of the place field aligned rotations across rats, see Figure 23. Those rats whose spatial firing patterns failed to rotate with the landmarks, however, were also those rats that performed most poorly on the water maze test (reported later in this text). The lack of rotation among the aged memory-impaired animals suggests that they either do not attend to the visual landmarks or, in concordance with theories proposed by McNaughton and colleagues (McNaughton et al., 1996) and by Redish and Touretzky (Redish and Touretzky, 1997; Redish, 1999; Touretzky and Redish, 1996), the aged rats require additional experience for the hippocampal representation to be bound to the visual landmarks by associative learning. To assess whether landmark encoding would become consistent with greater experience, four additional rats (2 young and 2 aged memory-impaired) were tested for up to eight rotation sessions with the novel environment.

During the initial session, place cells of the two aged memory-impaired rats again failed to rotate with the landmarks in the new environment, while the place cells of the two young rats clearly did rotate (rotation-aligned correlations: young = 0.32±0.06; aged = -0.04±0.04; non-rotation aligned correlations: young = 0.05±0.02; aged = 0.42±0.07). Figures 17 and 18 illustrate sample cells from ensembles recorded from each session for each rat. With further exposures to the hexagon arena and its rotation, the place cells of aged rats followed the landmarks during the second (rat R5)

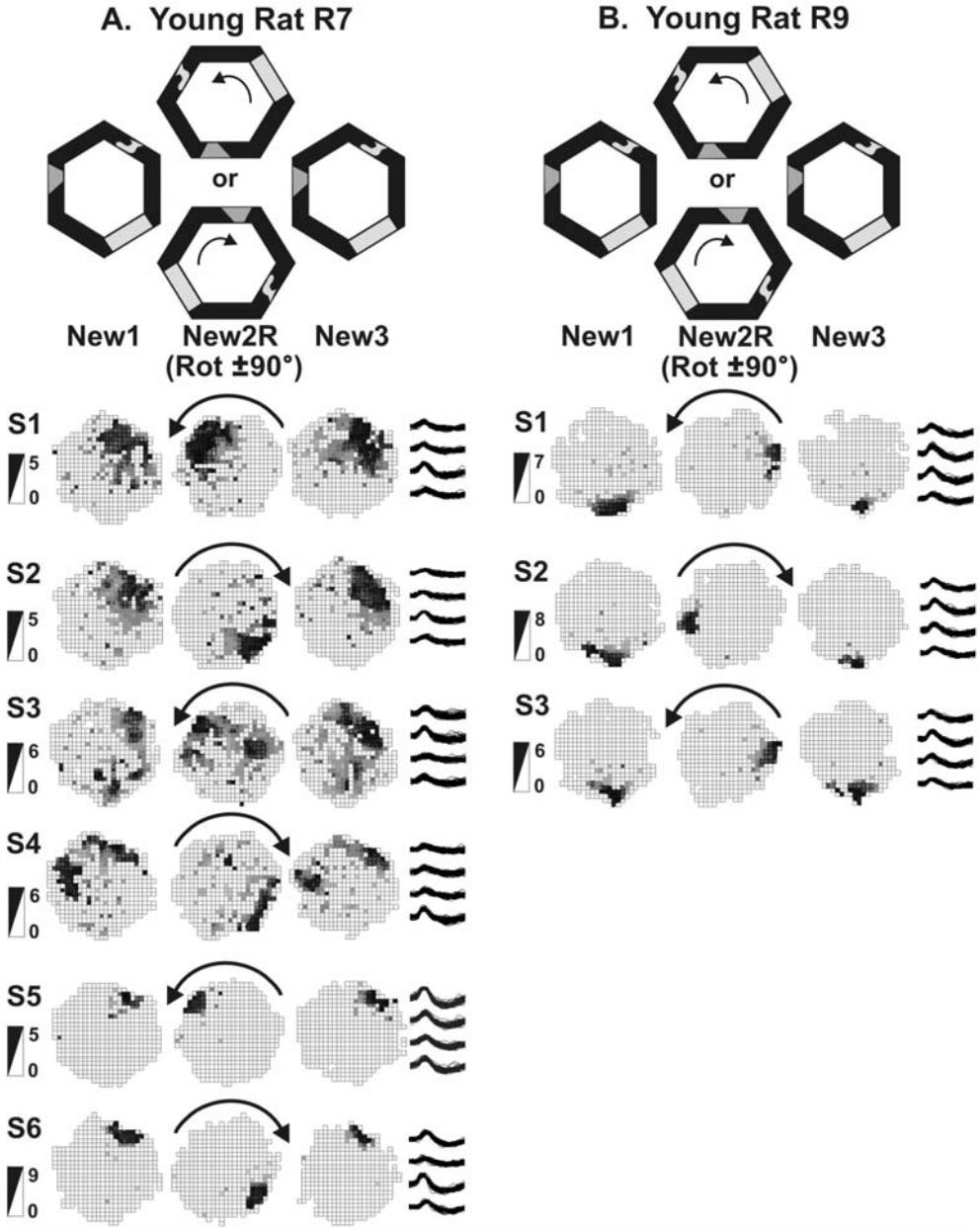

Figure 17

Place field responses of young rats to arena rotations over many exposures to a novel environment (from Experiment 2 of Study II).

One cell from each session is shown as representative of the entire ensemble. Mean responses of each ensemble for each session are shown in Figure 19. Sessions are abbreviated S1-S6, and arrows indicate the direction of the rotation.

A. Young rat R7 recorded for 6 sessions. The place fields rotated with the landmarks each time in trial New2R. Sessions 1 and 2 show recordings from the same cell, whereas sessions 3-6 involve different cells.

B. Young rat R9 recorded for 3 sessions. The place fields rotated with the landmarks each time in trial New2R. The same cell is shown across all sessions.

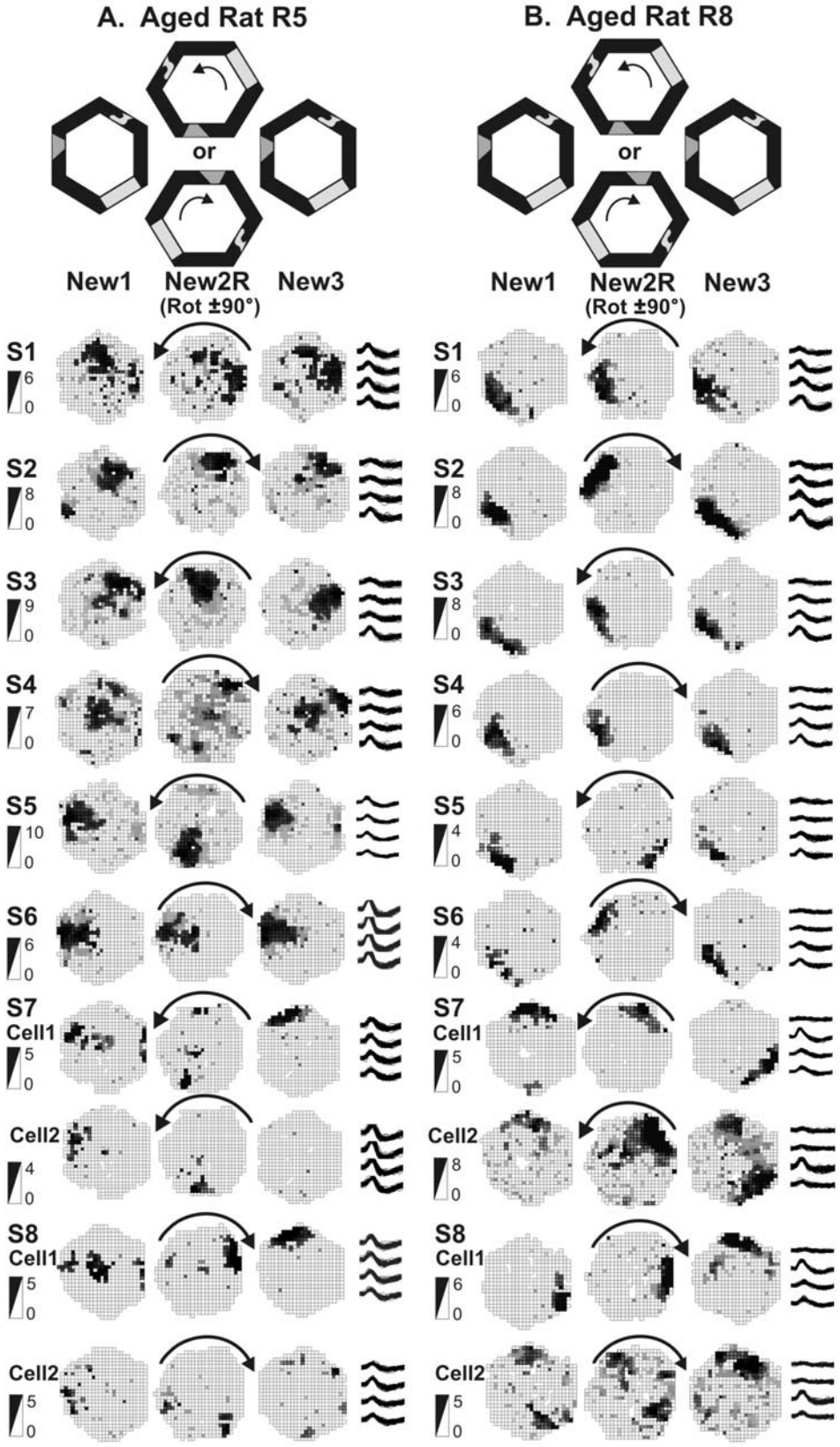
A. Aged Rat R5
B. Aged Rat R8
or
or
New1
New2R
(Rot ±90°)
New3
New1
New2R
(Rot ±90°)
New3
S1
6
0
S2
8
0
S3
9
0
S4
7
0
S5
10
0
S6
6
0
S7
Cell1
5
0
Cell2
4
0
S8
Cell1
5
0
Cell2
5
0
S1
6
0
S2
8
0
S3
8
0
S4
6
0
S5
4
0
S6
4
0
S7
Cell1
5
0
Cell2
8
0
S8
Cell1
6
0
Cell2
5
0

Figure 18 (previous page)
Place field responses of aged rats to arena rotations over many exposures to a novel environment (from Experiment 2 of Study II).
For explanations, see Figure 17. Sessions are abbreviated S1-S8. Illustrations for sessions 7 and 8 contain two cells to show remapping within the novel environment.
A. Aged memory-impaired rat R5 recorded for 8 sessions. The spatial representations did not rotate with the arena rotation in sessions 1, 2, 4, and 6, but in sessions 3 and 5 they did rotate. Sessions 2, 3, and 4 are illustrated with the same cell, whereas for all other sessions different cells are shown. Note that even the same cell in sessions 2, 3, and 4 responded differently on different days. In session 7 the place fields rotated, but remapped (new location or disappeared) in trial New3 instead of returning to their original positions. In session 8 the place fields were replaced by a new one in trial New2R that rotated in trial New3 with the arena frame. Note that Cell 1 (the same cell in both session 7 and 8) appears to have two different place fields, reflecting a kind of multi-stability.
B. Aged memory-impaired rat R8 recorded for 8 sessions. In sessions 1, 3, and 4, the spatial representations did not rotate with the arena, whereas they followed the arena rotation in sessions 2, 5, and 6. Sessions 1-6 all are illustrated by the same cell, again showing that individual cells sometimes rotated and sometimes did not. Another two cells are used to illustrate both sessions 7 and 8. Note that in sessions 7 and 8 the two cells appear to each have two representations of the same environment without regard to the landmarks. This is strongly suggestive of multi-stability.

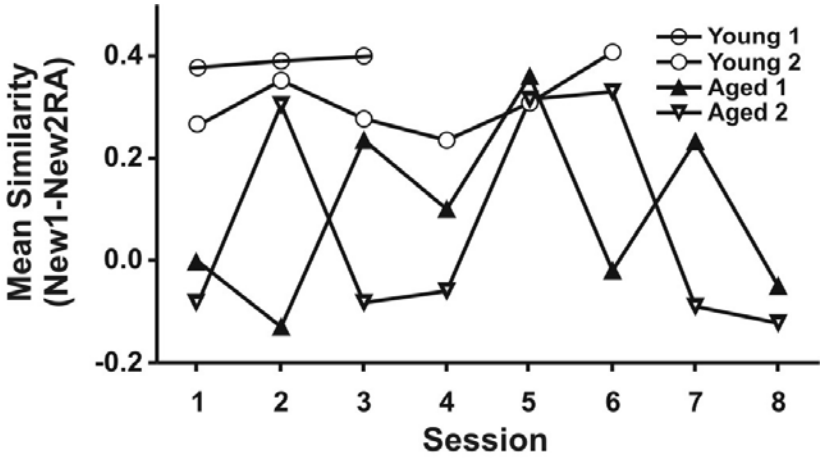

Figure 19
Reactions to the rotated novel arena (aligned) (from Experiment 2 of Study II).
The mean correlation of all place fields recorded simultaneously for each rat was calculated between the trials New1 and New2R-Aligned. Rats whose place fields did rotate with the arena have a high correlation (roughly 0.25 or above). Rats whose place fields did not rotate with the arena have a low correlation (roughly 0 or below). The spatial representations of young rats had high rotated-correlations on every occasion. The spatial representations of aged memory-impaired rats had low correlations in the initial session, but in the second or third session the correlations were as high as in young rats. In sessions thereafter, the place fields of the aged rats had low correlations on some occasions and high correlations on other occasions.

or third (rat R8) session, as determined by an aligned correlation score of greater than 0.25 (correlations similar to young rats). This suggested that the spatial representations of the aged rats had become bound to the environmental landmarks. In subsequent sessions, however, the place cells of aged rats did not consistently follow the landmark rotations, as evidenced by aligned correlations of around 0 (see Figure 19 for mean aligned correlations of each neuronal ensemble recorded in all four rats over all sessions). Thus, during repeated exposures to the novel environment, the place fields of aged memory-impaired rats did not rotate with the landmarks initially, but thereafter fluctuated with rotation on some but not all occasions, whereas the place fields of young rats always rotated with the landmarks. This inconsistency, along with the two alternating spatial representations shown in Figure 18, sessions 7 and 8 (see figure legend), is suggestive of multi-stable aged place cells.

The CA3 subregion, but not the CA1 subregion, of the aged hippocampus fails to rapidly encode new external environments.

With the repeated findings that the hippocampus of aged rats did not readily encode changes to the external environment (Study I, II, and (Tanila et al., 1997b)), it was important to determine if this failure could be attributed specifically to either subregion of the hippocampus. Study IV showed that the rigidity of place cells to environmental changes is characteristic of the aged CA3 hippocampus and not of the CA1 hippocampus. Analyses of the overall firing rate change and place field similarities indicated that the degree of change in response to a new environment is equivalent in the CA1 cells of young and aged rats (CA1 age effect: $F(1, 274) < 0.9$, $p > 0.35$; Figures 9D and 10D). In contrast, CA3 cells of young rats exhibited much greater change in firing rate and place fields (CA3 age effect: $F(1, 121) > 17.8$, $p < 0.001$).

These distinctions in place cell changes are also evident in correlations between the firing rates in the novel and familiar (Figure 20). Similar to the report by Leugeb and colleagues (Leutgeb et al., 2004), in young rats the firing rate of CA1 cells in the two environments in our studies were correlated ($rho = -0.42$, $p < 0.001$) whereas the firing rates of CA3 cells were not correlated across the two environments ($rho = 0.0$, $p = 0.98$). By contrast, in the aged rats the firing rates were correlated across the two

environments for both CA1 and CA3 cells (CA1: rho = -0.53, p<0.001; CA3: rho = -0.73, p<0.001). Thus, in contrast to young CA3 place cells which were active almost exclusively in one arena, aged CA3 place cells had similar levels of activity in both environments.

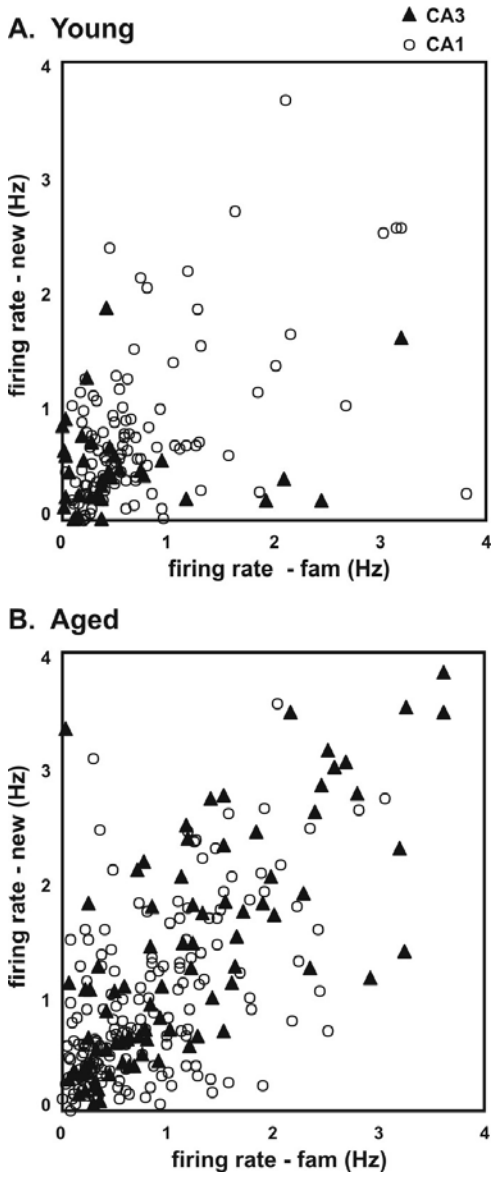

Figure 20
Firing rate correlation between familiar and novel environments for CA1 and CA3 cells (from Study IV).
A. young cells; B. aged cells
The firing rate in the familiar and novel environments were well correlated in young CA1 cells (p<0.001), aged CA1 (p<0.001) and aged CA3 cells (p<0.001). The firing rates of the young CA3 cells were not correlated between environments (p=0.98).

The aged hippocampus does process changes in self-motion information capably.

In addition to changes in the external environment, changes above a threshold level in self-motion information can also be sufficient to induce changes in the hippocampal spatial representation. To test whether the rigidity of aged hippocampal representations extended to occasions when self-motion information provided the impetus for change, in Study III the rat itself moved between two visually identical environments. In this situation the place cells of aged rats were equally likely as those of young rats to create separate spatial firing patterns for each compartment. In addition, the place cells of young rats with specific cholinergic lesions, a model for aging which has been found to possess rigid spatial representations when the external environment changed (Ikonen et al., 2002; Leutgeb and Mizumori, 1999), were also not different from the young control rats. Roughly half the cells of young rats, aged rats, and cholinergic-lesioned rats used the same spatial representations for the two compartments (Figure 21 cells Young 2, Ch-X 2, and Aged 2) and half created different representations (Figure 21 cells Young 1, Ch-X 1, and Aged 1) (for means see Figures 12C and 13C; place field similarity Trials A1-B2: $F(2,25) = 0.2$, $P = 0.83$; firing rate percent change A1-B2: $F(2,25) = 0.8$, $P = 0.45$). For distribution of the place field similarities across rats, see the Appendix, Study III, Table 1.

As a control, we compared the changes in spatial representations when the rat walked between the different compartments (trials A1 and B2) with those when the rat was lifted between the same compartment (trials B2 and B3). The change in spatial representations was significantly higher across all groups between the different visually identical compartments than within the same compartment (Figure 12C and 13C; place field similarity: $F(1) = 15.6$, $P = 0.001$; firing rate percent change: $F(1) = 7.2$, $P = 0.01$). Contrasting this to the distinctly rigid results of Studies I and II, the hippocampus of aged rats appears fully capable of processing internally-generated information, and it is the external cues which are incompletely processed.

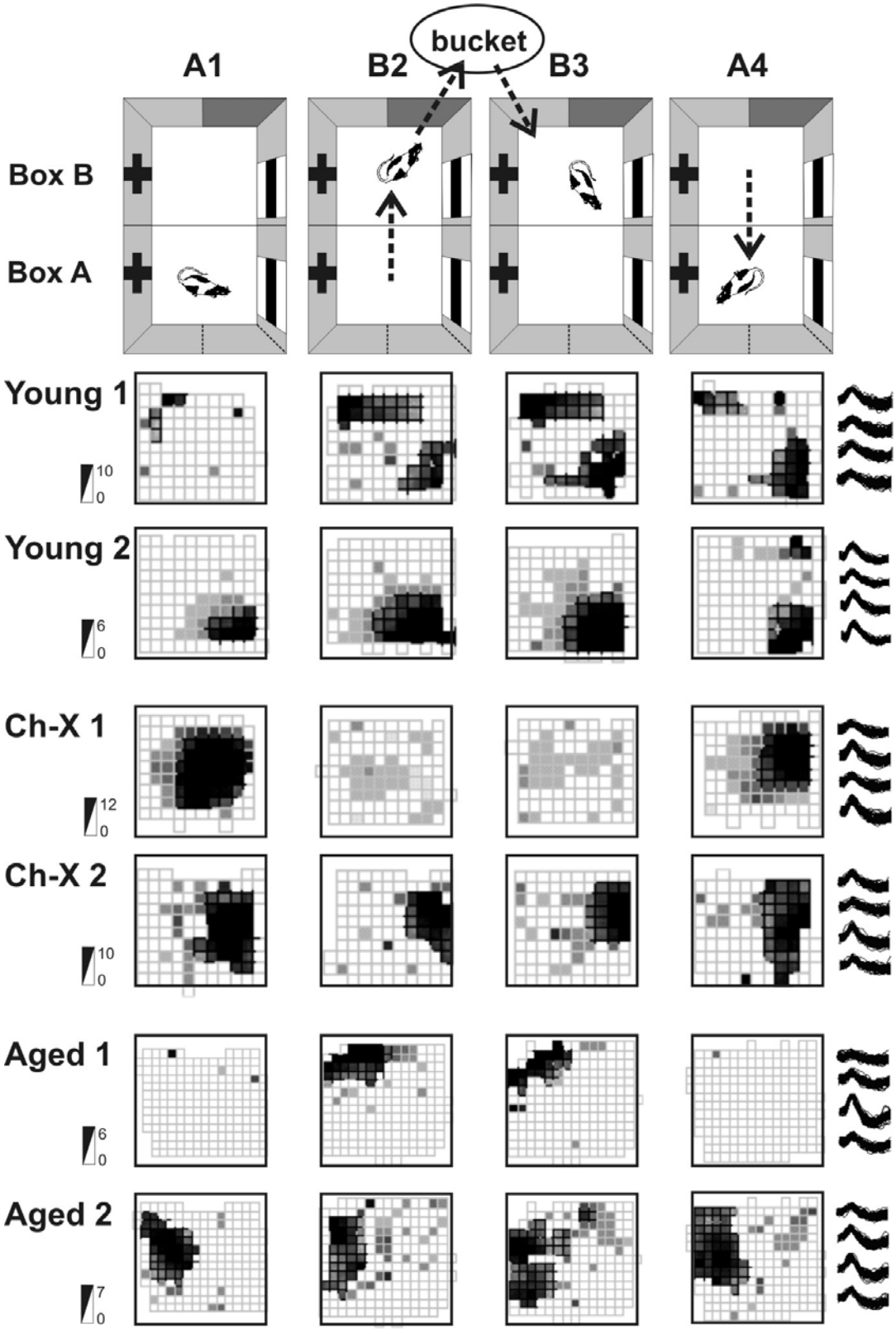
A1
B2
bucket
B3
A4
Box B
Box A
Young 1
10
0
Young 2
6
0
Ch-X 1
12
0
Ch-X 2
10
0
Aged 1
6
0
Aged 2
7
0

Figure 21 (previous page)
Spatial representations in visually identical compartments (from Study III).
Boxes A and B are separated by a wall with a hidden door. Dashed arrows indicate transitions between the trials: walking from A1 to B2, lifted passively by the experimenter from B2 into a hanging bucket and then to B3, and walking from B3 to A4. Each row depicts the spatial firing patterns of one cell across the experiment; note that only the explored arena (Box A or B) is shown for each trial.
Cell Young 1 shows different spatial representations in trials A1 and B2. The new representation is maintained into trial A4, demonstrating differences between A1-A4 spatial representations observed occasionally in young rats. Cell Young 2 shows a spatial representation that did not change across the trials.
Cell Ch-X 1 (from a young rat with a cholinergic lesion) had a low spatial correlation between the A and B compartments, whereas cell Ch-X 2 had a high correlation.
Cell Aged 1 was silent in environment A, but active in Box B. Cell Aged 2 had a similar firing pattern on all trials. These two cells were recorded simultaneously from the same rat and thus illustrate discordant responses observed within an ensemble.

The degree of failure of the hippocampus to process changes to the external environment predicts the magnitude of age-associated memory-impairments.

Having explored the characteristics of aged place cells as a group, we lastly examined what properties of place cells predicted spatial memory abilities on an individual rat basis. In Study I, the extent of place field rigidity despite changes in the surrounding environment correlated strongly with the magnitude of spatial memory impairment ($r(29) = 0.63$, $p<0.001$; Figure 22). Of particular interest was the correlation between spatial memory performance and hippocampal spatial representation within the subset of 27-month old rats, some of whom performed well on the water maze, whereas others performed poorly. Spatial memory index and similarity in spatial representation were significantly correlated within these nine rats ($r(17) = 0.55$, $p<0.05$). In contrast, performance on the visible platform task did not correlate with similarity in hippocampal spatial representations in the two environments ($r(29) = 0.1$, $p>0.10$; see Appendix, Study I, Figure 2b). Spatial memory impairment did not correlate well with the rigidity of place fields in Study II, Session 1 (Spearman's rho $(15) = 0.2$, $p>0.1$). This may be due to the novel environment produced less rigidity (fewer rats) than the more minor changes to the square arena.

In Study II we did find that those rats whose spatial firing patterns failed to rotate with the landmarks were also the rats that performed most poorly on the water maze test. Conversely, the rats whose place fields rotated with the cues performed best

on the water maze (all subjects: Spearman's rho(14) = -0.61, p=0.02; young only: rho(5)=0.1, p>0.8; aged only: rho(9) = -0.68, p=0.04). Figure 21 depicts the correlation between learning index and place field rotations. In contrast, rats whose place fields rotated with the landmarks did not perform any better on the visible platform water maze test than those whose fields did not rotate (Spearman's rho (14) = -0.3, p>0.20).

In Study III there was no correlation between spatial memory and place cell changes which were based on self-motion information. This supports the view that performance on the water maze depends on the use of external cues rather than on self-motion cues. In Study IV neither the hyperactivity nor the failure to rapidly encode new information by CA3 correlated strongly with spatial learning abilities. This may assert that abnormal processing in CA3 is a general feature of aging, or it may be that our experiment did not find a correlation due to a limited range of rats from which CA3 cells were recorded. The statistical power of the correlation analysis was limited since CA3 cells were recorded in only two of the aged rats which performed well enough on the spatial water maze to qualify as unimpaired, compared to six aged impaired rats.

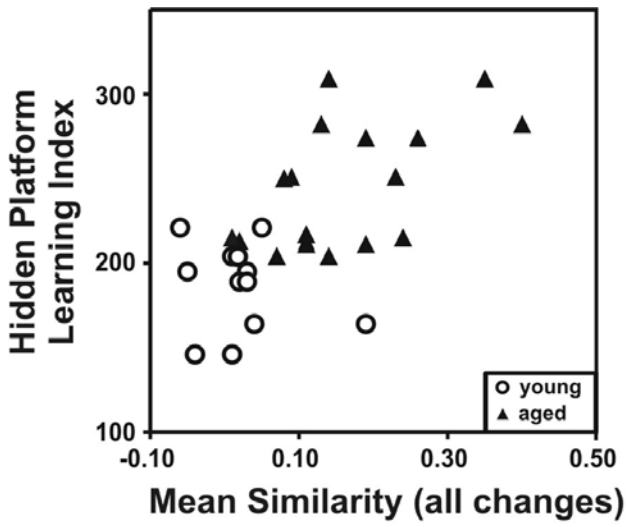

Figure 22
Place cell rigidity predicts magnitude of spatial learning impairment (from Study I). Similarity in spatial representation between exposures to the familiar cylinder versus altered-square are plotted against spatial learning performance in the water maze. Poor spatial memory is indicated by high learning index scores.

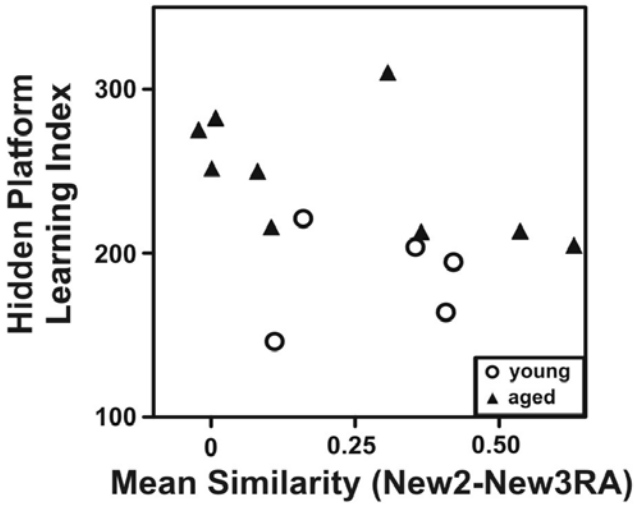

Figure 23
Place cell rotations predict magnitude of spatial learning impairment (from Study II). The spatial learning index correlated well with the similarity of spatial representations between the trials New2 and New3RA with the firing rate map of New3R rotated by $+90^{o}$ to bring the landmarks into alignment (hence the code New3Rotation-Aligned). Poor spatial memory is indicated by high learning index scores and is linked here to failures of spatial representations to follow landmarks. High similarity scores (rotation with the landmarks) predicted capable spatial memory performance.

6. Discussion

Place cells as a window into cognitive aging

These studies have examined how spatial information is processed by young and aged rats whose spatial memory abilities have been characterized as unimpaired or impaired. The hippocampal place cells of these animals have provided a window into how they encode spatial information, and thus provided a basis for investigating the differences between impaired and unimpaired processing the aging brain. The results indicate that the hippocampus of aged rats is impaired in the use of external cues, coinciding with a proclivity towards self-motion cues, and a hyperactivity and a weakened encoding of novelty specific to the CA3 subregion. These changes in hippocampal place cells reflect shifts in how information is processed in the aged brain. This discussion will focus on the implications of these shifts in hippocampal information processing for age-related memory impairments, and directions for future research aiming towards "bifocals of the mind" (Michela Gallagher, personal communication).

External visual cues are processed poorly by the aged hippocampus

There are three lines of evidence from current and past experiments which strongly suggest that poor performance by aged rats on the spatial navigation tasks arises from poor processing of the external visual cues. Firstly, hippocampal place cells of aged rats are rigid in that they show the same representation throughout despite changes in environment or task demands sufficient to induce new representations in young rats. Secondly, place cells of aged rats show delayed control by external cues. Thirdly, the cells of aged rats are multi-stable for the same environment. Although on the surface these results appear even contradictory, experimental data from Study II shows that they are different aspects of the same impaired processing of external cues.

Rigidity of aged hippocampal representations has been seen following manipulations of the spatial cues in an environment (Tanila et al., 1997a; Tanila et al., 1997b; Study I), exposure to a novel environment (Study II, Session 1), and changes in the behavioral demands (Oler and Markus, 2000). Furthermore the degree of such rigidity in spatial representation predicted the magnitude of the spatial performance deficit. It may be that even in aged animals this rigidity can be overcome with

additional exposure since the cells of aged rats in the second session in the novel environment of Study II did not show any carry-over of spatial representations from the familiar environment. Thus, spatial representations of aged animals fail to change under conditions which do produce change in the spatial representations of young animals, and this seems to play role in the ability to learn spatial tasks.

The hippocampal cells of aged rats are delayed in being controlled by the external cues. In the experiment of Rosenzweig and colleagues (2003) the place fields of aged rats were delayed in adjusting to external landmark control, and this delay correlated strongly with the rats' ability to locate the spatial goal during the place cell recordings. Consistent with these findings, place fields of aged rats in Study II were not initially controlled by the external cues, failing to rotate with the external landmarks. With additional experience, place fields of aged rats did rotate with the landmarks, again demonstrating that the hippocampal spatial representations in aged rats were delayed, but were eventually bound to the external cues.

Barnes and colleagues (Barnes et al., 1997) found that the place cells of aged rats represented the same environment with different place field arrangements on different occasions, in stark contrast to the consistent place field of young rats. The repeated exposures to the rotations of the novel environment of Study II also resulted in a kind of multi-stability. Even after numerous repeated exposures, the hippocampal spatial representations of aged memory-impaired rats rotated with the landmarks on some but not all occasions (Figure 19). Furthermore, in Study II the place cells seemed to develop two representations of the same environment without regard to the landmarks (Figure 18, Sessions 7 and 8).

Is it not contradictory for hippocampal place cells of aged rats to be overly stable, delayed in stabilizing to external stimuli, and unstable? Until now, largely theoretical considerations yielded two explanations for these differences. First, the conflicting results could arise from the weakened synaptic plasticity of the aged hippocampus (Barnes, 1994). In aged rats the existing synaptic connections may not be as strong, which would make recall of a given environment less likely to coalesce onto the correct stored pattern (Redish et al., 1998; Redish and Touretzky, 1999). Concomitantly, synapses which are less capable of change would make it more difficult for new spatial representations to be created (Redish et al., 1998).

Supporting this hypothesis is evidence that place cells of aged rats do not exhibit a synaptic plasticity characteristic of young rats. Namely, the place cells of aged rats do not undergo normal place field expansion as the rats run around a circular track (Shen et al., 1997). This provides evidence that the spatial representations of aged rats are affected by weakened synaptic plasticity. It is, thus, plausible that the multi-stable and rigid place cells of aged rats may be accounted for by the effects of weakened synaptic plasticity under different conditions.

An alternative reconciliation of the multi-stable and rigid place cells suggested that the limited scope of information encoded by the aged hippocampus may render the place cells unable to fully distinguish between environments (Rapp, 1998). Consistent with this view, the decreased connection in the aged rat between the entorhinal cortex and the hippocampus (Smith et al., 2000) likely decreases the environmental and contextual information reaching the place cells. In addition, the decreased cholinergic input may render the hippocampus less receptive to novel information. Both of these age-related changes would make the aged hippocampus less controlled by the outside world and therefore the aged place cells susceptible to rigid representations and multi-stability.

Of course, all three of these neurobiological changes (weakened synaptic plasticity, less entorhinal cortex input, and less cholinergic input) contribute to the age-related memory impairments and to the impaired information processing of the aged hippocampus. Accordingly, the hypotheses of failed plasticity and limited scope of information are not mutually exclusive: under different demands on memory processing, poor processing of external environmental information may manifest itself in different forms. Aged place cells which fail to change despite new environments provide evidence of failures during encoding. Aged place cells which do not readily adjust from self-motion to external landmark control provide evidence of failures during updating of the representation. The multi-stability of aged place cells upon return to a familiar environment provides evidence of failures during recall. Study II takes a significant step towards reconciling these divergent characterizations by observing that place representations of hippocampal cells in aged rats are rigid, delayed in acquiring control by external cues, and multi-stable at different sequential stages of spatial experience in the same experiment.

Self-motion information is processed capably in the aged rat hippocampus

The input to the hippocampus is multi-modal in nature, and therefore in addition to the external environment, self-motion information exerts important control over hippocampal place cells. Under normal conditions the cues interact coherently to produce spatial representations. For example, the representation may predominantly rely upon the self-motion information, but the visual cues may update or correct the representation at intervals (McNaughton et al., 1996; Redish and Touretzky, 1997; Touretzky and Redish, 1996). In conflict situations, however, the self-motion input can override the visual information. When the visual landmark is rotated in full sight of the rat, the place cells eventually fail to rotate with it, as if the hippocampus has learned that the visual landmark is unstable whereas the self-motion cues are reliable (Jeffery and O'Keefe, 1999). These conflicts between self-motion and visual cues for control of the hippocampal cells are reminiscent of the two navigation strategies, response and place learning, which can oppose each other for control of behavior under some experimental conditions. Because self-motion cues play an important role in response learning whereas visual cues play an important role in place learning, animals, such as aged ones, which favor response learning may have hippocampal information processing which favors self-motion information over visual information.

To test the ability of the hippocampus of aged rats to process self-motion information, Study III investigated whether the rigidity of aged place cells extended to conditions in which the self-motion provides the cues for environmental change. In Study III, the rats walked between two visually identical environments, pitting self-motion cues that indicated environmental change against visual inputs that indicated no differences between environments. Our results indicated that place cells in both aged and cholinergic-lesioned rats (another model of aging) were equally likely as those of young rats to create new spatial representations in the second compartment. These findings demonstrate that the hippocampal network of aged rats is able to process changes in internally-generated cues without rigidity. Furthermore and completely consistent with the findings of Rosenzweig and colleagues (Rosenzweig et al., 2003), this increased reliance on self-motion cues is accompanied by diminished use of external visual cues, and the resulting incomplete processing of external landmark cues may lead to impaired spatial learning.

What are the sources of weakened external cue control?

Considering the current data, there are four possible explanations for weakened control of external cues over hippocampal spatial representations as aged rats learn a new environment. First, one specific possibility is that the aged rats have impaired vision. However, a deficit in visual perception *per se* seems unlikely to account for the impaired landmark control for three reasons. Firstly, the place cells of aged rats did rotate on some occasions with the visual landmarks in both Studies I and II (Figure 14, cell A3 and Figure 18). Secondly, the aged rats in the current experiments performed normally in learning to find the visible platform in the water maze, contrasting with their impairment in the spatial task. Thirdly, in the experiment of Rosenzweig and colleagues (2003) the cells of aged rats did eventually switch to firing on the basis of the visual cues. These findings suggest that rather than impaired visual acuity, aged rats may suffer from impaired visual attention. Indeed, age-related attention deficits have been reported in humans (Coffey et al., 2001), and aged rats are known for poor performance on visual attention tasks (Muir et al., 1994). Fluctuations in attention to visual landmarks would account for fluctuations in place fields, such as multi-stability and failures to rotate with landmarks. It should be noted, however, that the dissociation between CA3 and CA1 seen in Study IV speaks against a general extra-hippocampal deficit unless it could selectively alter processing in subregion CA3 but not CA1.

Second, on each test aged rats may use only a subset of available cues. Tanila and colleagues (Tanila et al., 1997a) found that aged rats were more limited in the scope of information which they encoded. The spatial representations of aged rats rotated with only particular distal cues, rather than the entirety of spatial relationships used by the cells of young rats. Thus, sometimes the representations of aged animals may fail to incorporate the salient landmark cues. Even in young rats, place fields are sometimes determined only by particular cues, such as the floor, rather than prominent cues on the walls of an environment (Jeffery and Anderson, 2003). Furthermore, aged humans have also been shown to acquire less information than young subjects about environmental landmarks (Kirasic, 2000). In light of the current studies, perhaps all the available spatial information is not fully integrated into the spatial representations of aged

animals or, on some occasions, they are guided mainly by cues other than the salient visual landmarks.

Third, aged rats may learn spatial information at a slower rate than young rats. In the current Study II, aged rats required more experience to create new spatial representations and to anchor these representations to the external landmarks. These findings provide an important parallel to the well-documented delay in acquisition of spatial tasks in aged animals (for review, see (Foster, 1999; Rapp and Amaral, 1992)). Furthermore, it has been suggested that despite a weakened associative learning system, the aged rats could, through additional training, more often recall the correct hippocampal representation and improve water maze performance (Barnes et al., 1997; Redish and Touretzky, 1999). Study II provides evidence that impaired anchoring of spatial representations to visual cues does indeed relate to water maze performance and that this binding of the spatial representations to environment cues can be accomplished by aged memory-impaired rats through additional training. These results are consistent with models of hippocampal spatial representation in which new place fields for a novel environment are first created and then gradually the external cues are bound to the new representation (McNaughton et al., 1996; Redish and Touretzky, 1997; Samsonovich and McNaughton, 1997). Because aged rats in the novel environment of Study II constructed spatial representations more slowly, the two components appeared in sequence and not simultaneously as in young rats.

There is some behavioral evidence that these components may also exist separately in young rats. Cheng found that young rats preferentially navigated by geometric shape to a corner with food reward, even when clear landmarks existed on the walls of a rectangular enclosure (Cheng and Spetch, 1998; Cheng, 1986; Gallistel, 1990). The rats were prone to errors of searching in the 180° opposite corner for the food reward. Over many trials the rats did learn to discriminate the corners based on landmarks and reduced the search errors (Cheng and Spetch, 1998). Drawing conclusions from that study and the current Study II, it may be that aged rats do learn to distinguish between geometrically different environments (although not in Study I), but landmarks remain little used. Indeed in aged rats delayed learning alone, however, cannot account for the findings that, even in the later recording sessions in the novel environment, place fields of aged rats inconsistently followed the visual landmarks and

that, even in a familiar environment, place fields of aged rats were slow to realign themselves with the room cues (Rosenzweig et al., 2003). These two results may explain why some aged rats never reach the same level of accuracy in their spatial navigation as young rats (Gallagher et al., 1993).

Fourth, aged rats may have increased emphasis on self-motion (idiothetic) information instead of external cue information. Idiothetic cues, as well as external spatial cues, contribute strongly to hippocampal spatial representations (McNaughton et al., 1996; Redish and Touretzky, 1997; Touretzky and Redish, 1996) and participate in navigation through separate, competing learning systems (White and McDonald, 2002). In behavioral tests using a T-maze, Barnes and colleagues (Barnes et al., 1980) found that aged rats rely more on a response strategy than on a place strategy, consistent with an abnormal emphasis on idiothetic information. In support of this view, Study III found that the hippocampus of aged rats does process self-motion information capably. It is possible that mild disorientation prior to entering the environment (as done in Studies I, II, and IV) might further accentuate a bias of aged rats towards using idiothetic information. Reliance upon self-motion information could cause spatial representations of aged rats to remain rigid despite changes in the external environment or behavioral task (Studies I and II; Oler and Markus, 2000), to fail to rotate with landmarks (Study II), or to delay their switch from dependence upon internal to external cues (Rosenzweig et al., 2003).

Pattern completion and pattern separation in the hippocampus

According to a general view of hippocampal function in memory, the hippocampus dissociates distinct experiences (episodic memories) and links them into an organization of related memories (Eichenbaum et al., 1999). Consistent with this view, the rigidity of place cells may reflect an effect of aging on the balance of hippocampal processing of ambiguous information towards retrieval of a stored episodic information (pattern completion) and away from generation of a new episodic representation (pattern separation). According to models of the hippocampus (Redish and Touretzky, 1997; Treves and Rolls, 1994), presentation of a familiar situation, even when some of the cues are missing or modified, results in retrieval of a previously established representation (pattern completion). Conversely, presentation of a larger

number of changes in the environment outweighs the impact of any familiar features, resulting in generation of a new representation (pattern separation; Muller 1996). It has been suggested that pattern separation is accomplished largely by comparison of inputs in the dentate gyrus, whereas pattern completion arises through the auto-associative network of CA3 (Marr, 1971; O'Reilly and McClelland, 1994; Redish et al., 1998; Treves and Rolls, 1994; Redish 1999; McNaughton and Morris, 1987).

Recent evidence from young rats (Lee et al., 2004a; Lee et al., 2004b; Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004) has provided empirical support for these theoretical considerations on the role of each hippocampal subregion in information processing. Those data support a model in which the CA3 subregion is critical for rapid encoding of new information, while the CA1 subregion is used to compare outputs from the entorhinal cortex and the CA3 subregion (Lee et al., 2004a; Leutgeb et al., 2004). Furthermore, when the environment change is minor, the CA3 subregion employs auto-association via the recurrent collaterals to make the representations more similar (pattern complete), whereas, without this extensive recurrent system, CA1 shows less overlap in its spatial representations (Lee et al., 2004b; Vazdarjanova and Guzowski, 2004). When the difference between two successively experienced environments is great, the CA3 cells produce very different spatial firing patterns (pattern separation), whereas the CA1 cells maintain more similar place fields (Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004; for a recent review, see Guzowski et al. (2004)).

Since the hippocampal cells of aged rats often failed in the domain of CA3 cells, that is failed to rapidly encode new spatial information (Study I, II and Tanila et al., 1997b), Study IV examined the effects of aging on place cell firing patterns of the CA1 and CA3 subregions in familiar and novel environments. As would be predicted by the recent evidence implicating the CA3 subregion in the rapid encoding of new information (Lee et al., 2004a; Leutgeb et al., 2004; Nakazawa et al., 2003) and by the well-documented failure of aged animals to rapidly learn new information (Barnes and McNaughton, 1985; Gage et al., 1984; Gallagher and Burwell, 1989; Mizumori and Kalyani, 1997), age-related changes in information processing were particularly evident in the CA3 subregion. CA1 cells of aged rats had firing properties similar to those of the young adults: both groups had overall firing rates at similar values and made some adjustments in firing rates in response to a change in the environment. The CA3 cells of

aged rats, on the other hand, differed from young adults in two important ways: CA3 cells in the aged rats had abnormally high firing rates when compared to young rats and they did not show a robust change in response to the novel environment, indicating a failure to distinguish new information from old.

Guzowski, Knierim and Moser (Figure 24; Guzowski et al., 2004) have presented a model of information processing for young rats based on their results and on theoretical work (Marr, 1971; O'Reilly and McClelland, 1994; Treves and Rolls, 1994; Redish 1999; McNaughton and Morris, 1987) in which the CA1 subregion deals with differences between inputs (environments) in a linear manner. That is, the amount of change in the input leads to a similar amount of change in the output representation of CA1 cells. The CA3 subregion, in contrast, responds non-linearly to changes in input. That is, small changes in the environment do not alter the spatial representation until a threshold point in changes is reached, and then CA3 makes the representations even more distinct than reality. In light of the results of Study IV and of the age-related neurobiological changes to the hippocampal circuit (see below), the combination of two processing failures may lead to the excess rigidity in aged CA3. The aged CA3 may produce excessive pattern completion, and the aged dentate gyrus may fail to produce sufficient pattern separation. Accordingly, a graph of aging outcomes could be added to the model of Guzowski, Knierim and Moser (2004) (see Figure 24). Aged CA1 outputs should be similar to young CA1. Aged CA3 should skew towards pattern completion and (through influence from the dentate gyrus) away from pattern separation.

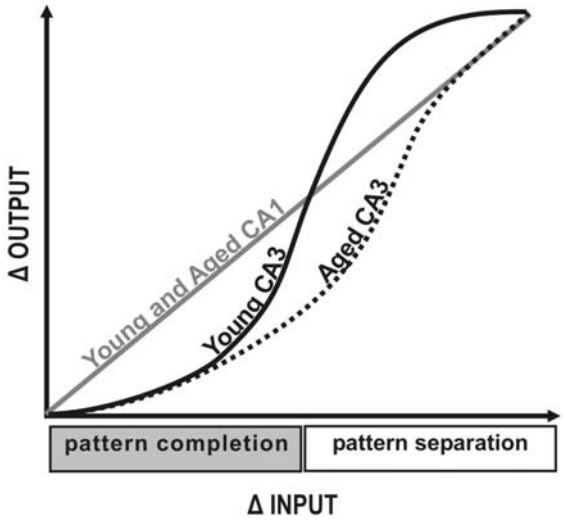

Figure 24
Model of CA1 and CA3 transformations of input (adapted from Guzowski, Knierim and Moser, 2004). In the model the CA3 subregion of aged rats has excessive pattern completion in comparison to the CA3 of young rats.

Information processing and the Neurobiology of Aging

Neurobiological investigations on aging have repeatedly stressed the regional specificity of changes within the hippocampus, affecting synaptic connections, physiology, and plasticity (Barnes, 1994; Rapp et al., 1999; Smith et al., 2000). Therefore, Study IV, which pointed to the failings of the aged CA3 subregion, provides an important means of tightening the connection between neurobiological changes and spatial information processing. Why would the aging CA3 be hyperactive and fail to rapidly encode new information about the external environment? Four age-related changes in the hippocampal formation may pave the way for the hyperactivity and impaired encoding of principle neurons in the CA3 subregion, where the circuit includes recurrent excitation by CA3 collaterals. Such sources could include less inhibition by interneurons, reduced cholinergic modulation, a diminished entorhinal cortex input to the dentate gyrus, and reduced synaptic plasticity. We have incorporated these age-associated changes into a model of hippocampal information processing in which the CA3 auto-associative network becomes disinhibited, such that pattern completion dominates function in this subregion of the hippocampus (see Figure 25).

First, in contrast to preserved number of hippocampal pyramidal cells (Rapp and Gallagher, 1996), the number of inhibitory interneurons or the intensity of glutamic acid decarboxylase (GAD) immunostaining in these neurons is decreased in aging (Cadacio et al., 2003; Stanley and Shetty, 2004; Vela et al., 2003). This effect may be particularly influential in the CA3 stratum radiatum, where the CA3 recurrent collaterals terminate (Freund and Buzsaki, 1996). Specifically, Stanley and Shetty (2004) noted a pronounced decrease in GAD-67 immunostaining in the stratum radiatum of aged brains. This reduction in inhibition could contribute to hyperactivity of the CA3 pyramidal neurons.

Second, the degree of cholinergic modulation is attenuated (Chouinard et al., 1995; Nicolle et al., 1999; Sugaya et al., 1998). The medial septum cholinergic modulation may set the stage for learning by focusing attention and switching the hippocampus from recalling stored information into a learning mode through altering the balance between entorhinal versus CA3 inputs to CA1 (Hasselmo et al., 1995). Hasselmo and colleagues (1995) have shown through slice recordings from activated recurrent collaterals that reduced cholinergic input releases the CA3 auto-association

fibers from inhibition, possibly resulting in a form of run-away excitation. Decreased cholinergic input in aged animals may reduce the relative influence of new information through the perforant path, and subsequently favor the reactivation of information in the CA3 network (Hasselmo and Schnell, 1994; Hasselmo and Wyble, 1997). In support of this notion, recent studies have shown that rats with selective cholinergic deafferentation of the hippocampus also have rigid place fields in response to a novel environment (Ikonen et al., 2002; Leutgeb and Mizumori, 1999).

Third, aging is accompanied by a substantial reduction in the synaptic innervation from entorhinal cortex layer II neurons into the dentate gyrus and CA3 region (Barnes and McNaughton, 1980; Geinisman et al., 1992; Smith et al., 2000). At the same time, markers for the connectional zone occupied by the CA3 recurrent excitatory synapses remain unaffected by aging (Rapp et al., 1999; Smith et al., 2000). Thus, the primary cortical input to the hippocampus is substantially reduced, which could impair the detailed processing of new sensory information and encourage similar spatial representations across distinct environments.

Fourth, synaptic plasticity, as measured by long-term potentiation, is generally weakened in the hippocampus of aged rats (Barnes, 1994; Foster, 1999; Wu et al., 2002). In long-term potentiation studies, aged rats generally require more stimulation to reach to same levels of synaptic enhancement as young rats, and the potentiation decays faster in aged rats than in young ones (Foster, 1999). These factors could make it more difficult for the aged rat hippocampus to learn new information. Interestingly, one of the bases of the weakened synaptic plasticity is that the pyramidal neurons of the CA1 subregion are less excitable by CA3 input in aged rats compared to young (for reviews see Rosenzweig and Barnes, 2003; Wu et al., 2002). Indeed it has been specifically shown that the CA1 dendrites of aged rats are not as depolarized by the CA3 inputs from the Schaffer collaterals as the CA1 dendrites of young rats (Rosenzweig et al., 1997). Nevertheless, most studies have found similar firing rates in CA1 cells of young and aged rats when recordings are made in vivo (current results; Barnes et al., 1983; Barnes et al., 1997; Markus et al., 1994; Mizumori et al., 1996; Mizumori and Kalyani, 1997; but see Shen et al., 1997). This seeming paradox could be explained by the hyperactivity of CA3 cells providing increased excitatory drive onto CA1 synapses. CA1 cells may compensate for the hyperactive CA3 cells by becoming less excitable, or

alternatively, the hyperactivity in CA3 cells may represent a compensation for a loss of CA1 excitability.

Why do the CA1 cells *not* show age-related firing abnormalities, as demonstrated in Study IV? In young rats the CA1 firing pattern can arise independently of CA3 activity (Lee et al., 2004b; Leutgeb et al., 2004), and CA1 place cells have been shown to encode environment-specific information despite a lesion of CA3 (Brun et al., 2002) or suppression of the CA3 output by septal inactivation (Mizumori et al., 1989). In aged rats, the preserved projection from entorhinal cortical layer III to CA1 (Smith et al., 2000) may extend the independence of CA1 cells so they display less rigidity in coding new information than CA3 cells (current data).

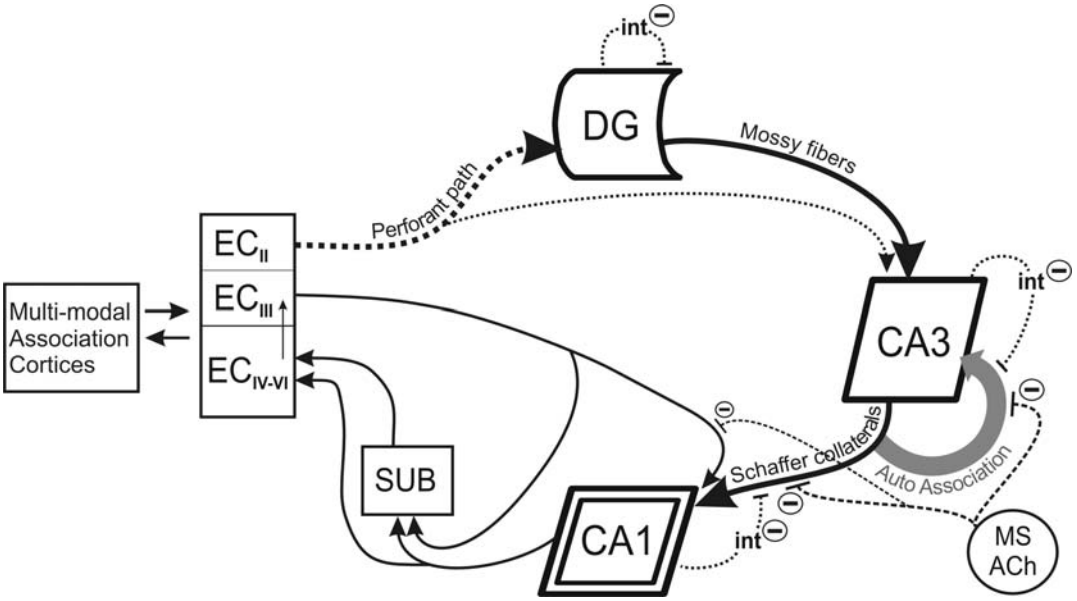

Figure 25
The neurobiology of the aged hippocampus.
See Figure 3 for a description of the pathway. Age-related deteriorations are denoted by dashed lines. Solid arrows remain intact. With aging the entorhinal cortex provides less input to dentate gyrus and CA3, the medial septum provides less cholinergic modulation, interneuron (int) activity is decreased, and the CA1 hippocampus is less excitable (depicted with a doubly insulated box). These changes may increase the activity of CA3 recurrent collaterals (in grey).

The model of the aging hippocampus presented here (Figure 25) incorporates these findings. The dentate gyrus and the CA3 subregion receive fewer details of the

external environment from the entorhinal cortex, perhaps already limiting changes in hippocampal representations. The reduced cholinergic and interneuron modulation may result in an increased CA3 firing rate while further limiting the learning of new information. The weakened synaptic plasticity and reduced excitability of CA1 neurons may limit new learning. Finally, the preserved connection from entorhinal cortex to CA1 may encourage the independence of CA1 representations from those of CA3.

How does spatial learning ability relate to the place cell findings?

It is important to consider how hippocampal spatial representations in a random foraging task could relate to performance on a spatial task. There were two general characteristics of spatial information processing which correlated strongly with individual performances on the spatial water maze task. Firstly, those rats whose spatial representations failed to change despite alterations in the external environment performed the poorest on the spatial task. Secondly, those rats whose spatial representations failed to rotate with the visual landmarks performed poorest on the spatial task.

In order to understand how the rigidity of hippocampal spatial representations in a foraging task might underlie the age-associated impairment in spatial learning within the single, constant environment used in spatial water maze training, it is important to note that training in this task involves beginning trials from any of four different starting points in the water maze. Thus a central demand of learning this task is to distinguish and ultimately reconcile the distinct swim paths associated with the four starting points. According to an episodic view of hippocampal function, during initial learning it might be expected that the rat would need to distinguish these different episodes, and eventually it would be required to reconcile them within a comprehensive network of path representations. Failure to distinguish the different types of trials, because of the large number of shared spatial cues involved among them, could retard the establishment of a cohesive representation that allows navigation from any starting point. Thus, aged place cells which are rigid across distinct environments may be illustrative of an impairment in distinguishing different types of trials.

Alternatively, according to a multiple maps view of hippocampal function, the problem of aged rats may be a failure to self-localize to the correct map (Redish and

Touretzky, 1999; Redish, 1999). For each trial in the water maze the rats must recall the correct internal map. Aged rats may have difficulties associating their internal (hippocampal) maps to external cues of the water maze due to the neurobiological deteriorations discussed earlier, and therefore the cues at the beginning of each water maze trial do not successfully drive the recall of the correct map. Thus, aged place cells which are rigid may be illustrative of a failure of the external cues to drive separation of hippocampal maps (Redish et al., 1998; Redish and Touretzky, 1999).

Successful performance on the water maze task is based on using the visual landmarks to navigate. It is therefore no surprise that the extent to which place cells followed rotations of the visual landmarks predicted the performance of the rat on the water maze test. Those rats whose place cells did not use the visual landmarks during the place field recordings were also those rats which did not use the visual landmarks to navigate during the water maze tests.

Limitations of place cells and of the current studies

It is the fantasy of every neurophysiologist to simultaneously monitor the activity of all neurons in the human brain with temporal and spatial precision whilst the subject is freely behaving. Such analysis would provide tremendous insight into the contribution of each neuron to the complex network of brain activity which produces behavior. Unfortunately, to this date it remains a fantasy.

Each neurophysiological technique must sacrifice some aspects of the fantasy in return for studying other aspects. For example, the fMRI technique can study activation of the entire human brain, while the subject thinks about a computer screen. However, the spatial precision is far from individual neurons, the trials must be averaged which reduces temporal precision, and the subjects are restricted in behavior.

The current studies chose the extracellular action potentials of hippocampal place cells of rats as a window into cognitive aging. The benefits of single-cell recordings are that they allow the study of information processing by individual neurons of a particular region within the brain with enough temporal precision to correlate activity of individual neurons with particular behaviors. The limitations of single-cell recordings are that the electrodes sample a very small selection of the neurons from only one subregion of the brain, monitoring numerous brain regions simultaneously is

difficult, and the anatomical locations and connections of the recorded neurons can be defined only in general terms. For example, the CA1 hippocampus contains roughly 400,000 pyramidal cells (Rosenzweig and Barnes, 2003); the published "world record" for number of place cells recorded simultaneously is 141 (Wilson and McNaughton, 1993).

The current studies recorded a range of 1 to 10 neurons at a time. This limited sample of cells is a drawback as individual cells may not provide a fair representation of the entire population. On the other hand, in these studies the aim was to sample cells from a broad range of rats, especially aged rats with varying spatial learning abilities. To this end, the Studies I-III used 8 young rats and 11 aged rats. Furthermore, the current studies averaged all the cells simultaneously recorded into one average value for each rat. Treating the animals as the unit reduces any bias due to greater contribution of cells from one animal.

Another weakness of the current studies, particularly for Study IV, is that cells of different subregions were not recorded simultaneously, but instead successively separated by at least one week. The advantage of one recording session with many simultaneous recordings is that all data is collected under the same conditions. Today it is possible to record from two brain regions at the same time, and certainly simultaneous recordings of aged CA1 and CA3 place cells is an important next step from Study IV. Even so, recordings of several brain regions are far from monitoring the entire neuronal network of activity, thereby limiting the power of the technique.

Spatial cells, non-spatial task

Place cells are a useful window into cognitive aging because they provide insight into mechanisms underlying a behavioral task on which aged subjects are impaired (spatial navigation). Clearly in the ideal case, hippocampal spatial representations would be recorded during the spatial learning task itself. To assess the spatial learning capacity of our rats, we selected the water maze task because there is a vast literature linking age-associated water maze impairments with the neurobiological changes of aging. However, the standard water maze is not suitable for recording studies due to inevitable undersampling of the arena, not to mention the fact that water and electrical recordings may cause electrocution of the rat (but see Hollup et al., 2001).

For this reason we chose to relate the best-characterized place cell recording task (random foraging) to spatial learning in the water maze in an attempt to find general characteristics of spatial information processing that may underlie the age-related memory-impairment. Nevertheless, random foraging does not demand hippocampal function, and place cells probably do encode information differently in hippocampal-dependent tasks than hippocampal-independent ones (Kentros et al., 2004).

Place cells are a useful window into cognition when they are placed in the proper behavioral and neurobiological contexts. Alone the spatial representation results of these studies are interesting but limited in value. By characterizing the spatial learning abilities of each individual rat, we can begin to identify which characteristics of aged place cells are important contributors to age-related memory impairments. Furthermore, analysis of how the place cell and behavioral results fit into the well-researched aging neurobiology provides an overall picture of how information is processed differently in the aged memory-impaired brain.

Predictions and Future Studies

Tasks on which aged rats will likely be impaired

Recently, several spatial memory tasks have been developed specifically to provide enough sampling of the arena area for tests of place cells (for example, see Kentros et al., 2004 and Ferbinteanu and Shapiro, 2003). Indeed, as discussed earlier, Rosenzweig and colleagues (2003) have already recorded from aged rats as they performed a task in which the goal location was predicted by the visual cues, which were in conflict with the self-motion cues. It will be interesting in future studies to record cells of aged rats performing spatial tasks in which these two sources of information are not in conflict with one another.

Judging from behavioral studies and these current results, one can predict that many aged rats would fail to learn a spatial task and the place cells of animals would fail to show any learning related changes. For example, Ferbinteanu and Shapiro (2003) found that some place cells of young rats encoded pure location, for other cells the location-specific firing depended on from where the rat came, and for still other cells the location-specific firing depended on to where the rat would go. One would predict from the rigidity results of Studies I and II that aged rats would possess fewer cells

which distinguish between the episodes, as long as the distinction was based on the external cues. If the distinction was based on internal, self-motion cues, then aged rats should possess equal numbers of retrospective and prospective cells as young rats.

Another recently developed task has been designed to study the speciality of the hippocampus, rapid encoding of memory. This task requires rats to learn paired associations between food and location in one trial (Day et al., 2003). Based on the failure presented in the current studies of CA3 cells of aged animals to rapidly encode new information, one would predict that aged rats would be especially impaired on this one-trial learning task. Furthermore, any changes which occur in young spatial representations due to learning of a paired-associate, such as increased encoding of the goal location (Hollup et al., 2001), may occur in aged unimpaired rats, but not in aged impaired rats.

Are there any tasks on which aged rats may outperform younger rats?

Several studies have shown that aged rats tend towards self-motion based navigation strategies in situations in which the young rats readily use spatial strategies (Barnes et al., 1980; Rosenzweig et al., 2003). The data from Study III support this view in that the hippocampal place cells were quite capable of producing new spatial representations when the self-motion cues indicated a change of environment. Possibly aged rats may perform better than young rats on tasks which tax self-motion information, in the same way that hippocampal-lesioned animals learned a response strategy on the T-maze better than intact animals (Packard and McGaugh, 1996). However, young rats are adept at learning response-based strategies also. The task must, therefore, be a difficult one, or set self-motion and place information in conflict, perhaps in a reverse of the task of Rosenzweig and colleagues (Rosenzweig et al., 2003).

The data presented in Studies I, II, and IV as well as in earlier research (Tanila et al., 1997a,b; Oler and Markus, 2000) suggest that the hippocampus and particularly the CA3 subregion of aged rats is impaired in separating distinct environments, relying upon the same representation for both. Two explanations for this result fit in well with the neurobiological data: the CA3 subregion may generate excessive pattern completion, and the dentate gyrus subregion may fail to generate sufficient pattern

separation. The hypothesis may be tested by a task which demands a great degree of pattern completion, such as was tested by Nakazawa and colleagues (Nakazawa et al., 2002). If the hypotheses hold true, then aged rats should perform better than the young rats precisely because they fail to separate the patterns. A small change in the environment may disrupt the performance of the young rats, whereas the aged rats may perform without difficulty.

Alleviating age-related memory impairments

The ultimate goal of cognitive aging research is, of course, to develop means for alleviating age-related cognitive impairments. The results from these studies, that CA3 cells show abnormal encoding of new external environmental information but CA1 cells did not differ from those of young rats, provides a potentially new starting point for understanding of and clinically intervening in age-related memory impairments. Furthermore, recently Small and colleagues (Small et al., 2004) have identified the dentate gyrus subregion as particularly vulnerable to normal aging; specifically, it showed less activation in both aged monkeys and aged rats in comparison to young animals. These findings point to the CA3 and dentate gyrus as potentially important targets for intervention.

As an experimental starting point, one can predict that processing based on the CA1-entorhinal connection should be mostly intact in aging. A study which lesioned the CA3 subregion of young rats recorded normal place cells from CA1 and found intact spatial recognition memory, but impaired recall of trajectories (Brun et al., 2002). Indeed, aged monkeys have been found to have impaired spatial processing but preserved recognition memory (Rapp et al., 1997). Furthermore, one would predict that a lesion of CA3 or severing the Schaffer collaterals would alleviate any rigidity in CA1 place cells, making their firing dependent upon input from the entorhinal cortex.

Currently drug interventions for Alzheimer's Disease have attempted to restore the function of the cholinergic system through inhibitors of the enzyme acetylcholinesterase AChE, which inactivates acetylcholine. Although success has been mild in Alzheimer's Disease patients, who have already lost the majority of their cholinergic input to the hippocampus (Davies and Maloney, 1976), the AChE inhibitor tacrine has improved the learning ability of aged rats on the water maze (Aura et al.,

1998). It would be interesting to test such an AChE inhibitor in aged rats in combination with place cell recordings to ascertain if the symptoms found in the current studies could be mitigated.

There is high hope today that medical treatments can become more and more specific to the desired target. With this in mind, clinical interventions which restore hippocampal function may alleviate memory impairments. Specifically, high firing rates and a failure to rapidly encode new information afflict the CA3 pyramidal neurons. Interestingly, a recent fMRI study showed that among aged humans with mild cognitive impairment, greater activations in medial temporal lobe cortex predicted greater subsequent decline over the course of three years (Dickerson et al., 2004). Treatments, therefore, that restrain the neuronal hyperactivity and reestablish normal CA3 function may provide hope for a novel mode of therapy for age-related cognitive decline.

7. Conclusions

This series of studies assessed the spatial information processing of hippocampal neurons from young memory-intact rats, aged memory-intact rats, and aged memory-impaired rats.

The following conclusions can be drawn:

I. The degree of rigidity of hippocampal spatial representation to alterations in the external environment predicts the magnitude of spatial performance deficit.

II. The extent to which place fields of aged rats followed rotations of the visual landmarks predicted the rat's ability to use the landmarks to navigate.

III. The aged rats were as capable as young rats at creating new spatial representations when self-motion information indicated environmental change but the external visual information remained the same.

IV. Aged CA1 cells possess similar characteristics to young CA1 cells, but aged CA3 cells are hyperactive and fail to rapidly encode new information in comparison to young CA3 cells.

What causes age-related memory impairments on spatial tasks?
Evidence from these studies indicates that it is a failure by the aged hippocampus to encode and to use external environmental cues, and that failures in the CA3 subregion may play a particularly prominent role.

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Appendix: Original Publications (I–IV)

I

Place cell rigidity correlates with impaired spatial learning in aged rats

Neurobiology of Aging

24(2): 297-305

March-April 2003

by

Iain A. Wilson,
Sami Ikonen, Robert McMahan,
Michela Gallagher, Howard Eichenbaum, and Heikki Tanila

II

Cognitive Aging and the Hippocampus: How Old Rats Represent New Environments

The Journal of Neuroscience

24(15): 3870-3878

14 April 2004

by

Iain A. Wilson,
Sami Ikonen, Irina Gureviciene, Robert McMahan,
Michela Gallagher, Howard Eichenbaum, and Heikki Tanila

III

Place cells of aged rats in two visually identical compartments

Neurobiology of Aging

26(7): 1099-1106

July 2005

by

Iain A. Wilson,
Sami Ikonen, Kestutis Gurevicius, Robert McMahan,
Michela Gallagher, Howard Eichenbaum, and Heikki Tanila

IV

Age-associated alterations of hippocampal place cells are subregion specific

submitted

by

Iain A. Wilson,
Sami Ikonen, Michela Gallagher,
Howard Eichenbaum, and Heikki Tanila

PUBLICATIONS
SERIES OF REPORTS, DEPARTMENT OF NEUROLOGY

1. **Juhani Partanen (1978):** Time-locked phenomena of human motor unit potentials. An electromyographic study of satellites and doubles.
2. **Eeva Leino (1981):** Clinical and biochemical studies on progressive myoclonus epilepsy.
3. **Hilkka Soininen (1981):** Senile dementia. A clinical, neurochemical and etiological study.
4. **Rolf Danner (1982):** Adverse effects of anticonvulsive treatment on peripheral nerve conduction and posterior dominant EEG rhythm.
5. **Markku Saksa (1982):** The autonomic nervous system in experimental allergic neuritis. A functional, morphological and biochemical study.
6. **Juhani Sivenius (1982):** Studies on the rehabilitation, epidemiology and clinical features of stroke in East Central Finland.
7. **Asla Pitkänen (1987):** Somatostatin in epilepsy. An experimental and clinical study.
8. **Esa Mervaala (1987):** Evoked potential in human epilepsy. A neurophysiological study.
9. **Kari Reinikainen (1988):** Neurotransmitters in Alzheimer's disease.
10. **Tapani Keränen (1988):** Epilepsy in adults. An epidemiologic study in Eastern Finland.
11. **Jukka Jolkkonen (1988):** Vasopressin in the central nervous system. A study based on cerebrospinal fluid measurements.
12. **Jouni Sirviö (1989):** The cholinergic system in ageing and dementia. With special reference to acetylcholinesterase.
13. **Hannu Koponen (1989):** Delirium in the elderly. A clinical, neurochemical, neuropsychological and neuroradiological study.
14. **Asla Pitkänen (1989):** Somatostatin in experimental and human epilepsy.
15. **Eeva-Liisa Helkala (1990):** Memory in patients with Alzheimer's disease and demented patients with Parkinson's disease.
16. **-**
17. **Paavo Riekkinen Jr (1990):** Animal models of age-related degeneration of subcortical regulatory systems. With special reference to cholinergic, noradrenergic and serotonergic systems.
18. **Toivo Halonen (1990):** Neurotransmitter amino acids in epileptic convulsions and during vigabatrin treatment.
19. **Ulla Lepola (1990):** Panic disorder. A clinical, neurochemical, neuropsychological, and neuroradiological study.
20. **Kari Murros (1991):** Stress reactions of brain infarction. A prospective study on 105 patients with acute ischemic brain infarction of internal carotid artery territory.
21. **Aarne Ylinen (1991):** Hippocampal reactions and their pharmacotherapy in experimental epilepsy.
22. **Antti Valjakka (1992):** The subcortical deafferentation of the hippocampus and noradrenergic lesions as experimental models of dementia. Hippocampal electrophysiology.
23. **Aimo Rissanen (1992):** Cerebrovascular disease in the Jyväskylä region, Central Finland.
24. **Reetta Kälviäinen (1992):** Newly diagnosed epileptic seizure disorder in adults. A prospective follow-up study on 100 patients.
25. **Maria Mazurkiewicz (1992):** The effects of the enhanced GABAergic transmission on cognitive functions: An experimental study.
26. **Pekka Jäkälä (1992):** Modulation of attention and working memory by noradrenergic, serotonergic and cholinergic systems. An experimental neuropsychopharmacological study.
27. **Kari Alhainen (1992):** Anticholinesterase drug, tacrine (THA), in Alzheimer's disease. Discrimination of responders and nonresponders.
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29. **Hannele Lahtinen (1993):** Hippocampus in experimental models of temporal lobe epilepsy. Amino acid-mediated neurotransmission and nerve cell injury following the transection of fimbria-fornix and the electrical stimulation of perforant pathway in rat.

30. **Päivi Hartikainen (1994):** Normal ageing. A neurochemical, neurophysiological and neuropsychological study with special reference to Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.
31. **Outi Heinonen (1994):** Neuropathologic and peripheral markers of Alzheimer's disease with special emphasis on β-amyloid accumulation.
32. **Minna Riekkinen (1994):** The interactions between cholinergic and serotonergic systems in the modulation of spatial navigation and passive avoidance behavior. An experimental neuropsychopharmacological study.
33. **Keijo Koivisto (1995):** Population-based dementia screening program in the city of Kuopio, Eastern Finland: Evaluation of screening methods, prevalence of dementia and dementia subtypes.
34. **Arja Tuunainen (1995):** Evaluation of epileptic patients for temporal lobe surgery and postoperative follow-up. An electrophysiological study with neuropsychological, psychiatric and clinical correlates.
35. **Mervi Pitkänen (1995):** The role and pharmacological modulation of the NMDA receptor/channel on hippocampal synaptic transmission and behavior.
36. **Olli Kosunen (1996):** A neuropathologic study on Alzheimer's disease with a special emphasis on diagnostic accuracy.
37. **Mikko Laakso (1996):** MRI of hippocampus in incipient Alzheimer's disease.
38. **Maarit Lehtovirta (1996):** Familial Alzheimer's disease. A clinical and molecular genetic study.
39. **Tuomo Hänninen (1996):** Age-associated memory impairment. A neuropsychological and epidemiological study.
40. **Vesa Savander (1997):** Organization of intrinsic connections in the rat amygdaloid complex with special emphasis on the lateral, basal and accessory basal nuclei.
41. **Heikki Sorvari (1997):** Neurons containing calcium-binding proteins in the human amygdaloid complex.
42. **Tiina Kotti (1997):** Excitotoxicity-induced neuropathological changes in the rodent hippocampus. Possible functional consequences and drug treatments.
43. **Sirja Ruotsalainen (1997):** Serotonergic system and its interactions with cholinergic receptor mediated mechanisms in the modulation of working memory. An experimental study.
44. **Seppo Helisalmi (1998):** Molecular genetics of Alzheimer's disease with special emphasis on presenilin, amyloid beta precursor protein and apolipoprotein E genes.
45. **Merja Hallikainen (1998):** Age-associated memory impairment, and apolipoprotein E. A population-based clinical, neuropsychological, neurophysiological and neuroimaging study.
46. **Matti Vanhanen (1998):** Cognitive function in glucose intolerance in the elderly: the role of hyperinsulinemia.
47. **Kirsi Juottonen (1998):** MRI-volumes of the entorhinal, perirhinal and temporopolar cortices in normal aging and in Alzheimer´s disease.
48. **Raimo Pussinen (1999):** An experimental study on the role of α_1-adrenoceptors and putrescine in the modulation of hippocampal plasticity and memory encoding - interactions with NMDA receptors.
49. **Tarja Puumala (1999):** Monoamines in the modulation of attention and response inhibition: development of a new animal model of attention deficit and impulsivity.
50. **Mia Mikkonen (1999):** The human entorhinal cortex. Anatomic organization and its alteration in Alzheimer's disease and temporal lobe epilepsy.
51. **Jukka Puoliväli (2000):** An experimental study on the cholinergic modulation of cortical arousal and cognitive functions. With special emphasis on apolipoprotein E.
52. **Kauko Pitkänen (2000):** Stroke rehabilitation in the elderly. A controlled study of the effectiveness and costs of a multidimensional intervention.
53. **Mikko Hiltunen (2000):** A molecular genetic study of factors involved in Alzheimer's disease.
54. **Sami Ikonen (2001):** The role of the septohippocampal cholinergic system in cognitive functions.
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57. **Tero Tapiola (2001):** Biological markers for Alzheimer's disease. With special emphasis on cerebrospinal fluid ß-amyloid and tau.
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61. **Pauliina Korhonen (2002):** Gene regulation in neuronal degeneration - Role of mSin3 and YY1 factors.
62. **Miia Kivipelto (2002):** Vascular risk factors in Alzheimer's disease and mild cognitive impairment. A longitudinal, population-based study.
63. **Margit Overmyer (2002):** Gliosis in relation to Alzheimer's hallmark lesions in aging and Alzheimer's disease. A postmortem immunohistochemical study.
64. **Marja Äikiä (2002):** Verbal memory in newly diagnosed partial epilepsy. A neuropsychological study.
65. **Li Liu (2003):** Cholinergic neurotransmission, amyloid-β peptide and the pathogenesis of Alzheimer's Disease. A study in the APP and PS1 double transgenic mouse model.
66. **Jun Wang (2003):** The role of Aβ-peptide on spatial memory, EEG, auditory evoked potentials and nicotinic cholinergic receptors in A/P transgenic mice.
67. **Juhana Aura (2003):** Interaction of muscarinic acetylcholine and N-methyl-D-aspartate –type glutamate receptors in the regulation of spatial learning and memory.
68. **Johanna Kuhmonen (2003):** Neuroprotection in experimental acute cerebral ischaemia: α2-adrenoreceptor agonism, MAO-B inhibition, and enhancement of GABAergic neurotransmission as neuroprotective strategies.
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71. **Maija Pihlajamäki (2004):** Functional MRI studies on human declarative memory.
72. **Chuan-sheng Zhao (2005):** Psychotropic medication and functional recovery following cortical stroke in aged rats.
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74. **Sinikka Peurala (2005):** Rehabilitation of gait in chronic stroke patients.
75. **Laura Parkkinen (2005):** Impact of α-synuclein pathology on aging.