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THE AGING HIPPOCAMPUS: 
NEURAL MECHANISMS UNDERLYING LEARNING AND 
MEMORY DEFICITS IN OLD RATS

by

Jiemin Shen

A Dissertation Submitted to the Faculty of the 
GRADUATE INTERDISCIPLINARY PROGRAM IN NEUROSCIENCE 
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1996
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Jiemin Shen entitled The Aging Hippocampus: Neural Mechanisms Underlying Learning And Memory Deficits in Old Rats and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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ABSTRACT

This dissertation focuses on the effect of aging on two functional aspects of rat hippocampus: cholinergic synaptic transmission and place specific firing of CA1 pyramidal cells. The effect of age on the cholinergic slow EPSP was studied in hippocampal slices of young, adult and old rats. The old rats were impaired on the spatial version of the Morris water task. The amplitude of the slow EPSP was significantly reduced in old rats in all hippocampal subregions (CA1 59%; CA3 55%; and DG 56%). Few statistically significant correlations, however, were found between the age-related deficit in spatial learning and the cholinergic deficit. In the subsequent study, effects of selective neurotoxic lesions of cholinergic afferents to the hippocampus on performance on two versions (spatial working memory and spatial reference memory) of the radial-8-arm maze task were examined. The lesioned rats were impaired in acquisition, but not retention, of the working memory task. There was no treatment effect, however, on acquisition of the reference memory task. The results suggest that the age-related deficits in hippocampal cholinergic function may contribute to behavioral deficits of old rats in working memory situations, but may not be primarily responsible for the spatial reference memory problem in the Morris water task.

The spatial and temporal firing characteristics of CA1 neurons were studied in young and old rats performing a simple spatial task on a rectangular track. The average place fields of young rats were larger than those of old rats. Precession of spike discharge relative to the theta rhythm proceeded faster in old rats, while the total phase change remained constant. These age-related changes were apparently due to a loss of experience dependent place field expansion of old rats during the first few laps around the track for a given recording session. The field sizes were not different between groups on lap 1.
Because experience-dependent place field expansion is a prediction of two recent theories which invoke asymmetric Hebbian LTP, the present observations point towards a substantial deficit in an LTP-like process in old rats.
As a society we are becoming older. Persons 65 years of age or older numbered 33.2 million in 1994, representing 12.7% of the U.S. population. This older population will continue to grow in the future, and is projected to reach 70 million by 2030, which will represent 20% of the total population. The oldest old, those 85 and older, is the fastest-growing segment of our population and is going to rise from the current 3.3 million to 9 million 25 years from now, and double again by the year 2050. This dramatic demographic shift has placed a great demand for more research on issues that surround aging, which received little attention before the 1970s. Currently, such research is widely recognized by the whole society as being necessary and extremely important (Institute of Medicine, 1991). Only through a greater effort to promote aging research will it be possible to control acute and chronic illness in old age and to achieve a major reduction in disability and suffering. It is important not only to extend life, but also to enhance it by improving the quality of life and independence of the whole old population as well.

One of the health problems associated with old age is the gradual decline in cognitive function, characterized initially by a disturbance in specific types of memory ability. This memory impairment is one of the most consistent age-related alterations in human cognitive function, and represents one of the most common neuropsychiatric complains of elderly people. Additionally, about 10% of people 65 years and older suffer from dementia, and approximately half of these have Alzheimer's disease. This disease produces an insidiously progressive cognitive loss typically leading to a requirement for long-term institutional care. There is substantial evidence that memory loss may occur with advancing age in many, and conceivably all, mammalian species (e.g., Bartus and Dean, 1987). Thus, the use of animal models becomes a useful approach to investigate
the neural mechanism underlying the age-related neural changes that might be responsible for memory loss in old age. Although animals other than humans do not contract Alzheimer’s disease, the study of aging in other species provides valuable information that will allow development of strategies to enhance the memory capacity in older organisms. These insights may eventually prove to be effective for the treatment of diseases, such as Alzheimer’s disease. One of the most frequently used animal models is the rodent, which shows spatial learning and memory deficits in old age (e.g., Barnes, 1979). A similar age-related impairment in spatial memory also occurs in humans (e.g., Light and Zelinski, 1983).

In 1957, a famous case study was reported by Scoville and Milner (1957). The patient, who was referred to as H.M., underwent bilateral medial temporal lobectomy, in order to treat his serious, intractable seizures. After the operation, although seizure activity was greatly reduced, the patient showed severe anterograde and retrograde amnesia, and lost the ability to store many kinds of new information into long-term memory. Because the hippocampus is one of the major brain regions that was removed in H.M., this report stimulated a wide interest in this brain area, that continues today. It is now well established that the hippocampus is essential for learning and memory (Cohen and Eichenbaum, 1993; Scoville and Milner, 1957; Squire, 1987), especially spatial learning memory in rodents (Jarrard, 1993; Morris et al., 1982; O’Keefe and Nadel, 1978; Sutherland et al., 1982). Interestingly, among the structures of the temporal lobe, the hippocampus is also one of the brain structures that undergoes age-related morphological changes during the aging process (Hasan and Glees, 1973; Landfield et al., 1977; Price et al., 1991), and is severely compromised in some age-related diseases, such as Alzheimer’s Disease (Ball, 1978; de Leon et al., 1989; Mann, 1985; Terry et al., 1981).
Because spatial memory is impaired in both aged humans and aged rats, and the hippocampus is the brain structure critical for spatial learning and memory in both species, the use of the aged rats as an experimental model has been a productive one, and intensive studies on the neural alterations in the aged hippocampus has provided important information concerning the neural alterations in this structure in old humans. In the past decades, many electrophysiological studies have been conducted in rat hippocampus, and basic biophysical parameters of single cells, cellular mechanisms in neural circuits, neuromodulatory effects as well as single unit activity in freely behaving animals have been examined extensively in young adult rats. These efforts have provided an enormous amount of data on normal function of the hippocampus, which has also greatly facilitated aging research on this brain region.

In the present dissertation, two lines of research have been conducted to search for age-related alterations in the hippocampus, that might be responsible for spatial memory deficits in old rats. One is a thorough study of the effect of age on cholinergic function in the hippocampus, and an investigation of its possible relationship to spatial learning impairments observed in aged rats (Chapter 6). In order to explore further possible behavioral consequences of the age-related change in hippocampal cholinergic function, a subsequent study, described in Chapter 7, was conducted to examine the effects of selective lesions of cholinergic input to the hippocampus on rat performance of different memory tasks. The other line of research focused on the effect of aging on hippocampal EEG theta rhythm and place specific firing of hippocampal neurons, which are the two prominent electrophysiological phenomena observed in the hippocampus of freely behaving rats, that may be involved in spatial memory. Chapter 8 is a study on the effect of aging on the theta rhythm and plasticity of place specific firing, and Chapter 9 examines the effect of age on the stability of place field maps after the animals were given an
intervention, during which they were allowed to explore in a novel environment. In addition to these chapters, Chapter 1 provides a background review of aging and memory research, emphasizing the role of hippocampus in normal learning and memory, and the use of rodent’s spatial learning and memory behaviors as a model system to search for the underlying neural mechanisms responsible for spatial learning and memory deficits in aging. Chapter 2 is a brief review on the anatomy of the hippocampal formation. Chapter 3 reviews the function of septo-hippocampal projection, mostly with respect to the cholinergic component of this pathway. This provides a basic background for the studies described in Chapters 6 and 7. Chapter 4 reviews what is known about the hippocampal theta rhythm and what is known about place specific firing of hippocampal neurons. This provides the basis for the studies described in Chapters 8 and 9. Chapter 5 reviews what is known about age-related changes in the hippocampus, and several important interventions (e.g., drugs) that may have a positive effect in enhancing memory. Finally, Chapter 10 summarizes the results from studies described in Chapters 6-9, discusses general principles emerging from aging research on hippocampus, and explores some possibilities for future research.
CHAPTER 1 AGING AND MEMORY

1.1 Introduction

It is common knowledge that as a society we are becoming increasingly older. One hundred years ago, only 2% of people in the United States were over 65 years of age. In recent years, one in eight Americans is 65 years old or older (U.S. Bureau of the Census, 1990). Within the total population of approximately 248 million, there are 31 million (12.5%) who are age 65 or older. Projections for the coming decades suggest that older people will comprise an even larger portion of the population. By 2030, one in five will be at least 65 years old. In particular, very old people (over 85 years old) constitute one of the fastest growing segments of population in this country. In 1900, there were only about 123,000 people 85 years and older, compared to an estimated 2.2 million in 1980. By the year 2050, there will be 16 million people in this very-old group, or 5% of the total population. The great increase in the life expectancy, largely due to the advances of medical sciences and public health in conquering life-threatening communicable diseases, is the major factor contributing to the rapidly growth of the older population.

The impact of this dramatic and unprecedented demographic shift is, in many ways, reshaping the demands placed upon medical institutions responsible for providing necessary treatment and care of society's health problems. In particular, it becomes important to investigate the mechanisms and to develop strategies to prevent a number of medical problems that are associated with old age. One of the medical problems that accompanies this demographic shift is the gradual decline in memory function associated with advanced age. Regarding this age-related dysfunction of memory, pathological or disease-related impairments are often emphasized, especially Alzheimer's disease, as the cognitive impairments in such diseases are particularly pronounced. In the United States,
approximately 5% of people currently over 65 years have Alzheimer's disease. The
prevalence rate of dementia doubles every 5 years over the age of 60. The absolute
number of dementia patients will continue to rise as the proportion of the population over
65 and of those very-old (over 85) people increases into the twenty-first century. This is,
indeed, an enormous burden on health care system as well as patient families. For this
reason, there has been a large increase in biomedical research directed toward
understanding, treating, and eventually preventing the devastating dementing diseases
which occur at an increasing frequency with age.

It is important to note, however, that the majority of elderly people do not contract
the Alzheimer's disease. Kral (1978) drew attention to the fact that there is a relatively
mild memory impairment in normally aged people, which is qualitatively different from
that observed in Alzheimer's disease or other types of dementing process. He defined
this type of senescent memory dysfunction as "benign senescent forgetfulness". He
separated old people into two subgroups, one including those suffering from an amnesiac
syndrome, the other including those with benign senescent forgetfulness. He found that
there were highly significant differences in the memory scores on psychological testing,
death rate and longevity between these two groups. He concluded, therefore, that the
benign type of senescent forgetfulness is an expression of physiological cerebral aging, a
senium naturale as far as memory is concerned, whereas the amnestic syndrome of
Alzheimer's dementia is an expression of pathological cerebral aging. The term "benign
senescent forgetfulness" (BSF), however, was not precisely defined. In 1986, a National
Institute of Mental Health (NIMH) work group outlined a proposed set of diagnostic
criteria designed to detect changes in memory in healthy individuals over the age of 50
(Crook et al., 1986). They critically evaluated the growing literature on age-related
changes in human memory, and confirmed that memory function is altered during normal aging after noncognitive, age-related contextual factors were controlled. They called this kind of change in memory “Age-Associated Memory Impairment” (AAMI), which can be normally expected as one ages. The development of the criteria to diagnose AAMI over the past decade has greatly facilitated the clinical distinction between memory deficits that should be expected in normal aging and those that occur due to pathological states such as Alzheimer's disease (e.g., McEntee and Crook, 1990).

Although the AAMI is relatively mild compared to the profound dementia that occurs in Alzheimer's disease, it is not trivial and may severely compromise the individual's ability to function in intellectually demanding activities and employment situations. Furthermore, memory is a critical factor in "fluid" intelligence and is an essential component for problem solving, concept formation, and informed decision making. Finally, while some elderly individuals accept age-associated memory loss as a normal component of their later life, many older individuals are greatly distressed by such impairment. Thus, the practical impact of memory loss with age may be profound in many elderly individuals, and the development of effective treatments for attenuating AAMI will improve the quality of later life, and benefit scores of millions of elderly people considerably.

1.2 Psychometric evidence of memory loss with age

The feeling that one's ability to remember and to retrieve information is not as good as it used to be is an universal complaint among middle-aged and elderly persons. This has stimulated an intensive research in psychological aging of memory. Most psychological studies in 1970s and 1980s were dominated by an information-processing
model (e.g., Murdock, 1967) that is based on three major assumptions. The model assumes that the memory system is a singular system, involving learning, storage and retrieval of various kinds of information, and that information flow can be traced through several hypothetical stages or memory stores. These stores are a modality-specific sensory memory, a short-term primary memory, and a long-term secondary memory that is a repository of newly learned information. Permanent or remote information is stored in a tertiary memory. There have been several reviews summarizing and evaluating the growing literature on age-related changes in human memory (Craik, 1977). The conclusions reached in these reviews, based largely on cross-sectional studies, have been highly consistent. They can be briefly summarized as: there is a significant but only modest decline in sensory memory, either no or rather mild impairments in primary (short-term or immediate) and tertiary (remote) memory. Secondary (recent) memory, however, shows significant age-related deficits when the performance of old and young individuals is compared. These deficits are apparent with both verbal and nonverbal information, and typically occur in free recall. Smaller age differences, however, are found in recognition or cued recall (Poon, 1985). The magnitude of the age difference in secondary memory is reduced considerably when 1) the pacing of the experiment is slower; 2) sufficient practice before testing is given; and 3) when the stimulus material is familiar to the subject (Poon, 1985). Overall, the available studies have reached a general agreement that the memory deficit in aging is due to encoding or retrieval processes. Although there are several studies indicating that there is no age difference in memory retention (Craik, 1968; Poon, 1985), the available evidence is still rather limited. Thus, the age effect on memory retention and forgetting rate in humans remains an open question. Encoding and retrieval are not necessarily independent processes, because retrieval efficiency is dependent on encoding and retrieval alone cannot account for all the processing deficits that have been
observed. It is possible that the memory deficit in aging is caused by age-related changes in neural mechanisms involved in both encoding and retrieval processes, although the neural mechanisms underlying both processes are still poorly understood.

Recently, investigators in the communities of neurobiology, behavioral neuroscience, cognitive neuropsychology and cognitive psychology are reaching another view that there actually exist multiple memory systems in the brain, each of which copes with a specific kind of information (see review, Nadel, 1994). This stands in contrast to the previous psychological view that there is a single memory system processing all kinds of information in different temporal scales. Although there remains some debate as to how many and what memory systems exist, the theory of multiple memory systems (Schacter and Tulving, 1994), nevertheless, points out a new avenue for future psychometric studies on memory changes during aging. If age-related alterations occur only selectively in memory of certain modalities, it would also, on the other hand, facilitate studies on the differentiation of multiple memory systems.

1.3 Animal models of normal age-related memory deficits

Because it is important to understand the mechanisms underlying age-related memory deficits, in order to develop effective means of treating AAMI, animal models of normal age-related memory deficits potentially become very important. There are clearly certain advantages in using animals rather than directly studying humans in that, in contrast to humans, a variety of attributable factors, including genetic background and past experience, can be easily controlled in experimental animals. Furthermore, it is possible to use invasive strategies in animals to gain deeper understanding of the neurobiological processes underlying the memory deficits. This would be impossible for humans.
An important approach to the study of animal models of memory aging is to choose a behavior in the species of study that has some analogue in human behavior, and which changes in a comparable manner in both species. In addition to this behavioral requirement, it is also important to determine whether or not the same neural structures are likely to be responsible for the production of these similar behaviors between these species. Only when a similar brain and behavior relationship is established in both species, is study in the animal model meaningful. Results from such studies can then provide valuable information for the development of potential treatment strategies for humans. One of the most extensively studied age-related behavior in rodents is the spatial memory deficits that occur both in elderly humans and in old rats, which is discussed in what follows.

1.3.1 Age-related spatial memory deficits in humans

One of the criticisms of experiments conducted in the laboratory is that the tests were administered in artificial situations and that, perhaps, if measures of cognitive ability were closer to "real life", people would perform better. Spatial memory is one type of everyday memory, which involves remembering the locations of objects, the sites of landmarks in larger environments, and routes through buildings and cities. This type of memory is useful for navigating effectively through space. In humans, it has been found that, in reconstructing arrays of everyday objects, older adults do not remember as much as the young do (Attig, 1983). In addition, younger adults perform significantly better than older adults in recalling the locations of "buildings" drawn on maps (Ohta and Kirasic, 1983) and recognizing correct building placements (Light and Zelinski, 1983). Under self-paced conditions, older adults studied the maps significantly longer than younger adults did, but still performed worse (Perlmutter et al., 1981). In an investigation
of spatial memory for a familiar downtown area, age differences were found in building recall and location accuracy (Evans et al., 1984). In two studies conducted in nursing homes, photographs of nursing home landmarks were used as the stimuli, and participants were asked to indicate where each one was located in the home (Herman and Bruce, 1981). The residents' performance was considerably worse than that of students who had taken a 40-min tour of the home. Overall, the above evidence suggests that there is a significant deficit in location and landmark recall in the elderly. No difference was found, however, in the recognition of landmarks, when adults and older individuals were instructed to go through the same route using the same procedure (Wilkniss et al., 1996). This is consistent with the results from laboratory studies. Uttl and Graf (1993) performed two experiments to examine the effect of age on memory for spatial locations in two "real life" environments. In the first experiment, 302 subjects with ages ranging from 15 to 74 were tested after visiting an exhibit room in a museum. Photos of 32 objects in the exhibit room were placed in two albums in a random order. The subjects were asked to indicate the locations of the targets shown by the photos on a copy of the floor plan of the exhibit room. Performance was similar across the young age groups but the accuracy of located items declined sharply for people in their 60s and 70s. In the second experiment, two age groups of subjects (young and old) were asked to do various "secretarial" tasks in a typical office, especially tasks involving moving and replacing things. The spatial memory of the subjects was than assessed using both a map test and a relocation test. The former test is the same as in the first experiment. The latter test required subjects to replace the targets back to the places where they appeared previously. In this experiment, the performance of the old group was also significantly worse than the young group in both tests. This study, therefore, strongly suggests that there is an age-
related deficit in spatial memory performance in the human case, which becomes evident during the sixth decade.

Recent evidence indicates that elderly people are also impaired in route learning. In a task of route learning through slide presentation, Lipman (1991) found that college-aged adults were significantly better than older adults at recalling landmarks, assigning an environmental "scene" to an appropriate route, and recalling critical route-maintaining scenes. Similarly, Kirasic, Allen & Haggerty (1992) showed that older adults, compared with young adults, selected fewer scenes with high potential as critical route-maintaining landmarks and were less accurate in estimating distance between landmarks when presented in scrambled order. In a recent study (Wilkniss et al., 1996), older adults and college students were asked to learn the routes by walking through them by themselves, because it was considered that sensorimotor integration in actual locomotion is critical for spatial learning. They found that older adults made significantly more errors during recall of the route than did young adults, and that the young adults were better than the older adults at ordering pictures of landmarks into correct sequential order of appearance along the route. In the second experiment, the subjects were asked to learn a 2 dimensional map first, before navigating the map space. It was found that older adults made significantly more route-maintaining errors than did young adults. Also, older adults required significantly more attempts than young adults to draw the route correctly from memory.

Taken together, the literature on the effect of aging on spatial memory in humans is consistent in that there is a significant age-related decline in spatial cognition. This deficit does not appear to be due to a less effective use of very salient contextual information in older adults, since it was found that distinctive contexts enhance spatial memory performance in both young and older adults, and there was no interaction between the age
and contextual variables (Zelinski and Light, 1988). Rather, the age-related spatial memory deficit is mostly likely caused by an impairment in the formation of correct spatial representations.

1.3.2 Age-related deficit of spatial memory in rodents

Spatial memory in rats can be tested in a number of ways, and because these animals are excellent foragers, it is relatively easy for them to learn these kinds of problems and perform well. Before interpreting the results from these animal experiments from the point of view of learning and memory, however, it is important to consider the potential artifacts or confounds that might arise unequally for different age groups in behavioral testing situations. Different behavioral manipulations and behavioral tasks have been used by many investigators, in attempt to separate 'pure' age-related learning and memory changes from the performance changes possibly caused by sensory, motor, emotional or motivational alterations that might occur with age. Clearly, proper control of these potential confounding factors is important for the study of age-related neural alterations in the brain that contribute to 'pure' learning and memory deficits in aged animals. Although these issues have not been completely solved in the spatial tasks that are discussed below, much care has been taken to control those potential confounds when spatial learning and memory abilities of aged animals were tested and compared with those of young animals.

1.3.2.1 Multiple-unit T-mazes

The first animal experiments that tested the memory of rats of different ages were conducted in the 14-unit T-maze (Fig. 1.1) by Stone (1929). In the 14-unit T-maze, the rat has to choose the most efficient route to travel through the maze with 14 points. Each
Figure 1.1 Diagram demonstrating the configuration of 14-unit T maze (Stone maze). S represents the start box; G represents the goal box containing reward; straight lines in maze represents guillotine doors. Adapted from Ingram (1988).
of these points had 2 options to choose from, and the rat has to make the correct decision at each binary choice point in order to reach the goal. In Stone's experiments, although a small proportion of the rats appeared to show some deficits, the older animals overall did not show significant cognitive impairments compared with his younger animals. Later studies conducted by other laboratories, however, consistently demonstrate that old rats require more trials to acquire the task, and make more errors compared to young rats (Goldman et al., 1987). Goodrick (1968) food-deprived older animals to 75% of their normal free feeding weight, while the young animals were restricted to 80% of free feeding weight. This led to a higher motivation for food reward in old rats than in young rats. The old rats, however, were still impaired compared to young rats. This suggests that the performance deficit of old rats was not caused by an age-related difference in motivation. The discrepancy between Stone and other investigators appears to be because Stone used a relatively long-lived strain of rat for his aging experiments (3-year average life span), while the rats used by later investigators had only an average life span of 2 years. Thus, the rats used in Stone's experiments (approximately 2-year old) were more equivalent to middle-aged rats, who often show little spatial memory deficit. A rat is considered to be “old” at the age at which 50% mortality for the strain in question occurs. For the Fischer-344 rats that have been used by most labs for aging studies, 24 mo is approximately the 50% motality point, and thus 2 year-old rats are considered to be old in this strain (i.e., essentially equivalent to 65 years old in humans).

In T-mazes with four choices or fewer, however, no difference is found in the performance between different age groups (Goodrick, 1972). While Goodrick suggests that task complexity may be the variable responsible for the age deficit, other interpretations have been suggested based on what strategies can be used by rats to solve
the task in question (Barnes et al., 1980). The 14-unit maze is surrounded by high wooden walls, such that only the ceiling and overhead fluorescent lighting are visible from the floor of the maze. The animal must presumably learn the correct pathway based upon left-right discriminations at 14 choice-points, because external sensory cues have been reduced to minimum. This maze, therefore, is essentially different from open mazes, such as radial, circular and water mazes, in which accurate performance of the animal is guided by its attention to visual landmarks distal to the maze. Unlike these open mazes, the 14-unit maze is most likely solved by an internalized response algorithm of right turns and left turns (Ingram, 1988). Thus, the underlying neural mechanisms necessary to solve open versus enclosed mazes may differ. Using an "open" T maze, Barnes et al. (1980) found that old rats tended to use response strategies to solve the problem, while the younger animals used spatial strategies a significantly greater proportion of the time. This suggests that the response learning on a simple task may be better preserved than spatial learning in old rats, such that age-related memory deficits can be observed only when a sequence of left turns and right turns need to be stored, such as in multiple T mazes that have more than 4 choices. Thus, Goodrick's interpretation that the task complexity may contribute to the age deficit in 14-unit T maze appears to be more plausible.

1.3.2.2 Eight-arm radial maze

The apparatus for the 8-arm radial maze consists of eight arms radiating from a central platform, with the end of each arm containing food reward. The procedure followed most often for training animals on this maze is the "working memory" version. The solution to this problem is to obtain a reward at each arm end without re-entering any of the arms where reward has been previously obtained within the current trial. Thus, the animal must remember which spatial location it has recently visited and go next only to
locations not yet visited on that particular trial. This short-term memory for where the rat has been for any given trial is called working memory. The animal is required to locate themselves in the room with respect to their position on the maze. Many experiments have shown that old rats take more trials to reach a given "criterion" of performance on the radial maze than do young animals (Barnes et al., 1980; de Toledo-Morrell and Morrel, 1985; de Toledo-Morrell et al., 1984; de Toledo-Morrell et al., 1981; Gallagher et al., 1985; Wallace et al., 1980; Wilig et al., 1987). Several studies have been conducted to control for potential confounding variables that might have contributed to the learning deficits of old rats on the 8-arm radial maze. Van Gool et al. (1985) tested brown Norway rats of 8 and 33 months of age on the radial 8-arm maze, and recorded visual evoked potentials from the same subjects. Consistent with other studies, the old rats performed more poorly than did the young rats. No correlation, however, was found between performance on the maze and visual evoked potentials in old rats. Thus, the impaired performance of the older rats is not likely caused by a change in visual sensitivity. In another study, Gallagher et al. (1985) trained old Long Evans hooded rats to reach the same level of accuracy as young rats did in one room. The two age groups were then transferred to a second room, and were required to learn the same maze as in the first room. It was found that the old animals still required more trials than did the young animals to reach criterion performance. Thus the behavioral deficits observed in old animals were not likely to be attributable to problems of learning the motor requirements of the task. Barnes et al. (1987a) examined the performance of old rats in a nonspatial working memory task on the radial-8-arm maze. In this task, strips of different textures, such as paper, wood, rubber, were placed on the floor of different arms. The locations of these cues, relative to the distal cues in the environment, were varied randomly following each trial. The rats were trained to remember which cues had already been sampled within
Figure 1.2 A photograph showing the apparatus of the radial-8-arm maze in an experimental room. A rat with chronically implanted electrodes was present on the maze. Adapted from Barnes (1991).
a given trial. In spite of the fact that this task was much more difficult to learn, it was found that old animals perform just as well as young animals on this task. Thus, it appears that old rats are selectively impaired in the spatial version of the working memory task. Taken together, these data suggest that the older rats' impairment on the radial maze is due to a deficit in spatial information processing.

1.3.2.3 Barnes circular platform

The Barnes circular platform is a spatial reference memory task first developed by Barnes (1979) for age comparisons of spatial memory. In order to avoid possible age differences in motivation level following food deprivation, this task utilizes the fact that rodents naturally prefer dark enclosed places to brightly illuminated open arenas. The apparatus is a large circular platform with 18 holes lining the periphery. Under one of the holes is a dark secure box. The platform surface can be rotated between trials, while the box remains at a fixed position relative to the distal cues in the environment. At the beginning of the task, the rat is placed in an enclosed start box in the center of the circular platform under strong illumination. The start box is then lifted off the platform by a pulley, which leaves the rat free to explore on the platform and find the reward tunnel. The most effective strategy for solving the task is spatial, because motor patterns (e.g., response strategies) and nonspatial cues (e.g., odor, texture of the platform) cannot be used. The old animals are impaired in learning the spatial location of the escape chamber compared to young rats (Rigter et al., 1984). Moreover, when old rats are given more trials to learn the task to comparable levels of performance as young rats, old rats forget the location of the box significantly faster than young rats (Barnes and McNaughton, 1985).
Figure 1.3 A photograph illustrating the Barnes circular platform. The arrow points to the position where the escape tunnel was located. Adapted from Barnes (1991).
1.3.2.4 Morris water task

The Morris water task (Morris et al., 1981) uses water instead of food deprivation to motivate the animal to find an escape platform in a large circular tank. The platform is submerged slightly under the water surface, and the water is made opaque by adding either powdered milk or white paint (Fig. 1.4). This is the task that has been used most often for assessing spatial learning abilities of aged rats, mostly because rats are highly motivated to escape, and learn this problem very quickly (usually 1 week). In the spatial version of this task, there is a hidden escape platform just below the surface of the water. Rats are released at various points around the periphery of the pool and trained to escape the water by locating the hidden platform. If random release site procedures are used, rats cannot use a response strategy. Nor can olfactory cues be used. The optimal strategy for solving this task is to remember the relationship of the goal platform to the configuration of distal cues in the environment. In this task, the old animals consistently show much slower acquisition of the spatial location of the hidden platform from day to day compared to young rats (Biegon et al., 1986) (see also chapter 6). In the probe test when the escape platform is removed and the rat is allowed to swim freely in the pool for a certain amount of time, young rats consistently search in the target quadrant that previously contained the platform. The old rats, on the other hand, tend to swim around the pool with much less bias toward the target quadrant. If the escape platform is raised above the surface of the water so that the task is now a visual discrimination problem, rather than a spatial navigation problem, old Long Evan rats' performance can be just as good as the younger rats' (Rapp et al., 1987). In the study described in Chapter 6, although the old rats showed an impairment in the acquisition of the cue task when only 6 trials were given, there was no correlation in the old rats' performance between the cue version and the
Figure 1.4 A photograph illustrating the apparatus of the Morris water maze in the center of an experimental room. Adapted from Barnes (1996).
spatial version of the task. Furthermore, when 12 training trials were administered instead of 6, the old rats eventually reached a performance level that was equivalent to that of the young rats. The slower acquisition of the discrimination version of the task in some groups of old rats may be due to the swimming strategy that the old rats adopted, such as a tendency to swim around the side of the pool. Possible contributions of other confounding factors to the impairment of old rats in the water maze have also been studied. Lindner and Gribkoff (1991) reported that aged rats can become hypothermic during testing in the Morris water task because of a loss of thermoregulatory control, and that warming the old rats between trials was able to significantly improve performance. Birren and Kay (1958) and Kay and Birren (1958) first noted that old rats were slower and became fatigued sooner than young rats in swimming situations, although with practice they improved their swimming performance. Thus, it is critical to place rats in an incubator between trials to prevent hypothermia in aged rats. It is also important for studies using the Morris water task to demonstrate that the total path-length measure is longer for the older animals as well as the latency measure, since the latter could have been a reflection of motor deficiencies. In another line of studies (Gage et al., 1984b), a variety of tests were conducted to test large groups of young and old rats on their motor abilities and spatial memory performance. Although aged rats show impairments in the spatial version of the Morris water task, and are also impaired in motor coordination, startle activity, locomotion activity and exploration, there are no correlations between these measurements. Their results suggest that these behavioral impairments in aged animals are likely independent from each other, which may reflect different components of the aging process with different mechanisms.
Another series of studies provided direct additional support for a spatial learning deficit in aged rats in the water task. Rapp et al. (1987) used an ambiguous version of the visual discrimination problem, in which the visible platform was always left in the same location over trials. The rats could therefore use either a cue or place strategy for accurate performance. After both young and aged animal reached an equal level of performance, a probe trial was conducted in which each animal was placed in the pool for 90 sec with the escape platform removed. In the absence of a local cue, the young animals still searched more in the quadrant of the maze that formerly contained the escape platform than in each of the remaining quadrants, whereas the aged rats did not direct their search to the training quadrant, and distributed their swimming evenly around the maze. This suggests that the old rats relied exclusively on a local cue strategy, rather than spatial ones, and implies that spatial information processing posed particular problems for the older animals compared to young animals. In another experiment (Pelleymounter et al., 1987), when a black arc was positioned on the inside wall of the maze as a distinct proximal cue, the aged rats still showed a prominent place learning deficit compared to young rats. Thus, the performance of aged rats on the Morris water task cannot be attributed entirely to age-related sensory changes, and, at least in part, can be attributed to a change in the way old animals process spatial information.

1.4 Involvement of the hippocampus in learning and memory

Since the discovery that hippocampal damage in humans results in severe amnesia, numerous studies have examined the role of the hippocampus in learning and memory, and numerous theories have been put forth regarding hippocampal function. Although the exact role of the hippocampus in learning and memory is still debated (e.g., O'Keefe and Nadel, 1978; Cohen and Eichenbaum, 1993; Eichenbaum, 1992; Jarrard, 1993; Rawlins,
1985; Sutherland and Rudy, 1989), all agree that the hippocampus is involved in spatial information processing. A comprehensive review of this field is beyond the scope of this dissertation. Thus, only the role of hippocampus in spatial learning and memory in both human and animal models are emphasized in the discussions that follow.

1.4.1 Human Neuropsychological Data

The wide interest in the hippocampus as a brain structure important for learning arises from a famous case study reported by Scoville and Milner (1957). The patient, referred to as H.M., began to have minor seizures at age 10 and had major seizures by the time he was 16 years of age. When H.M. was 27 years old, Scoville performed a bilateral medial temporal lobectomy, during which the prepyriform gyrus, uncus, amygdala, hippocampus and parahippocampal gyrus were removed, in order to treat his intractable seizures (Corkin, 1984). After the operation, seizure activity was greatly reduced (Scoville and Milner, 1957); however, H.M. showed a severe amnesia, and exhibited no obvious intellectual and cognitive deficits other than memory impairment. H.M.'s immediate memory span is normal. He can also remember and rehearse information for a short period of time; however, the information can not be stored into memory for a sustained period of time, suggesting that he is selectively impaired in long-term memory or secondary memory. H.M. not only has anterograde amnesia, which has seriously impaired his ability to acquire any new information in the past 40 years, but he also has retrograde amnesia for events which occurred up to 11 years before the surgery as well (Corkin, 1984). In addition, H.M. exhibits an inability to navigate normally in new environments, and is severely impaired in the recall of both absolute and relative locations of objects. Consistent with this, it has been reported that patients with right hippocampal damage are also impaired in learning a correct path through a stylus maze (Corkin, 1965;
Milner, 1965), and exhibit poor free recall of object locations (Smith and Milner, 1981). For example, Pigott et al. (1993) examined delayed recognition memory for different aspects of complex visual scenes in 65 patients with unilateral temporal- or frontal- lobe excisions. While all the patients with temporal lobe resections were impaired in memory for the visual characteristics of the objects in a scene, only patients with right temporal- lobe lesions including extensive hippocampal removal were impaired in detecting changes in the spatial location of specific objects relative to one another.

It should be noted that H.M. has extensive temporal lobe damage, which is not restricted to the hippocampus, but also includes other adjacent regions such as the amygdala and parahippocampal gyrus. Moreover, the extent of H.M.'s damage has not been finally confirmed by pathological examination since the surgery. More recently, a case study of another amnesic patient, referred to as R.B., has supported the view that the hippocampus is involved in memory. R.B. experienced an ischemic attack, following open-heart surgery, that resulted in bilateral damage restricted to the CA1 field of the hippocampus (Zola-Morgan et al., 1986). R.B. had a moderately severe anterograde amnesia while his intelligence and cognitive functions other than memory were intact. In contrast to H.M., however, R.B. had little detectable retrograde amnesia. Very recently, Rempel-Clower et al. (1996) reported three other amnesic patients with bilateral damage limited to the hippocampal formation. Like R.B., one of the patients had only bilateral lesions of the CA1 field of the hippocampus, and exhibited moderately severe anterograde and little retrograde amnesia. The other two patients, however, had bilateral extensive lesions of the whole hippocampal formation, including all CA fields, the dentate gyrus and some cell loss in the entorhinal cortex. They not only had moderately severe anterograde amnesia but also severe retrograde amnesia covering approximately 15 years and 25 years
respectively. Although the spatial memory of these patients (including R.B.) was not intentionally tested, the extensive neuropsychological examinations and careful anatomical studies of the postmortem brains have presented strong evidence indicating that, in humans, the hippocampus is necessary for memory formation. There is also evidence, however, suggesting that the degeneration of hippocampal CA1 region may produce abnormal cell activity in other related brain regions which CA1 pyramidal cells project to during the following prolonged period after the hippocampal damage. Thus, the memory deficits observed in R.B. may not be accounted for exclusively by cell death in hippocampal CA1 region (Duva et al., 1993).

1.4.2 Animal studies

While it is understandably difficult to have human accidents of nature that selectively involve the hippocampus, this can be relatively easily performed on experimental animals. Numerous lesion studies have been conducted to examine the role of the hippocampus in spatial learning and memory of rodents using a variety of spatial tasks. There were three methods that have been generally used to "lesion" the hippocampus: lesioning hippocampal afferents, electrolytic lesions and neurotoxic lesions. Although these are very useful techniques to investigate the role of a brain region in certain behaviors, they also can be problematic. For example, electrolytic lesions may cause damage not only to the hippocampus, but also to passing fibers and adjacent brain areas. In addition, the leak of neurotoxins from injection needles can cause damage to other brain regions through which the needles. Nevertheless, most lesion studies provide convergent evidence suggesting that the hippocampus is critical for the acquisition and processing of spatial information.
In 1978, after an extensive review of the literature of hippocampal lesion studies, O'Keefe and Nadel (1978) concluded that rat behavior was only impaired by hippocampal damage when the task involves a spatial problem. This supported their cognitive map theory that the hippocampus is involved in the acquisition and storage of spatial information. They predicted that damage to the hippocampus should impair spatial learning, but should not affect visual discrimination learning or response learning. Furthermore, they indicated that the latter two types of learning may actually be enhanced by hippocampal damage, as a consequence of the removal of the interference from the spatial strategy. These hypotheses have been supported by a number of subsequent studies using a variety of behavioral tasks.

Okaichi (1987) trained rats on a place version and a cue version of a T-maze task. In the spatial task, the maze was rotated from trial to trial and the rat was trained to go to the arm at a specific place relative to the distal cues in the room. In the cue task, the floor of one arm was painted black, with the other white, and the rat was rewarded only on the white arm. It was found that rats with radiowave-induced damage to the hippocampus were severely impaired on the spatial task, but performed equally well as the control rats in the cue task. When strategy was assessed, rats with hippocampal lesions used either cue or response strategies, even though neither of these strategies led to correct performance. These results, therefore, are consistent with the spatial map theory of the hippocampus. Consistent with this study, Aggleton et al. (1986) examined the effect of hippocampal lesions on rat performance in spatial (spatial alternation task) and nonspatial (delayed non-matching-to-sample task) working memory tasks on a Y-maze. Consistent with Jarrard et al. (1983), the lesioned rats were impaired in the spatial version of the task, but performed as well as the control rats in solving the non-spatial problem.
Nadel and MacDonald (1980) designed tasks to examine spatial and nonspatial reference memory performance of hippocampal lesioned rats on a radial maze with 7 arms. In the spatial version of the task, four arms were baited, and the other 3 were not. The position of these arms were maintained a fixed relationship with distal cues in the experimental room. In the cue version of the task, the arms of the maze were lined with textured floor inserts serving as local cues, while 4 cues were always associated with reward and the others were not. The maze was rotated from trial to trial, such that there was a relationship between the local cues and the spatial cues. It was found that the rats with hippocampal lesions were severely impaired in the spatial version of the task, but were mostly intact in the cue version of the task, although their performance was slightly worse than the control ones at the beginning of training.

It has been found that destruction of any of the major inputs to the hippocampal formation results in impairment on the standard working memory task of the radial maze (Olton et al., 1978b). Jarrard et al. (1983) used a combined version of the radial arm maze on which two kinds of learning (place and cue) and two kinds of memory (working memory and reference memory) could be tested concurrently. The design of the apparatus was similar to Nadel and MacDonald (1980), except that the radial-8-arm maze was used. For both place and cue tasks, if a rat enters an unbaited arm (never baited from day to day), then one can conclude that it fails to remember the reward location and the error would be of the reference memory type. If a rat makes a repetitive entry into a baited arm on the same trial, then the rat has failed to remember information that is only important for that trial, and the error would be one of working memory. It was found that rats with neurotoxic lesions of the hippocampus by ibotenic acid were impaired in both spatial working memory and spatial reference memory. These same rats however, performed as
well as control rats in the cued working and reference memory tasks (Jarrard, 1983). Interestingly, when working memory errors were controlled, lesioned rats acquired the cued version of the reference memory problem at a faster rate than did the control rats. This suggests that the lesioned rats were unable to use a spatial strategy as normal adult rats do, and therefore, would resort to a nonspatial strategy more quickly than controls, supporting O'Keefe and Nadel's prediction (O'Keefe and Nadel, 1978).

Rats with hippocampal lesions are also impaired in the spatial version of the Morris water task (Morris et al., 1982; Skelton and McNamara, 1992), but not in the visual discrimination version of the task. Although, in some cases, the lesioned rats also show some improvement over trials on spatial acquisition, this probably results from the fact that they tend to adopt a particular type of search strategy to solve the spatial task, such as swimming around the tank at a relatively fixed distance from the wall (DiMattia and Kesner, 1988). This is supported by the fact that, in the probe test, while the control rats preferentially swim in the target quadrant, lesioned rats fail to show a spatial bias and spend roughly equal amounts of time in each of the four quadrants. It has also been noticed in some experiments that lesioned rats acquired the cue task faster than control rats (DiMattia and Kesner, 1988). These results are consistent with the hypothesis that rats without a hippocampus adopt a non-spatial strategy rather easily, because of a lack of interference from the spatial strategy.

Using the Barnes circular platform, McNaughton et al. (1989a) found that rats with selective neurotoxic lesions to the granule cells the dentate gyrus were impaired in the acquisition of this reference memory task. In addition, repeated high-frequency stimulation of hippocampal afferents, which leads to a "saturation" of the synaptic
weights in the hippocampus, also results in an impairment of the rats' performance on this task (McNaughton et al., 1986; Barnes et al., 1994b).

In summary, performance in each of these spatial tasks is affected following damage to the hippocampus. These data, therefore, support the notion that the hippocampus is critical for spatial learning and memory. Nonspatial associative learning appears to be preserved in hippocampal lesioned rats (but see Bunsey and Eichenbaum, 1995), and rats tend to resort to these preserved abilities, such as using response strategy and cue associational strategy, to improve performance on spatial problems. Interestingly, these behavioral changes are more severe, but qualitatively similar to the changes in performance of old rats when tested in the same tasks. This implies that there may be age-related changes occurring within the hippocampus, which contribute to the memory deficits in old rats.

1.5 Summary

It is obvious from the forgoing review that the hippocampus is important for the acquisition of spatial information for both humans and rodents. It is interesting that, in both humans and rodents, aged subjects show a similar pattern of behavioral deficits in spatial learning and memory as subjects with damage to the hippocampus. Thus, it is reasonable to conjecture that hippocampal function may also be compromised in the aged brain, and that this may contribute to the spatial memory deficits in aging. Moreover, the subfield structure and general patterns of cell connections in the hippocampus are also rather similar and comparable between the two species (Rosene and Van Hoesen, 1987). Thus, the use of the aged rats as an experimental model to study age-related alterations in
the hippocampus that may be responsible for spatial memory deficits in old rats should provide important clues to the neurobiological changes underlying AAMI.
CHAPTER 2 ANATOMY OF HIPPOCAMPUS

2.1 Overall structures

The hippocampal formation is composed of a group of distinct regions, which are linked, one to the next, by unique and largely unidirectional projections (Fig. 2.1). It includes the entorhinal cortex, dentate gyrus, and hippocampus proper, which is subdivided into three fields (CA3, CA2, and CA1), subiculum, presubiculum and parasubiculum (Amaral and Witter, 1995). The cells in the superficial layers of the entorhinal cortex project to the dentate gyrus via the so-called perforant pathway, which is unidirectional because none of the cells in the dentate gyrus project back to the entorhinal cortex. Axons of dentate granule cells, called mossy fibers, project to the CA3 field of the hippocampus, while axons of CA3 cells do not innervate the granule cells, at least for the dorsal hippocampus. A similar, unidirectional pattern holds for the other major intrinsic connections from CA3 to CA1 and from CA1 to subiculum in the hippocampal formation. The existence of these one-way projections is one of the most prominent characteristics of the hippocampal formation, because in most other cortical regions, reciprocal connections are common.

In rat, the hippocampal formation appears as an elongated structure with its long axis extending in a C-shaped fashion from the septal nuclei of the basal forebrain rostrodorsally, at almost a 45 degree angle, over and behind the diencephalon, to the temporal lobe caudoventrally (Fig. 6.2A and 6.2B). The general longitudinal structure of the hippocampal formation, therefore, is defined as the septotemporal axis, and two ends as the septal pole and temporal pole, respectively. The cytoarchitectonic structure of the
Figure 2.1 A schematic diagram illustrating the major intrinsic connections of the rat hippocampal formation. Note that while many of the named pathways (e.g., the perforant pathway, mossy fibers, and Schaffer collaterals) are unidirectional, there are also parallel projections to different subfields, as well as intrinsic connections within some subfields.
Figure 2.2 The gross organization of the hippocampal formation in the rat brain. A. The C-shaped hippocampus is shown in a transparent shell of the rat brain. Note the columns of the fornix (f) extending first rostroventrally and then rostrocaudally from the septal pole of the hippocampus. Adapted from a drawing by Amaral and Witter (1993). B. On the top is the three-dimensional structure of the hippocampal formation, continued by the septal region in the medline. At the bottom is the laminar structure of a transverse section perpendicular to the longitudinal axis of the hippocampus, at the indicated level. FD, fascia dentata; H, hilus. Adapted from Gray (1982). C. The illustration of the fiber bundles associated with the hippocampal formation. Adapted from a drawing by Blackstad (1956b). CC, corpus callosum.
hippocampal formation is typically examined along its transverse axis (Fig 2.2B). If the hippocampal formation is cut transversely along its longitudinal axis, different fields are found to make up the structure at different septotemporal levels. At extreme septal levels, only the dentate gyrus and the CA fields of the hippocampus are observed. At about one third on the longitudinal axis away from the septal pole, the subiculum first appears, followed by the presubiculum and parasubiculum at progressive more temporal levels. The entorhinal cortex is located ventrally in the most caudal portion of the cortical mantle. In describing locations within the transverse axis of a hippocampal field, the portion of CA1 located close to the subiculum has been called the distal portion, and the portion closer to CA2 called the proximal portion. In addition, in discussing the radial organization of a particular field, regions located closer to the hippocampal fissure are usually called superficial, whereas those closer to the ventricle are called deep.

The ventricular surfaces of the subiculum and hippocampus are covered by a thin sheet of mainly myelinated afferent and efferent fibers called the alveus (Fig. 2.2C). The fibers extend obliquely over the surface of the hippocampus and collect in an increasingly thicker fiber bundle located at the lateral extreme of the hippocampus called the fimbria, which runs in the temporal-to-septal direction. As the fibers leave the hippocampus and descend into the forebrain, they are usually referred to as the fornix. The fornix and fimbria carry both efferent fibers from the hippocampal formation and subcortical afferent fibers to the hippocampal formation. A proportion of the fibers in the fimbria do not join the fornix, but cross the midline projecting to fields in the contralateral hippocampal formation. These fibers are referred as the ventral hippocampal commissure, which are mainly comprised of axons of dentate hilar mossy cells. There is a second commissural bundle associated with the hippocampal formation, called the dorsal hippocampal
commissure, which crosses the midline just rostral and ventral to the splenium of the corpus callosum. It carries mainly afferent and efferent fibers of the presubiculum, parasubiculum, and entorhinal cortex. The fibers of the dorsal hippocampal commissure are continuous laterally with a bundle of fibers, called angular bundle, which is located between the entorhinal cortex and the pre- and parasubiculum. This fiber bundle carries the efferent fibers from the ventrally situated entorhinal cortex, which project to the other hippocampal fields, particularly the dentate gyrus, hippocampus, and subiculum, at all septotemporal levels.

2.2 Dentate Gyrus

The dentate gyrus is also called the Fascia Dentata. This field is made up of three layers (Fig. 2.3). Closest to the hippocampal fissure is a relatively cell-free layer called stratum moleculare (or the molecular layer). The principal cell layer, called stratum granulosum or granule cell layer, lies deep to the molecular layer and consists primarily of densely packed granule cells. The granule cell and molecular layers form a U-shaped structure (depending on the septotemporal position) that encloses a cellular region, called hilus (or polymorphic cell layer), which constitutes the third layer of the dentate gyrus.

The molecular layer is mainly occupied by the dendrites of granule, basket, and various polymorphic cells as well as terminal axonal arbors from several sources, including a variety of interneurons in the hilus (Freund and Buzsáki, 1996). In addition, two types of "classical" interneurons can be found in this region. The first is a form of basket cell, with a multipolar or triangular cell body, which gives rise to an axon that appears to contribute to the basket plexus around the granule cells. The second type is the axo-axonic cell, known as the "chandelier" neuron, with highly collateralized axon
**Figure 2.3** Illustration of various histological view of a horizontal section through the rat hippocampal formation. **A.** The section stained by the Nissl method. The calibration bar equals 500 μm. **B.** The drawings of the same section in A, illustrating various regions, layers and fiber pathways of the rat hippocampal formation in A. ab, angular bundle; PsS, parasubiculum; PrS, presubiculum; ML, GL, and PoDG, molecular, granule cell, and polymorphic layers, respectively, of the dentate gyrus; so, stratum oriens; pcl, pyramidal cell layer; sl, stratum lucidum; sr, stratum radiatum; s l-m, stratum lacunosum-moleculare; alv, alveus. Adapted from Amaral and Witter (1995).
terminating exclusively on the axon initial segments of granule cells. Both types of cells are GABAergic because they are immunoreactive for markers of GABA, such as GAD and parvalbumin. There are a variety of other types of interneurons which have been classified, according to their dendritic distributions and axonal connectivities, or their neurochemical characteristics. Similar to basket and chandelier cells, they not only distribute in the dentate gyrus, but also in the CA regions as well. These cell types will not be described in detail in this Chapter. A comprehensive and excellent review can be found in Freund and Buzsáki (1996). The granule cell layer contains about $1 \times 10^6$ granule cells in each hemisphere (Boss et al., 1985), which is roughly equal to the total number of cells in all the other parts of the hippocampal formation. Each granule cell has a small soma lying in the granule layer, and a cone-shaped apical dendritic tree radiating into the molecular layer. The dendritic trees of the granule cells in the suprapyramidal blade, close to the hippocampal fissure, were significantly larger than those in the infrapyramidal blade. The former are also covered with more spines (about 5600 per cell) than the latter (about 3600 per cell) (Amaral et al., 1990). Seress and Pokorny (1981) reported that there were about 3500 basket cells situated at the interface of the granule cell layer and hilus for an average ratio of 1 basket cell for every 180 granule cells. Most of these cells are GABAergic and supply an inhibitory input to the granule cells. There appears to be many neuronal cell types in the hilus (Amaral, 1978). The most common cell type, and also the most impressive, is the mossy cell, which has a large cell body (25-35 μm). Their proximal dendrites are covered by very large and complex spines called "thorny excrescences" that are the sites of termination of the collaterals of the mossy fiber axon from granule cells (Frotscher et al., 1991). These thorny excrescences are more dense than the excrescences on the proximal dendrites of pyramidal cells in CA3, where the
mossy fibers also terminate. Another prominent cell type in the hilus is the neuron immunopositive for somatostatin, which also colocalizes with GABA (Bakst et al., 1986). These somatostatin/GABA neurons scatter throughout the hilus, and project densely to the outer thirds of the molecular layer at the same septotemporal level as the cell of origin, where their terminals form synapses on the distal dendrites of granule cells (Halasy and Somogyi, 1993). This pathway, therefore, may be involved in local inhibitory control of dentate granule cells. Seress (1988) found that there are approximately 32,500 cells in the hilus of the dentate gyrus. Of these, approximately 30 %, or about 10,000 cells, are immunoreative for somatostatin and GAD (Kosaka et al., 1988). The remaining 20,000 hilar neurons are heterogeneous but many are likely to be the mossy cells.

The granule cells give rise to distinctive unmyelinated axons, called mossy fibers. They have an unusual appearance under the light microscope, with alternating long narrow sections interrupted by large varicosities. The course of the mossy fibers is principally perpendicular to the longitudinal axis of the hippocampal formation, and the axonal collaterals arising from each septotemporal level of the dentate gyrus only minimally overlap with those arising from other septotemporal levels. This appears to be the only pathway in the hippocampal formation to distribute in a "lamellar" fashion. The mossy fibers are strongly stained by the Timm method, a stain for heavy metals (Timm, 1958). This is due to a high zinc content in the mossy fibers. As in the other principal intrinsic connections of the rat hippocampal formation, the mossy fibers are thought to use glutamate as a primary transmitter substance (Storm-Mathisen and Fonnum, 1972); however, it is also clear that some mossy fibers contains opiate peptides, such as dynorphin (Gall, 1984). Each principal mossy fiber (which is on the order of 0.2 -0.5 μm in diameter) gives rise to about seven thinner collaterals within the hilus before entering the
CA3 field of the hippocampus. These collaterals establish contacts with the proximal dendrites of the mossy cells with large (3-5 μm), irregularly shaped varicosities, also with the dendrites of other cells in the hilus with smaller (approximately 2 μm) varicosities (Ribak et al., 1985).

The inner third of the molecular layer receives a projection almost exclusively from cells in the hilus (Blackstad, 1965), and sparse subcortical inputs. This projection consists of fibers originating from both the ipsilateral and the contralateral sides, which are called the associational and commissural projections, respectively. The inner third of the molecular layer is, therefore, also called the associational/commissural zone. It appears that the majority of terminals of this pathway form asymmetric, presumably excitatory synaptic terminals on spines of the granule cell dendrites. Mossy cells contribute a considerable number of axons, which project both ipsilaterally and contralaterally (Laurberg and Sorensen, 1981). In addition to forming synapse with dentate granule cells, the associational fibers also terminate on the dendritic shafts of GABA-ergic basket cells (Frotscher and Zimmer, 1983). Thus, the associational and commissural projections may function both as a feedforward excitatory pathway and as a disynaptic feed-forward inhibitory pathway (Douglas et al., 1983). There are some unusual characteristics of these pathways that have attracted attention. The first is the powerful connection between the mossy fibers and mossy cells. Second is the divergent projection of mossy cells and other type of cells back to the granule layer. It has been reported that a mossy cell axon makes extensive contacts with dentate granule cells across two-thirds of the septotemporal extent of the hippocampus both ipsilaterally and contralaterally (Zimmer, 1971). This suggests that a single granule cell can be connected disynaptically with a large population of granule cells by mossy cells.
Due to the heterogeneous cell types in the hilus, and the multi-synaptic recurrent connections of granule cells through these cells, it has been speculated that the dentate gyrus may have a special function (e.g., memory) on its own (Buckmaster and Schwartzkroin, 1994). Clearly more anatomical and physiological studies are needed before a deeper understanding of its function is achieved.

### 2.3 Hippocampus proper

The hippocampus proper was first divided by Ramón y Cajal into two major regions, a proximal region containing large cells, called regio inferior, and a distal region containing small cells, called regio superior (Ramón y Cajal, 1911). The terminology of Lorente de Nó, however, has been accepted more widely (Lorente de Nó, 1933). He divided the hippocampus into three fields: CA3, CA2, and CA1. His CA3 and CA2 fields are equivalent to the regio inferior of Ramón y Cajal, and his CA1 corresponds to the regio superior (Fig. 2.3). In addition to differences in the size of the pyramidal cells in CA3 and CA1, the two regions can also be distinguished by their connections. The CA3 pyramidal cells receive a mossy fiber input from the dentate gyrus and the CA1 pyramidal cells do not. The CA2 field is a narrow zone of cells (less than 250 μm), interposed between CA3 and CA1, that has large cell bodies like CA3 but does not receive mossy fiber innervation like CA1. Although the identification of this region has been controversial, there is evidence suggesting that the CA2 has some distinguishing characteristics. For example, a number of immno-histochemical studies have demonstrated denser acetylcholinesterase staining (Paxinos and Watson, 1982) and much denser labeling for the calcium binding protein parvalbumin than adjacent regions of CA3 or CA1 (Baimbridge and Miller, 1982). Since the calcium binding proteins are considered to be protective of ischemic or excitotoxic cell death, the high level of calcium-binding protein may play a role in the
resistance of CA2 to epilepsy (Corsellis and Bruton, 1983). Nevertheless, much less is known about CA2 compared to CA1 and CA3, which, therefore, will not be reviewed further more.

2.3.1 CA3

CA3 contains five layers (Fig. 2.3). The principal cellular layer is called stratum pyramidale (or pyramidal cell layer). The narrow, relatively cell-free layer located deep to the pyramidal cell layer is called stratum oriens. There is a narrow cellular zone located just above the pyramidal cell layer which has few cells, named the stratum lucidum. This layer is occupied by the terminals of mossy fibers originating from the granule cells in the dentate gyrus. Superficial to the stratum lucidum is the stratum radiatum. The most superficial layer in CA3 is called the stratum lacunosum-moleculare, in which perforant pathway fibers from the entorhinal cortex travel and terminate.

There are estimated to be around 300,000 pyramidal cells in the CA3 pyramidal cell layer (Boss et al., 1987). The basal dendrites of pyramidal cells extend into the stratum oriens. The apical dendrites extend all the way through the stratum lucidum, stratum radiatum and stratum lacunosum-moleculare. There is also a population of basket cells of various sizes and shapes located in the pyramidal cell layer (Seress and Ribak, 1984). They also have apical and basal dendritic trees, which, however, have few dendritic spines. The axons of these basket cells extend transversely from the cell body of origin and form a basket plexus that innervates the cell bodies of the pyramidal cells. There are also a variety of nonpyramidal cell types in the stratum oriens, stratum radiatum, and stratum lacunosum-moleculare of CA3 (Freund and Buzsáki, 1996). The vast majority of these neurons are GABAergic local interneurons, many of which can also be
visualized with antibodies to other neuroactive substances, such as peptides (Morrison et al., 1982) or calcium-binding proteins (Baimbridge and Miller, 1982).

Mossy fibers, carrying the principal output of the dentate gyrus, make several en passant synapses on the proximal dendrites of the CA3 pyramidal cells in the stratum lucidum. The presynaptic expansion is unusually large (about 3-6 μm) and irregular in shape with several fine filopodial extensions. Their size and proximity to the soma suggest that these are "powerful" synapses, although in vitro recordings do not provide good evidence for this. Each mossy fiber contains approximately 14 expansions along its trajectory spaced approximately 140 μm apart. It has been estimated that each CA3 cell is innervated by approximately 46 granule cells and receives about 200 mossy fiber synapses (Amaral et al., 1990).

The CA3 pyramidal cells give rise to highly collateralized axons that project to the CA1 subfield and other CA3 pyramidal cells either ipsilaterally or contraterally, also, subcortically to the lateral septal nucleus. It is clear that each individual CA3 neuron gives rise to an axonal plexus that terminates over a broad region of ipsilateral and contralateral CA1, and make synapses on both apical and basal dendrites. Thus, the projections from CA3 to CA1 are not only highly associated with the apical dendrites in the stratum radiatum, as typically illustrated for the Schaffer collateral connections, but also contact the basal dendrites in the stratum oriens of CA1. There is also a topographic organization of the projections from CA3 to CA1 along the septal-temporal axis (Amaral and Witter, 1989). As illustrated in Fig. 2.4, those cells located proximally in CA3 (near the dentate gyrus) tend to project more heavily to the superficial part of the stratum radiatum in the distal portion of CA1 (near the subicular border). On the other hand, cells located more distally in CA3 (near CA2) give rise to projections that terminate deep in the stratum
Figure 2.4 The diagram illustrating the topography of projections from the CA3 pyramidal cells to the CA1 subregion of the hippocampus. The locations of cells of origin are indicated in the middle coronal section by triangles, and the distribution of fibers and terminals resulting from these cells is indicated by the same shading pattern as that in the corresponding triangles. Adapted from Amaral and Witter (1995).
radiatum and in the stratum oriens in the portions of CA1 located closer to the CA2 border. In addition, the highest density of CA3 fibers and terminals in CA1 shifts gradually along the septo-temporal axis, from the deeper parts of the stratum radiatum and stratum oriens in the portions of CA1 close to CA3 at septal levels, to more superficial parts of the stratum radiatum in the portions of CA1 close to the subiculum at temporal levels. This organization is an example that is in contrast to the classical view of the "lamellar" structure in the hippocampus, and that demonstrates a three-dimensional organization of the hippocampus. This information surely has some implications for work conducted with the \textit{in vitro} hippocampal slice preparation, especially on the interpretation of the results obtained from slice studies.

Besides the mossy fiber input, each CA3 pyramidal cell also receives about 10,000 other excitatory inputs, some 2000 of which come directly from the collaterals of perforant path from layer II of the entorhinal cortex, which also make synapses onto dentate granule cells. The remaining 8000 come from other CA3 pyramidal cells. The collaterals of a typical CA3 pyramidal cell axon project broadly to more than half the extent of CA3 on both sides of the brain, making contact with approximately 6000 other CA3 pyramidal cells or about 1.9\% of the entire population of CA3 pyramidal cells. Each CA3 pyramidal cell, therefore, receives approximately 80\% of its input from other CA3 pyramidal cells. This extensive recurrent excitatory circuit is a unique feature of the CA3 region, distinguishing it from other regions in the hippocampal formation. Thus, CA3 has attracted many speculations on its function and has been considered fundamental in neural network models of hippocampus (e.g., McNaughton and Morris, 1987).
2.3.2 CA1

CA1 has a similar laminar organization as CA3, except that CA1 does not have stratum lucidum (Fig. 2.3). Thus, CA1 has a pyramidal cell layer, consisting of 0.42 x 10^6 pyramidal cells (Boss et al., 1987) that have smaller cell bodies and dendritic trees compared to CA3 pyramidal cells. Deep to the pyramidal cell layer is the stratum oriens, which is a relatively cell free area and contains the basal dendrites of the pyramidal cells and Schaffer collaterals. Immediately above the pyramidal cell layer is the stratum radiatum, where Schaffer collateral synapses (from CA3 pyramidal cells) are located. The most superficial portion is the stratum lacunosum-moleculare, where the perforant pathway fibers from the layer III of the entorhinal cortex travel and terminate. Compared to CA3, CA1 pyramidal cells do not appear to give rise to many recurrent collaterals that are distributed within CA1. In addition, it appears that the CA1 field give rise to either very few commissural projections or none (Swanson et al., 1978; Van Groen and Wyss, 1990). The CA1 field gives rise to two intrahippocampal projections. The first is a topographically organized and also divergent projection to the adjacent subiculum. The second projection is to the deep layers of the entorhinal cortex. In addition, CA1 also project to the retrosplenial and perirhinal cortices as well as to the lateral septal nucleus and the nucleus of the diagonal band of Broca. The pyramidal cells in the portions of CA1 at midseptotemporal levels project to the medial frontal cortex. The temporal levels of CA1, on the other hand, project to the anterior olfactory nucleus, the olfactory bulb, the nucleus accumbens, the basal nucleus of the amygdala, and the anterior and dorsomedial hypothalamic areas (Van Groen and Wyss, 1990). It is apparent that the CA1 region gives rise to substantially more extrinsic connections than the CA3 field.
2.4 **Subiculum**

The subiculum contains a single diffuse layer of cells, most of which are pyramidal cells, mixed with a small proportion of interneurons. The cells are packed together less densely than in CA1 or CA3. Above the cell body layer lies a molecular layer containing mostly apical dendrites of pyramidal cells. The total number of pyramidal cells in the subiculum of the rat has been estimated at 128,000 per hemisphere (Amaral et al., 1990). The subiculum gives rise to a longitudinal associational projection that extends toward the temporal pole. It does not have commissural connections (Amaral and Witter, 1995). The subiculum receives its strongest inputs from CA1 and from layer III of the entorhinal cortex.

The subiculum is one of the major output regions of the hippocampal formation, and projects to a number of cortical and subcortical regions (Witter et al., 1989). The subiculum gives rise to a prominent projection to portions of the medial prefrontal cortex, as well as the prelimbic and infralimbic cortices. It also projects to medial portions of the anterior olfactory nucleus, the anterior cingulate cortex, and the retrosplenial cortex. In addition, the perirhinal cortex also receives a strong input from the subiculum, which terminates in both superficial and deep layers (Deacon et al., 1983). The subiculum also projects subcortically to the lateral septal nuclei and the mammillary nuclei (Amaral and Witter, 1995). Finally, the amygdala also receive inputs from subiculum (Price et al., 1987).

2.5 **Presubiculum and Parasubiculum**

Although the nomenclature of these two regions suggests a close relationship with the subiculum, from a cytoarchitectonic perspective, the pre- and parasubiculum are
completely different from subiculum. The subiculum has the typical cytoarchitectonic characteristics of the allocortex (i.e., three layers) as the other hippocampal subfields. The pre- and parasubiculum, however, are more similar to the entorhinal cortex, or adjacent parts of the retrosplenial cortex in that these cortical regions which are multilaminate (six layers). One of the most unique characteristic of the pre and parasubiculum is their strong interconnectivity with the lateral dorsal thalamic nucleus (van Groen and Wyss, 1990). This connection may reflect the anatomical basis for the fact that "head direction" cells were recorded from both presubiculum and thalamus (Ranck, 1984). In addition, the pre- and parasubiculum also send a major projection to the superficial layers of the entorhinal cortex, while receive innervation from the deep layers of the entorhinal cortex. Thus, it is generally thought that the pre- and parasubiculum are input structures, which is also in contrast to the role of the subiculum as a major output structure (Amaral and Witter, 1995). All in all, the pre- and parasubiculum appear to be very different from the subiculum in its cytoarchitectonic and connectivity perspectives. While the term "subicular complex" has been used by many authors to indicate a conglomerate of subiculum, pre- and parasubiculum, it appears that this terminology is not appropriate and is somewhat misleading.

2.6 Entorhinal cortex

The entorhinal cortex can be divided into 6 layers (Amaral and Witter, 1995; Ramón y Cajal, 1911) (Fig. 2.3). Layer I is the most superficial plexiform or molecular layer, which is relatively cell free, but is rich in transversely oriented fibers (Blackstad, 1956a). Layer II mainly contains stellate cells. A small number of pyramidal cell, multipolar and horizontal cells have also been seen in this layer. Layer III contains predominantly pyramidal cells. Layer IV (also called the lamina dissecans) is located close
to the rhinal fissure, and is devoid of neurons. Layer Va consists of a band of large, darkly stained pyramidal neurons. This layer is most prominent in the central parts of the entorhinal cortex. At other levels, the cell packing and is not easily distinguished from layer Vb. Layer Vb consists of scattered smaller pyramidal cells and polymorphic cell types. Layer VI contains a very heterogeneous population of cell sizes and shapes at low density. As for the intrinsic connection inside the entorhinal cortex area, the projections from layers 2 and 3 are much less compared to the projections from deep layers 5 and 6. The latter projections distribute and terminate extensively in layers 1 to 3. This suggests that cells in superficial layers not only project to dentate gyrus and hippocampal CA regions, but also receive feedback information from these regions via cells in deep layers of the entorhinal cortex.

It is generally accepted that the entorhinal cortex can be subdivided into two general areas, the lateral entorhinal area (LEA) and the medial entorhinal area (MEA). The LEA occupies the rostrolateral part of the entorhinal cortex; its base is oriented rostrally and its tip is located caudolaterally next to the rhinal fissure. The MEA occupies the remaining triangular area of the cortex. The major input to the dentate gyrus and a prominent input to the hippocampus proper and the subiculum arise from the entorhinal cortex. Fibers of the perforant pathway originate from stellate cells in layer II and pyramidal cells in layer III. Recent data suggest that many other cell types, including at least a few GABAergic neurons, in layers II and III, also project to various hippocampus subfields (Caballero-Bleda and Witter, 1993). The perforant path terminates in all subdivisions of the hippocampal formation (Köhler, 1985a; Köhler, 1985b; Köhler, 1986; Köhler, 1988; Ruth et al., 1982). The projection to the dentate gyrus arises mainly from layer II of the entorhinal cortex. In the molecular layer of the dentate gyrus, the terminals
of the perforant path fibers form asymmetric synapses on dendrites of granule cells at the superficial two thirds. Fibers originating in the LEA terminate in the outer one third of the molecular layer and fibers from the MEA terminate in the middle one third of the molecular layer (Hjorth-Simonsen, 1972). In addition, lateral parts of LEA project mostly to the suprapyramidal blade, while more medial parts of LEA project more heavily to the infrapyramidal blade (Wyss, 1981). The perforant path is mostly glutamatergic. The perforant path projection to the CA3 field also originates from the layer II of the entorhinal cortex and terminates in the stratum lacunosum-moleculare (Steward and Scoville, 1976). In fact, a recent intracellular labeling study by Tamamaki and Nojyo (Tamamaki and Nojyo, 1993) has demonstrated that collaterals of the same layer II cells reach both the dentate gyrus and fields CA3/CA2 in a similar fashion, such that projections from the LEA terminate more superficially than those from the MEA in the stratum lacunosum-moleculare. The entorhinal projections to CA1 originate in layer III rather than layer II (Steward and Scoville, 1976). In CA1, both the medial and lateral components of the perforant pathway terminate throughout the full radial extent of the stratum lacunosum-moleculare. The fibers from the MEA, however, project preferentially to the proximal part of CA1 (i.e., close to CA2). Fibers from the LEA, on the other hand, terminate in the portions of CA1 close to the subiculum. CA1 is the first hippocampal field that projects back to the entorhinal cortex, where the projection terminates predominantly in layer V (Finch and Babb, 1981). Subiculum also receives a strong projection from the layer III of entorhinal cortex, and a smaller proportion of collateral fibers from layer II cells (Tamamaki and Nojyo, 1993). Similar to CA1, the subiculum also projects back to the entorhinal cortex, where the projection also terminates in the layers deep to layer V.
The entorhinal cortex of the rat receives inputs from a variety of cortical regions. These cortical inputs form two groups: those that terminate in the superficial layers (I-III) and those that preferentially terminate in the deep layers (IV-VI). The first category delivers information to the entorhinal neurons, which are the source of projections to the dentate gyrus, hippocampus and the subiculum. The second group of inputs terminate on the deeper cells of the entorhinal cortex, which receive processed information from hippocampal CA1 and subiculum and also give rise to projections back to certain cortical regions. It is, therefore, possible that the second class of cortical inputs have influence on the output of the hippocampal formation. The prominent cortical inputs to the superficial layers of the entorhinal cortex arise from olfactory structures of the telencephalon, including the olfactory bulb, the anterior olfactory nucleus, and the piriform cortex (Haberly and Price, 1978), and the perirhinal cortex (Burwell and Amaral, 1993). Cortical afferents to the deep layers of the entorhinal cortex arise from a variety of cortical areas, including the agranular insular cortex, medial prefrontal cortex, anterior cingulate cortices and retrosplenial cortex (Amaral and Witter, 1995).

2.7 Subcortical Afferents

In addition to the pathways described above, a number of subcortical areas also send projections to all or part of the hippocampal formation. Most of these inputs are modulatory, probably not conveying detailed information, but rather profoundly influencing overall cell activity in the hippocampal formation.

2.7.1 Projections from the septal region

The septal projection to the hippocampal formation was first reported in the early 1950s (Daitz and Powell, 1954), followed by numerous studies conducted to further
define the projection. The septal projection arises from cells of the medial septal nucleus and the nucleus of the diagonal band of Broca. These fibers travel to the hippocampal formation via the fimbria and dorsal fornix, supracollosal stria, and ventrally through and around the amygdaloid complex. Septal fibers terminate in essentially all fields of the hippocampal formation and more densely in the hilus, a narrow infragranular band and a narrow supragranular zone just beneath the commissural projections in the dentate gyrus (Mosko et al., 1973). This projection is also topographically organized. Cells located medially in the medial septal nucleus project more heavily to septal levels of the hippocampal formation, whereas cells located laterally in the septal area project preferentially to temporal levels of the hippocampal formation (Gaykema et al., 1990). Lewis and Shute (Lewis and Shute, 1967) were the first to propose that the septohippocampal projection was cholinergic because fimbrial transactions led to a substantial loss of histochemical staining of acetylcholinesterase (AChE). Subsequent studies (Amaral and Kurz, 1985) indicate that only approximately 50% of the cells in the medial septal nucleus/diagonal complex that project to the hippocampal formation are cholinergic and stain positive to acetylcholinesterase (ChAT). Those additional noncholinergic cells were found to contain glutamic acid decarboxylase (GAD) and are presumably GABA-ergic (Köhler et al., 1984). The GABA-ergic cells project preferentially to the GABA-ergic cells in all subfields of the hippocampus proper and dentate gyrus (Freund and Antal, 1988), and many of the cells which receive GABAergic septal projections are also immunoreactive for neuropeptides (e.g., cholecystokinin, somatostatin, or VIP) (Gulyas et al., 1990) or contain calcium binding proteins (e.g., calretinin, calbindin, or parvalbumin) (Acsady et al., 1993).
In the dentate gyrus, septal fibers project heavily to the hilus, particularly in a narrow infragranular band, and terminate more lightly in the molecular layer. In CA3, the septal projection appears to terminate most heavily in the stratum oriens and to a lesser extent in the stratum radiatum (Gaykema et al., 1990). The CA1 field receives a substantially lighter septal projection than CA3, but as in CA3, the fibers are mostly densely distributed in stratum oriens (Nyakas et al., 1987). The projection originates from the medial septal nucleus and nucleus of the diagonal band terminates also in the pyramidal and molecular layers of the subiculum. Layer II of presubiculum also receives a prominent cholinergic input. In contrast, no septal-projections of the parasubiculum have been reported in the rat. The medial septal complex also projects robustly to the entorhinal cortex (Swanson, 1978). This projection is topographically organized such that cells in the horizontal limb of the nucleus of the diagonal band preferentially distribute fibers to the LEA, whereas the medial septal nucleus and the vertical limb of the nucleus of the diagonal band project to the MEA. Septal afferents terminate densely in the cell-sparse lamina dissecans and less densely in layer II (Amaral and Witter, 1995).

2.7.2 Monoaminergic projections from the brain stem

The pontine nucleus locus coeruleus gives rise to noradrenergic projections to the hippocampal formation. The hippocampal formation also receives a serotonergic projection that originates from several subdivisions of the raphe nuclei. Both types of projections terminate most heavily in the hilus region of the dentate gyrus (Fig. 2.5). In particular, it has been shown that the raphe serotonergic fibers preferentially terminate on a class of interneurons, which are likely somatostatin cells, in the dentate gyrus that primarily influence the distal dendrites of the granule cells (Halasy et al., 1992). As in the septal projection, many of the cells in the raphe nuclei that project to the hippocampal
Figure 2.5 Line drawing of a transverse section through the hippocampal formation, demonstrating the distribution of noradrenergic (A), serotonergic (B), and dopaminergic (C) fibers. Adapted from Swanson (1987).
formation are also nonserotonergic (Montone et al., 1988). There are also minor and diffusely distributed dopaminergic projections in the hippocampal formation that arise mainly from cells located in the ventral tegmental area. (Swanson, 1982).

2.7.3 Projection from thalamus

Herkenham (1978) demonstrated fairly prominent projections from midline (or "nonspecific") regions of the thalamus to several fields of the hippocampal formation. In particular, the small midline nucleus reuniens gives rise to a prominent projection to the stratum lacunosum-moleculare of CA1 (Dolleman-Van der Weel and Witter, 1992). Electromicroscopic studies demonstrate that the nucleus reuniens fibers terminate with asymmetric synapses on spines and thin dendritic shafts in the stratum lacunosum-moleculare, which suggests that the transmitter may be glutamate. There are also thalamic inputs to the portions of the subiculum and entorhinal cortex from the nucleus reuniens (Wouterlood et al., 1990). It is worthy to note that the dentate gyrus and CA3 are distinguished from the rest of the hippocampal formation in that they do not receive thalamic input.

2.7.4 Projection from supramammillary nucleus

The supramammillary area refers to the zone that caps and partially surrounds the mammillary nuclei in the hypothalamic region. A recent study (Magloczky et al., 1994) using anterograde tracing with Phaseolus vulgaris leucoagglutinin (PHAL) found that the supramammillary area projects to both CA2-CA3 and dentate gyrus in the hippocampus. Double-immunostatining for the tracer and different neuropeptides or calcium binding proteins revealed no multiple contacts between supramammillary afferents and labeled
inhibitory cells at both the light and electro- microscopic levels. The data, thus, suggest that principle cells in both CA2-CA3 and dentate gyrus are most likely the targets of supramammillary afferents in the rat hippocampus.

2.7.5 Projections from the amygdaloid complex

The basal nucleus of the amygdaloid complex and the posterior cortical nucleus and adjacent amygdalohippocampal area give rise to projections that terminate mainly at the CA1/subiculum border region, where they preferentially innervate the molecular layer of the subiculum and stratum lacunosum- of CA1 (Krettek and Price, 1977), primarily in the ventral portions of the hippocampus of the rat.

2.8 Conclusions

There has been rapid progress in increasing our understanding of the anatomy and connectivity of the hippocampal formation over the past decade. The concept of a "trisynaptic circuit, organized in a lamellar fashion" proposed in 1970s (Andersen et al., 1971) is no longer tenable. Rather, several new organizational principles of the hippocampal formation have emerged. First, many intrinsic hippocampal connections distribute as extensively in the septo-hippocampal axis as in the transverse axis, except the mossy fiber projection which indeed distributes in a lamellar fashion perpendicular to the longitudinal axis of the hippocampal formation. Second, the intrinsic hippocampal circuitry has both serial and parallel projections to several fields of the hippocampal formation. For example, the perforant pathway from the entorhinal cortex contributes many parallel projections to several fields of the hippocampal formation, while there is an unique serial pathway from dentate gyrus to CA3, to CA1, to subiculum. Finally, there is a significant difference in the topographical organization of the connections of the
hippocampal formation between the septal and temporal poles, which also implies a
difference in their function. The septo-temporal variance not only occurs in inputs that the
hippocampus receives, but also occurs in the sites of output terminations in the cortex
(Swanson and Cowan, 1977) and connections with subcortical structures (Witter, 1993).
It is interesting that only the ventral CA1 area projects to the medial prefrontal cortex
(Swanson et al., 1981) and only the ventral hippocampus is connected to the amygdala
(Van Groen and Wyss, 1990). Along with these anatomical data, functional differences
along the septal-temporal axis have been found in many other dimensions, such as the
seizure induction threshold and kindling of epileptic foci (Elul, 1964), event-related
potentials (Brazier, 1970), vulnerability to ischemia (Ashton et al., 1989), evoked field
potential responses (Gilbert et al., 1985) and firing characteristics of CA1 pyramidal cells
in vivo (Jung et al., 1994).
CHAPTER 3  CHOLINERGIC SEPTO-HIPPOCAMPAL PROJECTION

3.1 Introduction

As mentioned in Chapter 2, the medial septum/diagonal band complex provides one of the major afferents to all hippocampal subfields. A substantial component of this projection is cholinergic, and another major component is GABAergic. The medial septum/diagonal complex is a part of the basal forebrain, a structure composed of heterogeneous groups of neurons located in ventral and medial aspects of the telencephalon that are not clear components of other major forebrain structures, such as the basal ganglia, hypothalamus, or cortex. The most prominent feature of the basal forebrain is that it consists of large cholinergic neurons, which are found prominently within the medial septal nucleus, vertical and horizontal limbs of the diagonal band, ventral pallidum, magnocellular preoptic area, substantia innominata, basal nucleus, and so-called nucleus of the ansa lenticularis. The area around the latter 5 regions are also usually called the nucleus basalis of Meynert (nBM), where cholinergic neurons appear to be larger than those in the medial septum/diagonal band complex. While neurons in the medial septum and vertical limb of the diagonal band project to the hippocampus, the neurons in the nBM and horizontal limb of the diagonal band innervate the entire neocortical mantle and part of the amygdalar complex.

Acetylcholine (ACh) is one of the main neuromodulators in the brain. Its effects are slower, longer lasting and more spatially diffuse than neurotransmitters such as glutamate and GABA. Although ACh has clear neurotransmitter effects in the periphery, it does not appear to be involved in the direct transfer of information in cortical and
hippocampal structures. Rather, it appears to alter the processing characteristics of cortical structures through influences on physiological phenomena such as glutamatergic synaptic transmission and pyramidal cell adaptation, as will be discussed below. As shown by a recent EM study (Umbriaco et al., 1995), the cholinergic terminals in the hippocampus exhibit only 7% synaptic junctional specialization, while GABA varicosities appear entirely synaptic. This suggests that most terminals of cholinergic fibers do not form particular point-to-point synaptic relationships with hippocampal neurons, and that volume transmission may be the principal mode of transmission for ACh in adult rat hippocampus.

3.2 Pharmacological actions of ACh in hippocampus: Postsynaptic actions

As to the effect on the electrophysiology of single cells in the brain, ACh appears to be one of the most thoroughly studied neuromodulators. As early as 1971, Krmjévic et al. (1971) found that ACh has a pronounced excitatory effect in the cortex, which is mediated by muscarinic receptors. This excitatory action, also found in the hippocampus (Ben-Ari et al., 1981), was manifested by a depolarization of membrane potential, and significantly prolonged bursts of firing. Since an increase in input resistance was also observed at the same time, it was proposed that this excitation effect was mediated by a block of K⁺ conductance(s). With the advent of voltage-clamping techniques, various types of current could be examined more specifically, especially in hippocampal slices or cultures. ACh can block most of the known K⁺-currents generated by hippocampal neurons, including the voltage-dependent non-inactivating K⁺ current named I_M (Halliwell and Adams, 1982); the fast inactivating I_A (Nakajima et al., 1986); the Ca²⁺-activated current responsible for the slow after-hyperpolarization (I_AHP) (Benardo and Prince, 1982), and a voltage-independent "leak" K⁺ conductance (Madison et al., 1987). Thus,
the mechanism underlying the excitatory action of ACh on hippocampal principal neurons can be clearly understood. For example, suppressing the leak potassium conductance would result in a depolarization of the cell and facilitation of cell firing by increasing the electrotonic coupling between dendrites (where EPSPs are generated) and the cell body-axon hillock region (where spikes are most easily evoked). In addition, the cholinergic suppression of $I_A$ and $I_{AHP}$ current will remove rapid and slow accommodation to a depolarization and facilitate bursts of firing tremendously.

Recently, another line of evidence suggests that muscarinic agonists are able to enhance selectively NMDA but not AMPA currents in hippocampal CA1. This effect may be mediated through the activation of the IP3 second messenger pathway (Markram and Segal, 1990; Markram and Segal, 1992). In fact, it has been found that long-term potentiation is enhanced in CA1 in the presence of muscarinic agonists (Boddeke et al., 1992), which may be due to the cholinergic enhancement of the NMDA current. In addition, an imaging study (Kudo et al., 1988) demonstrated that muscarinic agents increase intracellular free Ca$^{2+}$ in hippocampal neurons. This increase in Ca$^{2+}$ was caused by the release of Ca$^{2+}$ from internal stores as well as increased Ca$^{2+}$ influx, possibly via a special, voltage-insensitive Ca$^{2+}$ channel.

In contrast to the intensive study of the muscarinic action on pyramidal cells in the hippocampus, its effects on interneurons have been much less studied, due to the difficulty of intracellular recording from these cells. Pitler and Alger (1992) reported that after the administration of muscarinic agonist on hippocampal slices, the spontaneous IPSP recorded from CA1 pyramidal cells was significantly enhanced. This effect was apparently not secondary to muscarinic increase in input resistance, since significant increases in both amplitude and frequency of spontaneous IPSPs were recorded using
either CsCl- or QX-314-filled pipettes, both of which can prevent the large increase in resistance caused by carbachol. This effect is apparently not mediated by other excitatory pathways, since it persists after the administration of both CNQX (antagonist of glutamic AMPA receptors) and APV (antagonist of NMDA receptors). These results of Pitler and Alger (1992), therefore, suggest that the increase in spontaneous IPSPs is due to a direct excitatory effect of muscarinic activation on interneurons. It implies further that there are also muscarinic receptors on hippocampal GABAergic interneurons, and that ACh is able to increase the excitability of interneurons, possibly through a similar mechanism as in pyramidal cells. Based on the fact that the septal GABAergic projection terminates mainly on GABAergic cells in the hippocampus, it remains puzzling why cholinergic and GABAergic cells in the medial septum have competing effects on hippocampal GABAergic cells.

3.3 Pharmacological actions of ACh in hippocampus: Pre-synaptic actions

In 1967, Yamamoto and Kawai (1967) found that perfusion of a hippocampal dentate gyrus slice with ACh can abolish a synaptically evoked granule cell discharge. Later studies also showed that EPSPs and recurrent IPSPs evoked in CA1 by stimulating Schaffer collateral, as well as EPSPs elicited in CA3 by stimulating mossy fibers, were depressed after the administration of ACh (Hounsgaard, 1978). These groups reached a consensus of interpretation of the finding by hypothesizing that the depression of synaptic transmission is not secondary to the depolarization or an increase in input resistance caused by ACh, but mostly likely mediated by a presynaptic mechanism. The evidence can be summarized as follows: 1) ACh applied onto the apical dendritic layer of the CA1 region reduces the size of EPSPs; while ionophoresis of ACh into the cell layer results in
an increase and prolongation of EPSPs and a transient decrease in the size of recurrent somatic inhibitory postsynaptic potentials (IPSPs) (Valentino and Dingledine, 1981); 2) in hippocampal tissue culture, excitatory and inhibitory postsynaptic currents (PSCs) evoked by short pulse applications of glutamate to other neurons are suppressed by the application of ACh near the recorded neuron. ACh, however, does not affect inward current responses to direct application of glutamate onto postsynaptic neurons, indicating that ACh may interfere with the release process (Segal, 1983); 3) the depression effect still exists when CsCl-filled electrode is used (Williams and Johnston, 1990); 4) The shape of the waveform, including rising time and decay time, of EPSPs and EPSCs are not altered by muscarinic agonists, and the reversal potential of EPSCs remains the same (Valentino and Dingledine, 1981; Williams and Johnston, 1990); 5) a study of a nine-compartment model of a CA3 pyramidal cell demonstrates that postsynaptic alterations in membrane resistivity have a much smaller effect on EPSP amplitude compared to the real change (Williams and Johnston, 1990). The precise mechanisms by which ACh diminishes glutamate or GABA release from presynaptic nerve endings is not certain. It could be by a direct depression of terminal Ca^{2+} current, comparable to the blockade of Ca^{2+} current in CA1 pyramidal cells, as reported by Gähwiler and Brown (1987); or less directly following depolarization of the terminal (Hounsgaard, 1978).

3.4 Actions of synaptically released ACh

To what extent are the above actions of exogenously applied ACh manifested in synaptically mediated actions of endogenous ACh? No central cholinergic pathway can be selectively stimulated, like the peripheral cholinergic nerves. Because of their slow time course, however, cholinergic synaptic actions can be identified in slices. As first demonstrated by Cole and Nicoll (1984), strong electrical stimulation of hippocampal
slices can evoke, in addition to the fast EPSPs and IPSPs, very prolonged "slow EPSPs" in CA1, whose cholinergic nature is revealed by the classical pharmacological criteria: enhancement of response by anticholinesterase (such as eserine) and their suppression by standard muscarinic antagonists (atropine and scopolamine). This slow EPSP was later also found in CA3 and dentate gyrus (Muller and Misgeld, 1986). This response is mediated mainly by the blockade of a leak K⁺ channel (Madison et al., 1987). During the response, the slow AHP is largely reduced, but with little or no detectable depression of Iₘ (Madison et al., 1987). Thus, suppression of Iₘ seems to be of lesser functional importance than the elimination of other K⁺-currents as the mechanism underlying the most prominent cholinergic effects, such as depolarization, excitation and facilitation of other depolarizing inputs and the generation of more or less prolonged repetitive firing (including bursts).

During the slow EPSP, Pitler and Alger (1992) also found that the spontaneous IPSP increased in both amplitude and frequency, which was very similar to the increase in spontaneous IPSPs after the application of carbachol. They suggest that synaptically released ACh may have a direct excitation effect on interneurons.

3.5 Muscarinic receptor subtypes and second messengers

Muscarinic receptors were initially classified into M1 and M2 subtypes according to their differential affinities for the muscarinic antagonist perenzepine, with M1 receptors having high affinity and M2 receptors low affinity for the compound. The use of a perenzepine derivative, AF-DX 116, helped in identifying diversity within the M2 subclass, which was further subdivided into M2-cardiac receptors having high affinity for AF-DX 116, and M2-glandular receptors having low affinity for the drug. The latter
subtype was subsequently called M3 (Ladinsky et al., 1990). A fourth subtype, M4, was later identified in cell lines as well as in brain and peripheral tissues (Lazareno et al., 1990). At the time when three pharmacological subtypes had been described, molecular cloning studies resulted in the identification, in both human and rat, of five genes or cDNAs encoding proteins with the characteristics of muscarinic receptors. These cloned receptors have been termed m1-m5, and studies on the pharmacological properties of these cloned receptors have shown that cloned m1, m2, m3 and m4 receptors correspond, respectively, to the previously pharmacologically identified native M1, M2, M3, and M4 receptors (see review, Hulme et al., 1990). To date, no native counterpart for the cloned m5 receptors has been described. It is thus evident that for muscarinic receptors, as is the case for many other families of neurotransmitter receptors, the molecular diversity is higher than that detected earlier by pharmacological means. In addition, it appears that m1, m3 and m5 receptors couple preferentially to stimulation of phospholipase C (PLC) through a Pertussis toxin-insensitive G-protein, with subsequent elevation of IP3, while m2 and m4 receptors couple through a Pertussis toxin-sensitive G-protein to preferentially inhibit adenyl cyclase (see Hulme et al., 1990).

Unfortunately, there are no single muscarinic antagonists with sufficient subtype selectivity to be clearly diagnostic for any particular receptor type. For example, pirenzepine is the most common muscarinic receptor antagonist that has been used. Its affinity to the M1 subtype, however, is only 100 times higher than that to the M2 subtype. In addition, because of the technical difficulty of recording voltage-clamped currents for prolonged periods, most attempts to define the muscarinic receptor subtypes involved the use of single concentrations of inadequately-selective antagonists. There are, therefore, some discrepancies as to which subtype is involved in a specific muscarinic function. It
has been consistently observed that the inhibition of leak K⁺ current can be easily blocked by low concentration of pirenzepine (Dutar and Nicoll, 1988). It has been concluded, therefore, that this effect is mediated by the M₁ receptor subtype. A more recent study (Pitler and Alger, 1990), using a battery of antagonists with different subtype preferences, demonstrates that the potency of M₃ antagonist, AF DX-116 in blocking the cholinergic slow EPSP is larger than that of pirenzepine, suggesting that the muscarinic inhibition of leak K⁺ current is more likely to be mediated by the M₃ receptor subtype.

Pharmacological characterization of muscarinic inhibition of Iₘ in neurons, has not provided a definitive picture of the receptor subtype involved: Marrion et al. (1989) suggested that M₁ receptors inhibited Iₘ in rat sympathetic neurons, while Constanti and Sim (1987) and Dutar and Nicoll (1988) proposed that M₂ receptors inhibit Iₘ in olfactory cortex and hippocampal neurons, respectively. Another study using cell lines transfected with a gene encoding a single muscarinic receptor subtype demonstrates that only the M-current induced in cells expressing m₁ and m₃ subtypes, but not m₂ and m₄ subtypes, can be inhibited by ACh (Robbins et al., 1991). In addition, the inhibition of the M-current in m₃ receptor gene-transfected cells was higher compared to m₁ receptor gene-transfected cells. This result might explain why muscarinic Iₘ inhibition seen in olfactory cortex neurons (Constanti and Sim, 1987) and hippocampal neurons (Dutar and Nicoll, 1988) are insensitive to pirenzepine, which may be due to the presence of m₃ receptors on these cells. Muscarinic inhibition of the slow AHP and Ca²⁺ have been suggested to be mediated by M₂ receptors, since they are pirenzepine-resistant (Muller and Misgeld, 1986). In both studies, however, only single doses of pirenzepine were used. These conclusions, therefore, remain to be confirmed.
The role of any further intracellular messengers such as Ca^{2+}, IP_{3}, and protein kinase C is even less clear, and several studies have produced conflicting results (Dutar and Nicoll, 1988). These will not be discussed further.

3.6 Nicotinic actions

The understanding of nicotinic actions is far less than of muscarinic actions. Binding studies have demonstrated that nicotinic receptors are distributed throughout the brain, including the hippocampus (Clarke et al., 1985). Molecular biological studies have identified different subtypes of nicotinic receptors composed of different combinations of α and β subunits (Boulter et al., 1987). In Alzheimer's disease, a decrease in the number of ACh nicotinic receptors has also been observed in a variety of brain regions (Araujo et al., 1988). The function of these nicotinic receptors, however, is far from clear. Perhaps the best known function of nicotine in the hippocampus is its facilitation of the release of neurotransmitters, including norepinephrine (Arqueros et al., 1978) and ACh itself (Chesselet, 1984), via action on presynaptic nicotinic receptors. There is also evidence suggesting that cholinergic activation of nicotinic receptors leads to vasodilation, resulting in an increase in local hippocampal cerebral blood flow (Cao et al., 1989). A similar effect has also been observed in neocortex (Biesold et al., 1989).

Another effect of nicotine in the hippocampus is its ability to increase neuronal excitability. It has been reported that nicotine elicits an increase in the population spike amplitude and in the number of population spikes in the CA1 region of hippocampal slices (Greund and Wehner, 1987), which is similar to the effect following the administration of the GABA-A antagonist bicuculline (Freund et al., 1988). The nicotine-elicited increase in excitability was blocked by nicotinic receptor antagonist, mecamylamine, or GABA.
agonists, suggesting that the effect is mediated by the activation of nicotinic receptors in the hippocampus, and possibly by a disinhibition of interneurons (Freund et al., 1990). Consistent with this, Miner and Collins (1989) examined nicotine-induced seizure sensitivity in 19 inbred mouse strains, and found a significant positive correlation between seizure sensitivity and the nicotinic receptor concentration in the neocortex and hippocampus, as revealed by alpha-bungarotoxin (α-BT) binding. A recent α-BT binding study conducted in the rat hippocampus revealed that most heavily labeled cells are GABAergic interneurons in both CA fields and dentate gyrus, which contain a variety of neuropeptides (Freedman et al., 1995). Interestingly, these interneurons are among those receiving medial septal GABAergic innervations (Freund et al., 1988). Therefore, the data are consistent with the hypothesis that cholinergic activation of nicotinic receptors may be involved in the regulation of neuronal excitability in hippocampal circuits by modulating the activity of GABAergic interneurons.

3.7 Effect of medial septal stimulation

Although septal stimulation does not produce observable responses in the hippocampus, a single stimulus to the medial septum facilitates the perforant path evoked population spike in dentate gyrus (Alvarz-leefmans and Gardner-Medwin, 1975). This septal prestimulation effect could not be blocked by a muscarinic antagonist, however, it could be blocked by a GABA antagonist (Bilkey and Goddard, 1985), suggesting that the facilitation effect may be through disinhibition of GABAergic interneurons in the hippocampus. A similar septal facilitation effect on cell excitability of population responses was also found in CA1 (Krnjévic et al., 1981), and this effect was mediated by disinhibition of interneurons in CA1 (Ben-Ari et al., 1981). In CA1, however, the facilitation effect could be partially blocked by atropine, and potentiated by hippocampal
administration of cholinesterase antagonist, eserine (Krnjévic and Ropert, 1982). It should be noted that the septal facilitation effect, both in CA1 and dentate gyrus, are transient, only lasting a couple of hundred milliseconds, which is in contrast to the conventional slow muscarinic effect mediated by second messenger systems. These findings were reported before the GABAergic component of the septo-hippocampal projection was known (Köhler et al., 1984). Thus, it is most likely that this facilitation effect is mediated by the activation of the septal GABAergic projection, resulting in the inhibition of inhibitory interneurons in the hippocampus. This disinhibition subsequently leads to a facilitation of the principal cell’s firing. It is not clear how the septal facilitation effect in CA1 is also partially blocked by atropine. It is possible that the GABA release from inhibitory interneurons may be suppressed by ACh through a presynaptic mechanism, and the blockage of muscarinic receptors may lead to an increase in neuronal excitability. Unfortunately, no evidence is available yet to address the question of whether hippocampal applications of GABA receptor antagonists block the facilitation effect of septal stimulation in CA1 as is found in the dentate gyrus.

3.8 The role of the hippocampal cholinergic input in learning and memory

The projection from the medial septum is one of the major subcortical inputs to the hippocampus. It is well known that damage to the medial septum mimics the behavioral effects observed following damage to the hippocampus (see review, O’Keefe and Nadel, 1978). For example, rats with medial septal damage are impaired on T-maze alternation (Rawlins and Olton, 1982), performance on the spatial version of the Morris swim task (Hagan et al., 1988), and reference and working memory measures of performance in the radial eight-arm maze (Crutcher et al., 1983). Thus, it appears that the integrity of the
septo-hippocampal projection is necessary for the normal operation of the hippocampus. Because it is well established that a substantial component of this projection is cholinergic, until recently, it had been thought likely that these lesion effects were due to the disruption of cholinergic cells in the medial septum. Alternate interpretations are also possible, however, due to the lack of specificity of older lesion methods, and the fact that at least some of the deficits observed may be caused by damage to GABAergic cells in the medial septal area, which project intrinsically as well as to the hippocampus (Freund and Antal, 1988).

Very recently, a promising new tool has become available for selectively lesioning cholinergic basal forebrain neurons (Wiley, 1992). Many cholinergic basal forebrain neurons express high levels of the low-affinity p75 NGF receptor (NGFr) relative to other cholinergic and non-cholinergic neurons in nearby regions (Gage et al., 1989a). Virtually all cholinergic neurons in the medial septum are NGFr positive. When a monoclonal antibody to the p75 NGF receptor (192 IgG) is coupled to the ribosomal-inactivating protein saporin, it selectively destroys neurons bearing NGFr in vivo in rats (Book et al., 1992). Most of the cholinergic cells within the basal forebrain are vulnerable to this toxin, with the possible exception being those neurons that send an efferent projection to the amygdala (Heckers & Mesulam, 1994). It has been reported that medial septal injections of this immunotoxin selectively kill cholinergic cells, but spare NGFr-negative non-cholinergic neurons in this area (Heckers et al., 1994). Thus, it is now possible to examine explicitly the septal cholinergic projection to the hippocampus, and to assess its effect on spatial learning.

Nilsson et al. (1992) were the first to examine the effect of selective immunotoxic lesions of cholinergic cells in the basal forebrain on rat behaviors on a learning and
memory task (Nilsson et al., 1992). They found a substantial spatial learning impairment on the Morris water task, following intraventricular (ICV) injection of 192 IgG-saporin. Berger-Sweeney et al. (1994) using injection of immunotoxin directly into the basal forebrain system, however, found that rats with medial-septal immunotoxin lesions were only impaired mildly in the initial acquisition and reached normal asymptotic levels of performance. In contrast, ICV injection of immunotoxin produced a significant deficit on the water task, which is consistent with Nilson et al.'s results. This deficit appears to be due to non-cholinergic damage after immunotoxin administration by the ICV route, because analysis of ICV-injected brains in both studies (Nilsson et al., 1992) revealed quite extensive cerebellar damage where p75 NGF receptors are also known to be localized to non-cholinergic neurons. In addition, Berger-Sweeney et al. found that rats with ICV immunotoxic lesions were impaired in both spatial learning and a non-spatial cue-guided task. Baxter et al. (1995) also found that selective lesions of cholinergic cells in the medial septum (about 90%) do not have any apparent effect on the rat's place discrimination on the Morris task when compared to control rats. The lesioned rats were, however, mildly impaired on a trial-unique matching-to-place problem in a delay-independent fashion. Consistent with this, Torres et al. (1994) found that injections of immunotoxin into either the medial septum or the diagonal band failed to produce any behavioral deficits in the acquisition in the spatial version of the water task. These data, therefore, suggest that lesions of the cholinergic septal-hippocampal projection are not sufficient to cause a behavioral deficit in spatial learning and memory on the Morris water task.

Previous studies (Decker, Radek, Majchrzak, & Anderson, 1992; Miyamoto et al., 1988) indicate that septal lesions can produce qualitatively different effects on rats'
performance on the Morris swim task and the radial 8-arm maze. It remained to be examined whether selective neurotoxic lesions of the medial septum have the same effect on rats' behavior on the radial-8-arm maze. The study described in Chapter 7 will address this question.
CHAPTER 4 HIPPOCAMPAL PHYSIOLOGY

4.1 Theta rhythm

In 1938, Jung and Kornmuller (1938) first reported rhythmical slow wave activity in the hippocampus of rabbits induced by the stimulation of peripheral nerves. It was Green and Arduini, however, who first systematically studied the hippocampal theta rhythm in both acute (curarized) and chronic conditions, using three species, rabbits, cats, and monkeys (Green and Arduini, 1954). They reported a large regular sinusoidal oscillation under some conditions with a frequency range of 3-6 Hz in acute animals, and 5-7 Hz in chronically implanted animals. This oscillation was most obvious in cats and rabbits, but rarely observed in monkeys, possibly because the conditions under which it has been studied is under restraint. They also explored the mechanism of theta rhythm and found that stimulation of midbrain tegmentum elicits theta rhythm, and lesions of septal areas or disconnection of the hippocampus from medial septum by cutting the dorsal fornix abolished the theta rhythm. These important findings provided an essential basis for intensive studies on hippocampal theta rhythm for the following decades. Although the theta rhythm has intrigued many investigators and numerous reports have been published in the past decades, the mechanism of this prominent hippocampal EEG pattern is still far from completely understood, and its functional significance remains even more elusive. The following section is a brief summary of what is currently understood or generally believed about the hippocampal theta rhythm. More detailed reviews can be found in Bland (1986), Bland and Colom (1993), Gottesmann (1992), Vinogradova (1995), and Skaggs (1996a).
4.1.1 Two types of theta

In the 1950s and early 1960s, investigators proposed that the theta rhythm may be related to arousal, orienting, motivation, learning or attention processes (e.g., Green and Arduini, 1954). Vanderwolf is credited with being the first person to carry out studies correlating hippocampal theta with observable behaviors, i.e., he studied what animals did rather than inferred processes. He found that the strongest theta was observed during locomotion, and concluded that the theta rhythm is related to “voluntary” movement (Vanderwolf, 1969), or “type 1” behavior (Vanderwolf et al., 1975). This was supported by several other reports in the literature around that time, and by numerous publications in the ensuing years in a variety of species (e.g., rat, rabbits, guinea pigs, Mongolian gerbils, dogs, see review, Bland, 1986). Cats are special in that they can walk around without any theta activity accompanying this behavior. On the other hand, there was another line of evidence suggesting that theta also occurs during alert immobility and during anesthesia, however, with a lower frequency range. Based on the accumulating evidence (Green and Arduini, 1954), Kramis et al (1975) published the first paper arguing that there were two types of theta. This was also later supported by a number of behavioral and pharmacological studies (see review, Bland, 1986). Currently type 1 theta is also called movement-related theta, which is associated with the type 1 behaviors, including walking, running, swimming, rearing, jumping, digging, manipulation of objects with the forelimbs, isolated movements of the head or one limb and shifts of posture. In rats and rabbits, type 1 theta has a frequency range of about 7-12 Hz. It cannot be abolished by systematic administration of large doses of atropine sulfate, which can cross the blood-brain barrier. It can, however, be abolished by anesthetics such as ethyl ether, urethane and pentobarbital, all of which also abolish locomotion. Type 2 theta is operationally defined as the theta that occurs in the complete absence of movement. It is
the type of theta that appears to vary the greatest across species with respect to the ease with which it may be elicited. All species investigated thus far appear to possess type 2 theta. The type 2 theta is most easily elicited in rabbits by sensory stimuli of any modality. Guinea pigs also produce type 2 theta in response to sensory stimuli, but the range of effective stimuli is narrower. On the other hand, type 2 theta is rarely seen in the unrestrained rat. It has been observed that type 2 theta occurs when the rat is presented with shock stimuli in a fear conditioning situation, or presented with a cat or a ferret moving around (Sainsbury, 1987). Thus, it has been hypothesized that type 2 theta may occur only in a high state of arousal in rats. In anesthetized states, however, type 2 theta can be easily elicited by pinching on the rat tail or stroking its fur. In rats and rabbits, type 2 theta has a slightly lower overall frequency range of 4-9 Hz. In contrast to the type 1 theta, it is resistant to most anesthetics but is abolished by cholinergic antagonists like atropine sulfate and scopolamine. Type 2 theta can also be elicited by the administration of eserine, a cholinesterase antagonist.

It has been found that entorhinal cortex lesions do not eliminate theta, but render it atropine sensitive (Montoya and Sainsbury, 1985). In other words, the theta remaining after the lesion is of the type 2 variety. Ylinen et al. (1995) have shown that the depth profiles of theta in entorhinal-lesioned and urethane-anesthetized rats are similar in shape. These results suggest that the defined type 1 theta may actually co-exist with type 2. In fact, Bland (1986) has argued that type 2 may be always active when the type 1 theta is active, based on the evidence that acetylcholine release is increased in both the neocortex and hippocampus during both sensory stimulation and movement (Dudar, 1975); and that the administration of atropine sulfate abolishes theta cell rhythmicity during the type 2 theta behavior conditions and reduces the number of rhythmic discharges during the type 1 theta behavior condition (Bland et al., 1984). In the discussion that follows, it can also be seen
that in most cases type 1 and type 2 theta can be distinguished physiologically, and that this can also assist classification and distinction of type 1 from type 2 theta.

4.1.2 Generators

The amplitude of the theta rhythm in the hippocampus is one of the largest amplitude EEG signals in the brain. This amplitude can be as large as 1mV in the rat, depending on where the recording electrode is positioned. Its regular, large waveform indicates that it is caused by synchronous actions of a large population of cells, presumably principal cells, in the hippocampus. Before going into a detailed discussion of the generators, the dipole mechanism for the generation of field potentials should be understood.

Because of the regular laminated structure in both the CA1 region and dentate gyrus, large field potentials can be recorded in both regions by the stimulation of afferent fibers. Field potentials reflect the sum of extracellular currents generated by populations of individual neurons. Electrical stimulation of the afferents (perforant path in the dentate gyrus, and Schaffer collaterals in CA1) results in synchronous, monosynaptic activation of dendrites of principle cells in interneurons. Synaptic currents from the distal parts of the dendrites will flow toward the cell body inside the cell. Correspondingly a current will flow from cell body to the place of synaptic inputs in the extracellular space. Thus, from the perspective of an extracellular electrode, current appears positive at the soma (source) and negative at the active synapses (sink). The “source” and “sink” constitute a dipole for the generation of the field potential. Thus, if stimulating Schaffer collaterals, an extracellular recording electrode gradually moved down through the CA1 region records a positive potential at the cell body layer, and a negative potential in stratum radiatum. A
Figure 4.1 Dipole mechanisms in the generation of the theta rhythm in the hippocampus. 

A. A schematic drawing of a layer of CA1 pyramidal cells with extensive dendritic trees. When a population of cells receive synaptic inputs at their distal dendrites, the positive current would flow inside the cell toward the cell body. Correspondingly, in the extracellular space, positive current would flow from the cell body layer (sink) to the distal portion of the dendritic area (source). Thus, with a gradual penetration of a recording electrode, as represented by the dashed line, two maxima of the extracellular potentials would be encountered at places around the distal dendrites and the cell body layer respectively, which are 180° out of phase. A transitional area with virtually zero potential occurs somewhere in between. 

B. The depth phase profile of hippocampal theta generated by the dipole located in CA1 and the dipole in dentate gyrus. A schematic drawing of a transverse section of the hippocampus is illustrated, superimposed on which are drawings of two phase profiles generated by CA1 and dentate gyrus dipoles respectively, as represented by thin lines. The thick line represents the summed phase profile of the theta rhythm in the hippocampus. (Adapted from Skaggs, 1996).
similar result will be obtained from the granular and molecular layers of the dentate gyrus. Out in the molecular layer, negative field potentials induced by stimulation of the perforant pathway will be recorded, while positive field potentials will be recorded from the granule cell layer (see fig. 4.1A).

Some early studies suggest that the depth profile of the theta rhythm could be explained entirely in terms of a single generating dipole located in the CA1 cell layer (Green et al., 1960). Winson (1974) and Bland et al. (1975) first proposed that the theta rhythm is generated by two generators in the hippocampus, one located in CA1, the other in the dentate gyrus. This is supported by the following lines of evidence: 1) there are at least two maxima of theta rhythm in the hippocampus, one in the stratum oriens of CA1, the other in the superficial dentate gyrus molecular layer near the fissure. The theta rhythm at the two maxima are 180° out of phase; 2) theta can still be observed in the dentate gyrus after manipulations eliminating theta in CA1 (Bland et al., 1975); 3) cells recorded in both CA1 and dentate gyrus are modulated by theta; 4) there appears to exist a third amplitude peak in the molecular layer of the lower blade of the dentate gyrus (Bland et al., 1975); 5) rats that have had neonatal X-radiation to prevent development of granule cells show theta rhythm in the CA1 area, while the theta amplitude peak in the lower blade of the dentate gyrus is absent (Willshaw et al., 1978). An excellent illustration of the dipole mechanisms of hippocampal theta by two generators (Skaggs, 1996) is shown in Fig 4.1 B. The summed activity of two dipoles, one generated in CA1, the other in the dentate gyrus, results in a relatively larger maximum near the hippocampal fissure and two smaller maxima located at CA1 stratum oriens and the lower blade of the dentate gyrus, respectively. In the hilus, no theta rhythm is recorded, possibly because the amplitude produced by two dipoles are approximately the same, but 180° out of phase. There has
been some controversy regarding the theta rhythm in CA3. Petsche and Stumpf (1960) reported a theta maximum in CA3, but later studies by Bland et al. (1975) and Bland and Whishaw (1976) failed to find theta in this area. The cells in CA3 have been observed to be modulated by theta (Skaggs, 1996), but this may be due to the fact that the CA3 region receives rhythmic inputs from dentate gyrus and entorhinal cortex, both of which exhibit clear theta.

Winson (Winson, 1976a) found that in rats, the depth profile of the theta rhythm in the hippocampus is different for the type 1 theta and type 2 theta. In urethane-anesthetized and curarized rats, the theta recorded from CA1 and dentate gyrus generator regions were separated by a short “null zone” where no theta was recorded (Bland and Whishaw, 1976). In freely moving rats, however, the depth profile is characterized by a gradual phase shift occurring over 400 μm in CA1 stratum radiatum and no null point below the pyramidal cell layer (Winson, 1976b). Leung (1984) provided a model that successfully explained the type of theta profiles one would expect to see under various experimental conditions. This model proposed that the theta field potential seen in the urethane-anesthetized or curarized rat is generated by rhythmic somatic (proximal) inhibition, which results in a dipole with two amplitude maxima at the basal dendritic and the distal apical dendritic layers respectively, and a distinct null zone and phase reversal at the apical side of the CA1 pyramidal cell layer. The gradual phase shift seen in CA1 stratum radiatum of freely moving rats can be accounted for by the addition of another dipole, which is produced by rhythmic excitation at distal dendrites of CA1 pyramidal cells with some time-delay to the somatic inhibition.

The hypothesis of the above model has been supported by recent data. Heynen and Bilkey (1991) applied theta rhythmic stimulation to afferents in the CA1 region of the
hippocampal slice preparations, and found that only the rhythmic activation of stratum lacunosum of CA1 (near the distal tips of the dendrites), but not stratum radiatum nor stratum oriens, produced theta of an amplitude and phase profile similar to that of naturally occurring theta. This is consistent with the hypothesis that the rhythmic excitation of the distal dendrites of pyramidal cells plays an important role in the generation of type 1 theta. It should be noted that it is the entorhinal projection that synapses on the distal dendrites of CA1 pyramidal cells. Leung and Yim (1986) reported that in the urethane-anesthetized rat, intracellular theta potential has the same properties as the evoked IPSP. Consistent with this, Ylinen et al. (1995) found that the theta recorded after bilateral entorhinal cortex lesions lacks the usual large amplitude peak at the level of the hippocampal fissure. In addition, intracellular recordings during urethane-induced theta, which has a phase profile very similar to that associated with entorhinal lesions, reveal that rhythmic subthreshold potentials could be blocked by injection of Cl⁻. This indicates that the intracellular potentials are GABA-A-mediated rhythmic IPSPs. These results are therefore consistent with the hypothesis that type 2 theta may be generated by rhythmic inhibition at the soma of pyramidal cells in hippocampal CA1.

4.1.3 Afferent Systems

4.1.3.1 Brain Stem

As mentioned previously, in the first detailed report on theta rhythm, Green and Arduini (1954) demonstrated that hippocampal theta could be elicited by direct electrical stimulation of the brain stem reticular formation (RF). Virtually every later study concerned with the effect of brain stem stimulation on the hippocampal EEG has implicated the reticular formation in hippocampal theta generation (see review, Vertes, 1982). Three laboratories (Macadar et al., 1974a) have systematically compared various
reticular nuclei with respect to their relative efficacy in generating theta. Vertes (1980, 1981) and Macadar et al. (1974) found that pontis oralis (RPO) stimulation was most effective in generating theta compared to the stimulation of other reticular nuclei. Robinson and Vanderwolf (1978), however, reported essentially equivalent effects from stimulation of the three main reticular nuclei of the brain stem (nucleus gigantocellularis, NGC; nucleus pontis caudalis, RPC; and nucleus reticularis pontis oralis, RPO). The discrepancy appears to arise from the fact that the former studies were conducted in anesthetized animals, whilst the latter was carried out in freely moving rats. Vertes (1982) argued that the RPO is the principal brain stem site involved in the generation of theta activity. Vertes and his colleagues have also reported that microinjections of carbachol into RPO were able to generate theta immediately, while injections into the adjacent areas in the brain stem had either no effect or produced theta rhythm with a much longer latency (Vertes et al., 1993). It has been found, however, that lesions of the PNO have no effects on hippocampal theta nor on the behavioral correlates of theta (Faris and Sainsbury, 1990). This suggests that the PNO may be part of a network of reticular sites involved in the generation of theta rhythm, but is not essential for it.

An unequivocal intriguing finding of all studies examining the effects of brainstem stimulation on hippocampal EEG has been that after the first second or so of stimulation, the theta frequency increases with increasing amplitude of the stimulus (see review, Bland, 1986). If the stimulation remained, however, the frequency declines to an asymptotic level within about 30-50 seconds, which is still positively correlated with the stimulation intensity (Bland and Vanderwolf, 1972). These results imply that besides the direct control of the reticular formation on the frequency of the theta rhythm, there may also be a dynamic process occurring somewhere in the complex neural circuits involved in theta generation, which leads to a final stable state for the hippocampal theta rhythm. It is also
interesting that both the stimulation intensity and the theta frequency are positively correlated with running velocity of animals, indicating that the central motor control system is closely related to the theta generation process.

### 4.1.3.2 Medial Septum / Diagonal Band Complex

The importance of the medial septum in the generation of the theta rhythm was already reported by Green and Arduini in 1954. They found that lesioning the medial septum eliminates theta in both freely moving and paralyzed animals, i.e., medial septal lesions abolish both type 1 and type 2 theta. This effect is not due to damage to passing fibers, because infusions of muscimol, a GABA-A agonist, into the medial septum eliminate the theta rhythm in the hippocampus in both waking and anesthetized conditions (Allen and Crawford, 1984). Electrical stimulation of the medial septum also results in theta activity in the hippocampus (Brücke et al., 1959). In contrast to the high frequencies (100 Hz) needed for stimulation of the brain stem, optimal septal stimulation frequencies lie in the range of naturally occurring theta. Each stimulus pulse or pulse train in the medial septum produces a corresponding wave in the hippocampal EEG, resulting in the induced theta frequency being identical to the stimulus frequency. On the other hand, high frequency stimulation of the medial septum results in desynchronization of electrical activity in the hippocampus. Thus, it is generally thought that the medial septum acts as a transducer which transforms the intensity of tonic input from the brain stem into a frequency for the hippocampal theta rhythm, hence, may be a "pacemaker" for hippocampal theta. Petsche et al. (1962) were the first to record from a group of cells in the medial septal region that discharged in rhythmic bursts during the presence of the theta rhythm in the hippocampus. They also found that these rhythmic bursts always occurred during a certain phase of the hippocampal theta wave, and was not affected by the post-
ictal depression of hippocampus following a seizure. This indicated that rhythmicity of the septal units was not dependent on output from the hippocampus. This is supported by a later finding (Stewart and Fox, 1989) that disconnecting the hippocampus from the medial septum by cooling the fornix abolished the theta rhythmicity of hippocampal cells but had little or no effect on the rhythmicity of septal cells.

As mentioned in the Chapter 2, there are mainly two groups of cells in the medial septum, cholinergic cells and GABAergic cells. Because the action of ACh in the hippocampus take places for a course of seconds, it does not appear possible for the cholinergic projection to “pace” the theta oscillation. In fact, this is supported by recent evidence that selective lesions of cholinergic neurons in the medial septum by a neurotoxin does not affect the theta frequency in the hippocampus, although it reduces greatly the theta amplitude (Lee et al., 1994). It has been found that stimulation of the medial septal area does not give rise to evoked responses in the hippocampus, but results in an increase in the population spike elicited by the subsequent stimulation of the perforant path. In other words, it increases the excitability of dentate granule cells (Alvarz-leefmans and Gardner-Medwin, 1975). This increase in excitability is a transient effect, lasting only for around 150 msec. The effect is blocked by local hippocampal administration of picrotoxin, a GABA-A antagonist, but not cholinergic antagonists. Septal stimulation also increases the excitability of CA1 pyramidal cells within the same time scale, which appears to be due to a disinhibiton effect (Krnjévic and Ropert, 1982). This line of evidence suggests that the GABAergic septal projection may lead to a disinhibiton of principal cells by its inhibition of hippocampal interneurons. This conclusion was later supported by the anatomical evidence that the GABAergic septohippocampal projection terminates mostly on interneurons in the hippocampus (Freund and Antal, 1988).
Simultaneous intracellular recordings from GABAergic interneurons and pyramidal cells from \textit{in vitro} slice preparations demonstrate that individual GABAergic interneurons can effectively entrain the phase of spontaneous firing and subthreshold oscillations in hippocampal pyramidal cells at theta frequencies (Cobb et al., 1995). This frequency entrainment is due to the interaction of GABA\textsubscript{A}-receptor-mediated hyperpolarizing synaptic events with intrinsic oscillatory mechanisms tuned to this frequency range in pyramidal cells. The authors suggested that the basket cells in the hippocampus may be entrained by GABAergic inputs from the medial septal inputs. The question that remains is that although a proportion of neurons in the medial septum discharge in phase with hippocampal theta, the phase relationship between those cells appears to be random (Petsche et al., 1962). It is thus not clear how the inputs from septal neurons are able to maintain the firing of hippocampal basket cells at the same theta phase, if one assumes that at least some of these rhythmic firing septal neurons are GABAergic neurons.

It has been found that application of a cholinergic agonist, carbachol, into the hippocampus induces theta rhythm both \textit{in vivo} (Malisch and Ott, 1982) and \textit{in vitro} (MacIver et al., 1986). It is, therefore, possible that the neural network in the hippocampus has its own capacity to oscillate at the theta frequency when receiving certain inputs. It should be noted that all those \textit{in vitro} studies used hippocampal slices from juvenile rats, in which interneurons have not been fully developed. Heynen and Bilkey (1991) reported that in hippocampal slices of adult rat, carbachol itself could not elicit theta rhythm. Only the presence of both carbachol and a GABA receptor antagonist resulted in the appearance of the theta rhythm. Moreover, they found that the presence of both excitation and disinhibition also elicited theta rhythm in \textit{in vivo} hippocampus without any septal influence (i.e., after fornix transection). In another study (Kononpacki and Golebiewski, 1993), it was also found that GABA-A antagonists facilitate the effect of
low concentrations of carbachol for inducing theta-like oscillations in hippocampal slice preparations. These findings, therefore, indicate that septal cholinergic and GABAergic efferents may act synergistically to generate theta in the hippocampus, and that the hippocampus is capable of generating theta-like oscillation without an external source of rhythmic device.

It remains controversial as to whether the medial septum is the real pace maker. If this structure were a true pace maker, manipulation of the medial septum should result in a change of theta frequencies in the hippocampus. So far, all the data suggest that manipulation of medial septum either results in a complete elimination of theta, or leaves the frequency intact. It has been found that the microinfusion of muscimol, a GABA-A agonist into the medial septum/diagonal complex resulted in a progressive reduction in the theta amplitude and finally the total loss of theta activity. In contrast, theta frequency remained unaffected during the entire postinfusion period when the theta field activity was present (Bland et al., 1996). A similar result has also been observed using the microinfusion of lidocaine into the medial septum (Kirk and McNaughton, 1993). Smythe et al. (1992) reported that reversible blockade of the medial septum/diagonal band complex by microinjection of procaine abolished the theta rhythm. During the post-drug period, while the amplitude of the theta rhythm gradually recovered within one hour, the theta frequency appears to recover abruptly within 20 minutes. Therefore, the medial septum appears to be more involved in the determination of the amplitude of the theta rhythm, likely by the synergistic actions of both cholinergic and GABAergic projections to the hippocampus, and may not be directly involved in the determination of theta frequency. The frequency properties could be determined by the intrinsic properties of the neural circuit in the hippocampus. Alternatively, the medial septum may relay a frequency from an upstream region.
4.1.3.3 The supramammillary area

As discussed above, high-frequency stimulation of the brain stem results in the appearance of theta rhythm in the hippocampus. Anatomical studies, however, suggest that there are no direct projections from the pontine nucleus to the medial septum. The pathway, therefore, is most likely a polysynaptic one (Vertes, 1982), and the supramammillary area in the posterior hypothalamus may be at least one of the relay areas between the brain stem and the medial septum. A number of studies on the theta rhythm have found that electric stimulation of posterior hypothalamus evokes theta rhythm in the hippocampus. Furthermore, Bland et al. (1994) found that local administration of either atropine or procaine into the posterior hypothalamus blocked both the theta elicited by brain stem stimulation and rhythmic activity of cells in the medial septum in urethane-anesthetized rats. Kirk and McNaughton (1991) reported that theta rhythm can be recorded in the supramammillary nucleus, whose frequency was not affected by the inactivation of medial septum using a local anesthetic. This finding suggested that the supramammillary nucleus may lie somewhere upstream of the medial septum for the generation of theta. In a later study (Kirk and McNaughton, 1993), the effect on the theta frequency and amplitude of inactivation of various points along the axis starting from nucleus reticularis pontis oralis of the brain stem, through the supramammillary area, to the medial septum was examined in urethane-anesthetized rats. It was found that inactivation of areas either below or including the supramammillary nucleus reduced both the amplitude and frequency of the theta rhythm elicited by brain stem stimulation, while inactivation of areas above the supramammillary nucleus, including the medial septum, reduced only the amplitude but not the frequency. This finding strongly implicates the supramammillary nucleus in the role of a relay station between the brain stem and the medial septum for the generation of type 2 theta. Furthermore, these authors have
suggested that the real pacemaker, at least for type 2 theta, may reside in the supramammillary nucleus itself. Consistent with this hypothesis, Kocsis and Vertes found that a proportion of neurons in the supramammillary nucleus discharge in synchrony with hippocampal theta rhythm in anesthetized rats (Kocsis and Vertes, 1994). Two additional studies (McNaughton et al., 1995), however, were inconsistent with the view that the supramammillary nucleus is the pacemaker for type 1 theta. In contrast to the obvious effects of inactivation of the supramammillary body on type 2 theta induced by brainstem stimulation in anesthetized rats, the same inactivation resulted in little change in theta frequency in freely moving rats. This indicates that the supramammillary nucleus may not be critical for the generation of type 1 theta. It should be noted that lesioning the medial septum abolish both types of theta. Thus, it appears that the supramammillary body may be one of multiple upstream regions that play a role in controlling the medial septum in the generation of hippocampal theta.

4.1.3.4 Entorhinal Cortex

As stated above, Vanderwolf and Leung (1983) reported that lesions of the entorhinal cortex in rats abolished type 1 theta, while leaving type 2 theta intact. The evidence for this supposition was that the subsequent administration of atropine or scopolamine to these lesioned animals resulted in the complete disappearance of slow wave theta activity. Without the administration of atropine or scopolamine, the rats continued to produce some theta activity during activities like walking and rearing. In another study (Vanderwolf et al., 1985), the same results were observed using the technique of entorhinal isolation rather than entorhinal lesions. Montoya and Sainsbury (1985) have investigated the effects of entorhinal cortex lesions on type 1 and type 2 theta in the guinea pig, and found that the administration of atropine to the lesioned animals
abolished all theta, in agreement with Vanderwolf and colleagues. They also noticed a reduction in the occurrence of type 2 theta in response to sensory stimuli following entorhinal lesions in the awake guinea pig. Consistent with this finding, Heynen and Bilkey (1994) found that entorhinal lesions reduced type 2 theta in anesthetized rats. The entorhinal cortex, therefore, appears to be involved in the generation of both type 1 and type 2 theta in the hippocampus. The fact that lesions of entorhinal cortex abolish completely the type 1 theta, but only partially reduce type 2 theta, suggests that the inputs from entorhinal cortex are more critical for the generation of type 1 theta.

Shortly after the discovery of the theta rhythm in the hippocampus (Green and Arduini, 1954), theta oscillation was also recorded in the entorhinal cortex (Adey, 1960; Holmes, 1960), the region providing one of the major inputs to the hippocampus. A recent study using the anesthetized rats demonstrates that the theta rhythm in the entorhinal cortex has an amplitude maximum in the outer half of layer I, and an amplitude minimum in the outer third of layer III and the inner half of layer I (Alonso and Garcia-Austt, 1987a). The discharge patterns of entorhinal cortex units have also been studied (Alonso and Garcia-Austt, 1987b). Three types of units were recorded, including cells firing rhythmically with theta rhythm at low rates, cells firing rhythmically at high rates, and non-rhythmic cells whose firing was modulated by theta. Most rhythmic cells were located in superficial cell layers (II-III), which consist of the cells projecting to dentate gyrus and CA regions of the hippocampus. Thus, along with the evidence reviewed above, the entorhinal cortex may provide rhythmic inputs to distal dendrites of CA1 pyramidal cells, which may play a critical role in the generation of type 1 theta in the hippocampus.
4.1.4 Relations between hippocampal theta and movement

As mentioned above, the relationship between movement and theta was first proposed by Vanderwolf (1969), who found that the strongest theta occurred during locomotion. Klemm (1971), using rats chronically implanted with EMG electrodes and electrodes in both hippocampus and brain stem reticular formation (BSRF), found that hippocampal theta rhythm and an increase in multi-unit activity BSRF were always associated with movements, and also occurred during any phasic increases in muscle electrical activity even without the presence of any overall movements. Several later studies have found a general linear relationship between the frequency of theta and the speed of movements (McFarland et al., 1975; Recce, 1994). It should be noted that there was only a small change in theta frequency corresponding to a relatively large change in velocity. Whishaw and Vanderwolf (1973) observed that hippocampal theta increased greatly in frequency when the rat made a jump. This increase in theta frequency occurred even before the initiation of the movement, suggesting that the theta rhythm more likely occurs before the movement, possibly reflecting an "intent" to move rather than the sensory consequences of movement. This hypothesis was supported by Morris and Hagan (1983), who also found that the frequency was strongly correlated with the height of the jump, and that the theta was more likely to occur before movement.

As mentioned above, brain stem stimulation induces theta rhythm in the hippocampus and locomotion at the same time. The intensity of the brain stem stimulation, theta frequency and running speed are all positively correlated. Consistent with this, Klemm (1971) found that there was a strong correlation of multiunit activity in the brain stem with hippocampal theta rhythm and muscle movement. It has also been observed that a short train of stimulation of the medial septum at 4-12 Hz elicited theta
rhythm in the hippocampus and orienting behavior (searching, rearing, sniffing, exploration) at the same time (Wetzel et al., 1978). Thus, it appears that the whole theta generation system involving the brain stem, medial septum and hippocampus are associated with movement when type 1 theta is generated. The underlying mechanism, however, is poorly understood. It appears that the theta rhythm is not necessarily always associated with movements. Klem (1971) noticed that the theta rhythm often outlasted muscle activity by a few seconds or more. Wetzel et al. (1978) reported that a sustained septal stimulation resulted in periodic orienting behavior alternating with grooming behavior, even though a continuous hippocampal theta rhythm was present. On the other hand, except during REM sleep, movement is always associated with hippocampal theta rhythm. Thus, it is unlikely that the theta rhythm in the hippocampus controls movement, rather, it may modulate or be involved in the central control of movement. Because there are regions in the brain stem that control locomotion (e.g., mesencephalic locomotion region [MLR])(Johnels, B. and Steg, 1982; Nicolopoulos-Stournaras and Iles, 1984), and because cells of the medial magnocellular reticular formation discharge at high rates of activity during waking-movement (Vertes, 1977, 1979), it is possible that the correlation between the theta rhythm and running velocity may be caused by a brain stem synchronizing process, rather than by a direct relationship between them.

Komisaruk (Komisaruk, 1970) observed in chronically implanted rats that there is a correlation between hippocampal theta and rhythmic movement of the rat vibrissae during sniffing. Just because these two processes exhibit frequency ranges very similar to one another does not prove a causal relationship between them. Macrides (Macrides, 1975) studied this phenomenon quantitatively. He found that the rhythmic frequencies of vibrissae movement and hippocampal theta were largely independent, but that there was a significant tendency for the two rhythms to entrain each other, exhibiting a fixed phase.
difference for a given animal. This suggests that the hippocampal and vibrissea oscillators were weakly coupled. Again, similar to the relationship between running velocity and theta rhythm, this weak coupling may be caused by an ascending system in the brain stem, whose output controls both rhythmicities via different pathways.

4.1.5 A hypothesis on the generation of hippocampal theta rhythm

As reviewed above, although the study of theta rhythm has a relatively long history in hippocampal physiology, the generating mechanism of theta is still far from completely understood after forty years of intensive investigation. The main reason for this possibly has to do with the fact that there appears to be many systems in the brain involved in theta generation. Here, I will summarize most experimental data and integrate them in a unified model.

Type 2 theta appears to be caused by the activation of nuclei in the brain stem, which belong to the ascending arousal system and whose efferents are mostly of cholinergic nature. The ascending output from the cholinergic nuclei are relayed by the supramammillary body, and reaches the medial septum / diagonal band complex. The quasi-rhythmic signals most likely originate in the supramammillary area and cause a group of neurons in the medial septum, most of which are presumably GABAergic, to discharge at theta frequencies. These GABAergic cells in the septum, then pace the GABAergic cells in the hippocampus, which in turn synchronize the membrane potential of principle cells in the hippocampus, resulting in the generation of theta rhythm. This pathway, therefore, is sensitive to atropine, because it would block the connection between the cholinergic ascending system and the supramammillary nucleus.
The mechanism for the generation of type 1 theta is much less understood. It is clear that type 2 theta is always coactive with type 1 theta. Because type 1 usually has a higher frequency, it usually dominates the oscillation. Only when type 1 theta is removed, does type 2 theta take over. Type 1 theta rhythm is tightly correlated with movement. Theta occurs during REM sleep in the absence of movement, because there is a descending pathway in the brain stem that is active during REM to inhibit the spinal cord from producing muscle movement. Thus, it can be hypothesized that the rhythmicity of type 1 theta is associated with the activity of “locomotion centers” in the brain stem by neural connections. One of the locomotion centers corresponds to the mesencephalic locomotion region (MLR), which overlaps somewhat with the cholinergic ascending arousal system (Garcia-Rill et al., 1987). This pathway is apparently not cholinergic. There is evidence showing that injection of GABA agonists into MLR blocks locomotion (Agmo and Tarasco, 1985). Anesthetics may have a similar effect. The efferents from this system appear not to pass through the supramammillary area, and end in the medial septum via an unknown region, which subsequently “paces” the GABAergic interneurons in the hippocampus by the same mechanisms as used in type 2 theta. At the same time, entorhinal cortex (and possibly other adjacent cortical areas) are also more strongly involved in this “locomotion” loop. It is possible that type 1 theta reflects a resonance of neural circuits in the brain that is involved in locomotion. Clearly, this model may be just naive conjecture. More effort is needed to decipher the exact neural mechanisms underlying the theta rhythm.
Figure 4.2 Schematic diagram of the hypothesis on the brain regions involved in the production of theta rhythm in the hippocampus.

4.1.6 The median raphe nucleus (MR) and EEG desynchronization

It has been shown that in both the rat (Assaf and Miller, 1978) and cat (Macadar et al., 1974b), median raphe (MR) stimulation produces hippocampal desynchronization (asynchronous, fast EEG activity). Additionally, Assaf and Miller (1978) report that MR
stimulation inhibits the discharge of the septal rhythmical bursting cells that pace hippocampal theta. This effect is blocked by pre-treatment with 5-HT synthesis inhibitors. Vertes (1981) has shown that MR stimulation not only desynchronizes hippocampal activity, but results in a significant decrease in the amplitude of the hippocampal EEG. This effect contrasts with the strong synchronizing response elicited with stimulation approximately 1 mm dorsal to the median raphe in the medial longitudinal fasiculus (MLF) and with the lack of an effect obtained with dorsal raphe stimulation and with stimulation just ventral to MR. Correspondingly, MR lesions produce a continuously synchronous pattern of hippocampal EEG activity (i.e., a continuous theta rhythm) independent of behavior (Maru et al., 1979). This effect has been shown to persist for at least 20-30 days post-lesion, which was temporarily interrupted by intraperitoneal injections of the 5-HT precursor L-5-hydroxytryptophan (L-5-HTP) (Yamamoto et al., 1979). It should be noted that the theta rhythm occurring under these conditions is in the lower theta frequency range (6-9 Hz). Blockade of neuronal activity in the median raphe using a variety of pharmacological means, such as microinjections of procaine hydrochloride, serotonin agonists (Vertes et al., 1994), excitatory amino acid antagonists, and GABA agonists (Kinney et al., 1994), also have been found to produce theta rhythm in the hippocampus. Overall, these data suggest that MR may play a role in hippocampal EEG desynchronization, whose effect appears to be serotonin-mediated. This is consistent with another line of evidence provided by McNaughton and his colleagues. McNaughton et al. studied the effects of pharmacological manipulation of 5-HT on theta elicited by electrical stimulation of the septal cells (McNaughton et al., 1977). As mentioned above, stimulation of this kind generates a phase-locked hippocampal rhythm with a frequency corresponding to the septal stimulation frequency. When the threshold current for driving theta in this way is plotted as a function of stimulation frequency in the free-moving rat, a
characteristic function is observed, with a minimum threshold at an inter-pulse interval (IPI) of 130 msec, corresponding to a frequency of 7.7 Hz (James et al., 1977). They have found that 5-HT receptor blockers, 5-HT synthesis blockers (McNaughton et al., 1977), or neurotoxic lesions of serotoninergic neurons in the MR (McNaughton et al., 1980) all shift the minimum threshold in this ‘theta-driving curve’ to an IPI of 145 msec (6.9 Hz), by selectively lowering the threshold at this frequency.

Thus, the above body of evidence indicates that the MR normally exerts a suppressive effect on theta rhythm of the hippocampus, which stands in contrast with the view originally proposed by Vandervolf and colleagues that the type 1 theta rhythm is generated by serotoninergic mechanisms (Peck and Vanderwolf, 1991). A careful examination of Vanderwolf et al.’s data reveals that severe 5-HT depletion itself did not produce any reduction in the amount of theta. Only when 5-HT depletion was made in conjunction with a virtually complete block of cholinergic transmission was the theta rhythm significantly altered. It is also possible, on the other hand, that the serotoninergic cells in the medial raphe may play a role in the suppression of type 2 theta. They may, on the other hand, be involved in the generation of type 1 theta. There is so far no plausible explanation for the discrepancy between the results of Vanderwolf’s groups and those of other studies.

4.1.7 Function significance of theta rhythm

There have been a number of studies that argue that the theta rhythm may be associated with learning. For example, Winson (1978) observed that, among rats with septal lesions, only those with elimination of the theta rhythm were impaired on a spatial memory task which required the rat to use distal spatial information to remember the spatial location of one of eight cups on an elevated circular platform. Givens and Olton
(1990) also reported a strong inverse correlation between the rat's performance on a spatial alternation task, and the magnitude of reduction in the power of hippocampal theta rhythm following microinjections of either muscimol or scopolamine into the medial septum. These correlations, however, do not prove causation. It is always possible that it was changes in some other aspects of hippocampal function, caused by septal lesions, that led to the behavioral learning deficits. A major problem in answering the question of what is the function of the theta rhythm is that theta cannot be selectively removed.

A different line of evidence relates the theta rhythm to long-term potentiation (LTP), which is regarded as a good candidate for the cellular mechanism underlying certain forms of learning and memory. In the early experiments, LTP was mostly induced by long-lasting trains of high frequency stimuli (usually higher than 100 Hz, and longer than 0.5 sec). Rose and Dunwiddie (1986) were the first to demonstrate that it is possible to induce LTP of the Schaffer collateral inputs to CA1 using two brief bursts of stimuli separated by a time interval of around 200 msec. Because this time interval is approximately equal to the duration of a theta cycle, this phenomenon was called "theta burst potentiation" or "primed burst potentiation", and has been confirmed by later studies, both in CA1 (Larson and Lynch, 1986) and in the perforant path projection to the dentate gyrus (Greenstein et al., 1988). It appears that the first burst leads to a temporary reduction of feedforward inhibition mediated by GABA-B receptors. This results in an enhancement of NMDA currents induced by the second burst of stimulation, and thereby effective induction of LTP (Mott and Lewis, 1991). Thus, it has been suggested that the membrane voltage changes during the theta rhythm might be able to facilitate the induction of LTP in the hippocampus in natural behavioral conditions. Conceivably, septal GABAergic inputs to the GABAergic interneurons in the hippocampus could lead to rhythmic disinhibition of groups of pyramidal cells, hence, providing conditions
conducive to synaptic modification through LTP-like mechanisms. This hypothesis is supported by Pavlides et al. (1988), who found that LTP of the perforant-path to dentate gyrus projection could more easily be induced if stimuli were delivered at the positive phase of locally recorded theta than if they were delivered on the negative phase. Consistent with this, a recent study illustrates that LTP could be induced with low frequency stimulation (0.1 Hz) during theta in hippocampal slices, induced by bath administration of carbachol, if the stimuli were timed to coincide with the peaks of the theta waves, but not if they coincided with the valleys (Huerta and Lisman, 1993). A later study using the same preparation found that a single burst of stimulation (4 pulses, 100 Hz) could induce LTP when the burst was given at the peak of the theta rhythm, but long-term depression (LTD) was induced if the burst was delivered at the trough of theta (Huerta and Lisman, 1995).

Finally, the recent finding of theta phase precession of place specific firing suggests a functional role of theta rhythm in the “timing” of place information, which may play an important role in coding of space (O'Keefe and Recce, 1993). This will be discussed in more detail in the third section of this chapter.

4.2 Place specific firing of hippocampal single units in freely moving rats

Under some behavioral conditions, the majority of single cells recorded from the hippocampal CA1 region in freely behaving rats discharge bursts of two or more spikes with interspike intervals of 4-6 msec and declining spike height. These have been called "complex spike cells" (Ranck, 1973). Because these cells are densely encountered when the electrode passes through hippocampal CA1 cell body layer, and because these cells
can often be activated antidromically by stimulation of the fimbria, it has been accepted universally that complex cells are pyramidal cells. On the other hand, a much smaller proportion of single units encountered in the hippocampus exhibit mostly single spikes, fire rapidly whenever theta rhythm is present in the hippocampal EEG, and are usually very obviously modulated in synchrony with the theta rhythm. These cells have been called "theta cells". Because they are encountered more rarely and have a different anatomical distribution, and because this kind of unit cannot be antidromically activated by stimulation of fimbria, these units are proposed to correspond to GABAergic interneurons of the hippocampus. The activity of hippocampal cells is clearly different between different behavioral states. During slow-wave sleep, or when a rat is quiet or eating, the hippocampal EEG exhibits large irregular activity (LIA). At this time, the probability of hippocampal cell firing is uniformly high. During movement, when theta rhythm occurs, approximately 50% of pyramidal cells in CA1 are active in a certain environment, and the rest become virtually silent (see Chapter 8). Thus, the firing rate variance increases in the population. The following review will focus on complex spike cells and the place specific firing during spatial behavior of rats. Among the three subregions of the hippocampus, single units have been recorded from CA1 and CA3 regions in most studies that have been published. Thus, the data reviewed below are mostly based on the data from these regions, although the activity of single units in the dentate gyrus is also mentioned when appropriate. Although there are also many data reported in monkey hippocampus, most of these studies were conducted when the monkey was restrained. Thus, only the rat data are reviewed here.

Single unit recording techniques have been frequently confronted with the question of whether one is recording from a single cell. This problem is especially prominent when recording from hippocampal principle cell layers where cell bodies are densely packed.
The classical solution to this problem is to use a spike height window discriminator, also usually with an associated waveform duration window discriminator. This method, however, has several problems. Because complex spike cells produce bursts with a monotonically decreasing amplitude, setting of a spike height threshold always compromises losing the spikes of the cell in question, and including spikes from other cells or noise signals. In addition, this method allows only one cell to be recorded from an electrode at one time, and biases the sampling to cells with the largest extracellular spikes, many of which may reside at the borders or even just outside of the cell body layer. McNaughton et al. (1983) proposed a multielectrode technique, which can overcome these problems. The multielectrode that was developed was called "stereotrode", consisting of two wires twisted together, with the two tips lying at the same level. Two channels, therefore, would most often acquire different voltage amplitudes from a single neuron depending on their distance from the cell. When the peak to peak amplitude recorded on one channel is plotted against the peak to peak amplitude on the second channel, distinct clusters corresponding to each cell would be observed on the plot, because different cells have different amplitude profiles on two channels. This technique was extended by using 4 twisted wires, called the "tetrode", for which unit isolation is conducted within a four dimensional parameter space (McNaughton et al., 1983b). This greatly enhances the unit isolation and the yield of simultaneously recorded single units (O'Keefe and Recce, 1993). Recently, the ensemble recording technique has been developed, in which 12 tetrodes are used to record single units simultaneously, and allows isolation of up to at least 150 cells (Wilson and McNaughton, 1993). The literature reviewed below involves all these recording methods, from single electrode with window detectors, to stereotrode and tetrode recordings, and finally ensemble recording techniques. Presumably these techniques should give rise to data that are qualitatively
Figure 4.3 An example of multiple single unit recording with a tetrode for hippocampal CA1 region of a freely behaving rat. Each point in the scatter-plot represents a signal that exceeded the experimenter-defined threshold. The x and y axes represent peak amplitudes of spike signals recorded by channel 1 and channel 3, respectively, of the four channels for a tetrode. As shown, individual cells tend to form clusters, which therefore can be separated from each other.
similar, when a reasonably large sample of neurons are recorded. Therefore, the recording technique will not be specially emphasized, except when a related issue is encountered.

4.2.1 Spatial correlates of CA1 pyramidal cells

O'Keefe and Dostrovsky (1971) were the first to report the characteristic firing properties of hippocampal cells in freely moving rats. In this study they reported that 8 out of 76 units recorded from CA1, CA4 or dentate gyrus fired "solely or maximally when the rat was situated in a particular part of the testing platform facing in a particular direction". In 1976, O'Keefe described the spatial firing properties of hippocampal cells in much more detail, which has now been confirmed by many other laboratories. O'Keefe called the place where the cell increased its firing rate the "place field" and those cells which have place fields "place cells". Furthermore, it was recognized that all place cells were complex spike cells. Several studies supported the observation that the firing of the complex spike cells is a function of place, and is not caused by the animal's motivation nor other psychological states nor its particular behaviors (e.g., left turn or right turn) (Olton et al., 1978a). In a review paper, O'Keefe (1979) gave several definitions of place cells with increasing rigor. In his first definition, place cells were referred to all cells "whose firing rate or pattern consistently discriminated between different parts of an environment". This definition appeared to be too broad, and many purely "sensory" cells could be included in this category. His second definition was "a place cell is a cell whose firing rate or pattern varies as a function of the animal's location in an environment but cannot be shown to be dependent on a single specific sensory input." His third definition was that "a place cell is a cell which constructs the notion of a place in an environment by connecting together several multisensory inputs each of which can be perceived when the animal is in
particular part of environment". This last definition consisted of several assumptions which were not supported by later experimental studies. Thus, the second definition of a place cell is probably closer to the current consensus, although single landmarks can have a powerful effect on individual cells (Gothard et al., 1996b).

The firing rate of a CA1 pyramidal cell is highest at the center of its place field, usually in the range of 2-20 Hz, and becomes minimal at the edge of the place field. It appears that when the sampling of cell firing in a given environment is large enough, place fields scatter everywhere in the environment. It remains unclear, however, whether these place fields are distributed evenly, or tend to cluster in some spots in the environment, although Leonard (Leonard, 1990) observed that some fields tend to be more concentrated near the boundaries of the apparatus.

It has been observed that place fields vary in size and shape to some extent in correspondence to the geometry of the environment (Muller et al., 1987). A cell can have two or more place fields in the same environment, which do not appear to have any regularity in their relationship for a given cell (Muller et al., 1987). Although it can be also argued that the two fields may be associated with different cells which were indistinguishable during extracellular recordings, this, however, cannot account for all the cases of multiple fields. In addition, even with techniques with the best resolution of single cells (e.g., stereotrode or tetrode methods), there is a substantial proportion of cells that have two or more place fields (see also chapter 8). Muller et al. (1987) examined a cell with two place fields by advancing the electrode in small steps (15μm). If the two place fields were contributed by two cells, the spike waveform should become different at certain levels. They found that a single spike profile was recorded at all levels, and that
two place fields were not affected. This result is consistent with the view that a single place cell can have two or more place fields.

Supported by the finding of place cells in the hippocampus, O'Keefe and Nadel (1978) proposed the "cognitive map" theory, which states that the hippocampus acts as a cognitive map, which is used by animals to know where they are when navigating in an environment. It was of interest to determine whether the anatomical layout of CA1 pyramidal cells was really like a map, on which adjoining cells code adjacent spaces in the environment. This hypothesis has not been supported by experimental data. In fact, the consistent finding has been no obvious relationship between the place fields of closely recorded place cells, i.e., these place fields can scatter around the environment even when the cells are close together in the CA1 pyramidal layer (O'Keefe, 1976). Thus, the spatial map in the hippocampus appears to be nontopographic. In addition, when a cell is tested in multiple environments, it is common for the cell to fire in more than one environment, with no recognizable relationship of firing patterns between environments (O'Keefe and Conway, 1978).

It should be noted that place cell firing is not solely dependent on space, but also on the movements made in space. McNaughton et al. (1983a) reported that the firing rate of pyramidal cells increases with increased running velocity of rats. Foster et al. (1989) found that when a rat is trained to be in an immobile state while wrapped in a towel, the place-dependent firing of CA1 pyramidal cells was abolished, even when the rat was passively placed at the location where the pyramidal cell previously exhibited high firing rates when the rat was allowed to move freely. This result indicates that the place specific firing depends on motor inputs.
4.2.2 Sensory control of place fields

The first systematic study of the sensory control of place fields was performed by O'Keefe and Conway (1978). The apparatus used was a T-maze in a curtained area with four distal cues: a light, a card, a fan, and a buzzer. It was found that place fields in the controlled cue area usually persisted at the same absolute locations if the T-maze was rotated, and most place fields remained intact if any two of the four distal cues were removed. This suggested that place fields are more strongly influenced by distal visual cues than by local cues, and that the global configuration of environmental cues under these conditions were more important than any individual cue.

Muller et al. (1987) examined the effects of manipulation of various cues on place-related firing of hippocampal pyramidal cells in an open foraging task. Rats were trained to seek scattered food pellets inside a 76 cm diameter plywood cylinder, with 51 cm high walls painted gray except for a white cue card occupying 100° of arc and running the full height of the wall (MKR cylinder). They found that rotating the cue card caused equal rotations of place fields. Changing the dimensions of the cue card, however, had no noticeable effect on the size, shape or radial positions of place fields, although small rotations were occasionally observed. Complete removal of the cue card left the shapes and radial locations of the fields unchanged but caused them to rotate to unpredictable angular positions. Doubling the size of the apparatus in both diameter and height caused some cells to scale by about one third. Most of the others changed in unpredictable ways, and a small proportion of the cells became silent. Transforming the cylinder into a rectangle led to a complete rearrangement of place fields. In this study, they also observed that whenever a barrier is placed at the center of a field, firing within the field was abolished, even when the barrier occupied only a small fraction of the place field area.
The barrier itself, on the other hand, had no effect if it was located outside the place field. In another experiment performed in the MKR cylinder (Sharp et al., 1990), rats were tested with a second identical cue card placed on the wall diametrically opposite the first cue, such that the cylinder became visually symmetric. It was found that the majority of CA1 and CA3 pyramidal cells (13 out of 18) maintained asymmetrical fields in the symmetrical environment, whose relative relation to one of the two cue cards varied between sessions, depending on the point at which the rat was placed into the cylinder. Whenever the entry position was rotated 180°, the field position also rotated by 180°. The other five cells showed complex changes over time which suggested “remapping”. It should be noted that most of the cells recorded in this study were from different sessions. It is not clear whether all the cells that were active in one session underwent “remapping” conjointly.

In a very recent study (O'Keefe and Burgess, 1996), 27 place cells were recorded from hippocampal CA1 or CA3 of 4 rats navigating in four rectangular boxes that differed solely in the length of one or both sides. It was observed that a group of cells fired at a fixed distance from one or two walls, or fired at a fixed distance from one wall, and at a fixed ratio of distance between the other two walls. In other words, these cells appeared to code absolute or relative “distance”. For another category of cells, the same place fields were either stretched, doubled or disappeared in the linearly stretched rectangular environment. Interestingly, for the place fields that were doubled, each place field became directional, with the preferred directions of each half oriented towards each other. When the environment is stretched in both directions, a place field would become tripled or vanish. The authors suggested that it was the geometric shape of the environment that determined these firing properties of the place fields. These results, however, can also be interpreted as a consequence of changes in reference frames, hence, different place field
maps may be used when the rat encountered different reference frames. This interpretation will become more obvious when we come to sections 4.2.6 and 4.2.7.

One of the most direct ways to study the effect of visual cues on place-specific firing is to turn off the lights and to test the effects on cell firing when all visual cues are excluded. All studies in which this experimental manipulation has been performed are consistent in that most, but not all, place fields are maintained if the lights in the recording area are turned out when the animal is in a familiar environment (O'Keefe, 1976). The reliability of place fields, however, can be reduced in the darkness (Markus et al., 1994). In addition, Hill (1979) tried to eliminate entire modalities by surgical means. Animals were either blinded, deafened, had their vibrissae removed, or their olfactory receptors destroyed. Place cells were still found in all these animals and no qualitative difference was observed compared to those of animals with all their senses intact.

In summary, these data suggest that although the visual cues can have control over place-specific firing, vision cannot account for all factors controlling place cell firing.

4.2.3 Dependence on head direction system

In a study of the spatial properties of electrophysiologically identified granule cells of the dentate gyrus, Jung and McNaughton (1993) reported that in several cases well-defined place fields recorded on the radial-8-arm maze that were stable over several trials (sometimes over days) occasionally rotated relative to the prominent visual landmarks. All cells recorded together exhibited the same relative rotation, and the relative relationship between the fields were not altered. Field orientation usually back to the original orientation on subsequent trials. These results also suggested that there are other factors beyond the global visual cues in the room that also control the place fields, and raised the
possibility that place field rotation may be due to inconsistent associations between the internal directional reference system and the visual landmarks. The internal directional reference system is presumably mediated by head-direction cells, which have been found in the presubiculum (Ranck, 1984), anterior (lateral dorsal) nuclei of the thalamus (Mizumori and Williams, 1993), the retrosplenial cortex (Chen et al., 1994) and the striatum (Wiener, 1993). A head-direction cell is defined as a cell which fires at a high rate when the rat's head is oriented in a specific absolute direction in the environment, regardless of either the spatial location or the position of the head with respect to the body. The relationship between head-direction cells and place fields has been directly studied by Knierim et al. (1995). Cells were recorded both in the hippocampus and in the thalamus. During the training period, one group of rats was completely disoriented before they were transferred to the recording room, and the control group was transported to the recording room with no intentional disorientation. Both groups of rats received the same amount of experience in the MRK cylinder with the cue card maintained in a fixed orientation. During subsequent recording sessions, the cue card was reoriented to one of four directions by rotating the cylinder, and all the rats were disoriented prior to entry into the cylinder. It was found that the rats that were originally disoriented during training exhibited frequent conjoint rotations of place and head-direction cell firing preferences relative to the cue card, while cells of rats that had been trained without disorientation exhibited strong binding of place and head-direction cell firing to the cue card during the first several days of recording under disorientation conditions. With repeated disorientation, however, the behavior of these cells tended to destabilize. The general conclusion from these data is that inertial orientation can control the orientation of place fields. When the relationship between the external visual cues and rat's internal sense of orientation is disrupted, place fields sometimes follow the internal orientation sense. This
explains a number of results discussed above in which fields changed independently of visual stimuli (Muller et al., 1987). It is generally believed that the head direction system receives its main inputs from the vestibular system (McNaughton et al., 1994). Recent data, however, suggest that the control of head direction system is more complicated, and the vestibular signal input may be just one of the factors, and cannot account for all the mechanisms (Sharp et al., 1995).

4.2.4 Directionality

Cell firing in a place field depends not only on place but also on the direction the rat is facing. This was first noticed by O'Keefe and his coworkers in their early studies that some place cells only fired when the animal traversed the place fields toward certain directions, but not the others (O'Keefe and Dostrovsky, 1971). This phenomenon was confirmed by McNaughton et al. (1983a), who recorded hippocampal units in rats running on an eight-arm radial maze. They found that many CA1 and CA3 complex spike cells with place fields on a specific arm fired only when the rat was moving in one direction, i.e., either inward or outward. Directionality is more difficult to detect, however, when rats are recorded in an open field such as the MKR cylinder, and most cells appear to fire in many directions of motion through the place field (Muller et al., 1987). Markus et al. (1996) conducted an experiment to identify the possible factors that contribute to directional cell firing. The directionality of place fields was compared when rats performed different tasks on different apparati, including searching for randomly scattered food in the MKR cylinder, searching for scattered food on an large, open, circular platform, searching for food at 4 fixed locations on the platform, and a task on the radial-8-arm maze. Except for the MKR cylinder, which had high walled surroundings, the other apparati were in the same room with the same arrangement of distal cues. It was
found that the directionality was the least in the MKR cylinder, and became more evident when the rat moved on the open platform, but only when the rat searched for food at fixed locations in a stereotypic and directed manner. Place fields were even more directional on the radial arm maze than on the circular platform, regardless of the width of each arm. These results indicate that the directionality appears when animals plan or follow a stereotyped route between points of special significance. It appears that, under these conditions, the forward and return journeys are encoded by different groups of cells.

4.2.5 Dynamics of place fields

There is convincing evidence to suggest that experience may not be required for the formation of place fields. Hill (1978) first noticed that place fields appear at the first time when an animal is placed in or enters a novel environment (Wilson and McNaughton, 1993). During the rats' first experience on a T maze, 10 out of 12 cells fired on the very first passage of the rat through the place field, and he did not detect changes in the characteristics of the fields when the rats had appreciably more experience. The place fields in this early study were only examined qualitatively. Very recently, Mehta et al. (1996) found that the field size expands rapidly during the first few trials when the animal traverses the place field in either a familiar environment or a novel environment. This field expansion could not be explained by any behavioral or velocity changes, but is consistent with the hypothesis proposed by a number of connectionist models, which state that LTP-like mechanisms cause the asymmetrical strengthening of synapses in hippocampal neural networks during initial experience of animals in an environment. This strengthening should lead to an enlargement of place fields. Wilson and McNaughton (1993) also examined effects of novel environments on hippocampal spatial representation using ensemble recording techniques. Rats were trained to forage for randomly scattered
chocolate pellets in one half of a rectangular box, which was separated from the other half by an opaque barrier. After the rat foraged for the pellets in the familiar half of the box for about 10 min, the barrier was removed, and the rats were allowed to enter the other novel half of the box for another 10 min. Finally the barrier was returned, the rat’s movement was restricted to the familiar half again. The development of the hippocampal place representation was examined in the novel situation by measuring the accuracy of position reconstruction on the basis of the activity of a large population of simultaneously recorded CA1 pyramidal cells. It was found that the accuracy of population reconstruction gradually improved over the course of the first 5-10 minutes in the novel environment. It is possible that the field expansion observed by Metha et al. (1996) may correspond to the novelty effect on the population reconstruction reported by Wilson and McNaughton (1993), with larger field sizes resulting in more accurate population coding.

Bostock et al. (1991) conducted a study examining the effect of substituting a new stimulus for a familiar stimulus in a familiar environment. The apparatus was again the MKR cylinder with a white cue card affixed to the wall. Once a place cell was recorded in the presence of the white card, the white card was replaced by a black card of the same size and shape. It was observed that when the black card was substituted for the white card, place cells showed time-variant changes in their spatial firing patterns: from a similar pattern as that with the presence of the white card, to a complete “remapping”. It was interesting that once the differentiation of firing patterns had occurred in a given rat, all place cells subsequently recorded from that rat had different firing patterns in the presence of the white and black cards. This suggested that a whole new map for the black card was formed in a time-dependent manner. The author argued that this remapping could not be explained by purely sensory changes, nor behavioral-related changes. It was concluded, therefore, that this alteration in the pattern of place fields following exposure to a novel
stimulus is an experience-dependent process. The real reason for this experience-dependent change was not clear, but could reflect a "learning" process, which took time to develop. In fact, this result may be consistent with the novelty effect reported more recently by Wilson and McNaughton (1993).

4.2.6 Switches between spatial cognitive maps

Markus et al. (1996), in their study on place field directionality, observed that a substantial proportion of place cells exhibit a change in place field locations with the shift in the task demand on the same apparatus. For example, on the open circular platform, when the task changed from the random search for food to the directed search for 4 fixed locations, the same cell could have different field locations in two tasks. In addition, they provided evidence indicating that the change in place field location occurs abruptly when the animal behavior changed. This indicates that the place fields may be task- and/or behavior-dependent, and there may exist different cognitive maps in the hippocampus for different tasks, which can be switched instantaneously.

In a very clever experiment done by Gothard et al. (1996b), the behavioral correlates of rat hippocampal CA1 cells were examined in a landmark-learning task designed after Collett et al. (1986), in which two cylindrical landmarks predicted the location of food. The landmarks were maintained at a constant distance from each other, but were moved from trial to trial in a random manner within the same global environment surrounded by static background cues. On each trial, the rats were released from a box, allowed to find food reward between the landmarks, then returned to the same box which was placed at a different location. Both initial and end locations of the box varied between trials. Three categories of cells were identified: cells firing in relation to the static background in the environment (i.e., the classical place fields); cells that fired in the
vicinity of the goal or landmarks; and cells that fired either when the rat was in the box or as it was leaving or entering the box. Because the locations of the box and the goal were changed in an unpredictable way from trial to trial, the latter two classes of cells apparently encoded relative location with respect to different reference frames which were associated with the task relevant, mobile objects. It was also noted that box-related cells fired at different distances in relation to the box. This binding of place-specific firing to different reference frames in the same global environment was substantiated by a subsequent study (Gothard et al., 1996a), in which the rat shuttled on a track between a fixed reward-site at one end and a moveable reward-site, mounted in a sliding box, at the opposite end. While the rat ran toward the fixed-site, the box was moved to five evenly distributed locations on the track in a random order. It was found that on the initial part of all journeys, cells fired at fixed distances from the origin, while on the final part of the journey, cells fired at fixed distances from the destination. Thus, it appeared that the spatial representation shifted between the reference frames with respect to the sliding box and the fixed reward location, respectively.

4.2.7 Cognitive mapping and path integration: A unified theory

The local view theory, which proposed that hippocampal spatial representations are formed by the encoding of the immediately sensory information (McNaughton et al., 1989b) is clearly an untenable in view of the fact that place fields are sustained in the dark, and can be formed in the dark in the absence of visual input (Quirk et al., 1990). These results, on the other hand, support the view that there is a stable spatial representation, or a spatial “model”, in the hippocampus which is resistant to sensory changes. This is supported by the observations that the place cells fire consistently with each other, while
the orientation of the entire map can be changed following reorientation (Jung and McNaughton, 1993). As mentioned above, the orientation of the hippocampal "spatial map" is controlled by the head direction system, which presumably receives input from vestibular system and is subject to cumulative errors when the animal is disoriented. The study by Knierim et al. (1995) indicates that stable visual cues in the environment may serve as landmarks to anchor or correct the internal sense of orientation. It is worth mentioning that the cognitive map theory has not been able to explain some of the recent findings, such as the task-dependence of the place fields (Markus et al., 1996), the directionality (McNaughton et al., 1983a), or the shifts between different reference frames between local and global cues (Gothard et al., 1996b).

Recently, Samsonovich and McNaughton (1996a, 1996b) proposed an internal dynamic cognitive model, which apparently can explain most of the phenomena that have been described for place cells. By assuming that the internal map is based on an abstract preconfigured model of space implemented in CA3, which is considered as an autoassociative attractor neural network, the environment in this model is represented in CA3 by a quasi-continuous, two-dimensional set of point-like attractors, which they call an "attractor map" (AM). Active points on the AM represent the animal's currently "perceived location", and all points of the AM, which represent a population of place cells coding for a given environment, are assumed to be bound to the environment primarily by binding to different landmarks, and their overall orientation is controlled by the head direction system (Fig. 4.4). In this view, a cognitive map for an environment is a dynamic attractor state of cell assemblies. In addition, it is also proposed in this model that multiple representations of different maps can be maintained simultaneously within the same synaptic matrix, and used to represent different environments, or the same environment in different contexts. This model also incorporates another important concept
besides dynamic attractor properties, which is path integration. This refers to the ability of animals to keep track of their position by integrating ideothetic (self-motion) information (Barlow, 1964). The idea that the hippocampus may be involved in path integration is actually not new, and was first considered by O'Keefe (1976) and elaborated more by O'Keefe and Nadel (1978). In Samsonovich and McNaughton's model (1996a, 1996b), the hippocampus itself works as an inertial guidance system, or path integrator, which integrates the velocity vector during motion in order to obtain a representation of the expected location (with respect to the starting point). The strongest data supporting the existence of a path integration mechanism are the results reported by Gothard et al. (1996b), which show that when the rat shuttled on a linear track between a fixed reward position and a box whose position was changed from trial or trial in an unpredictable manner, the place cells fired at a fixed distance from the point which it departed, on both inbound and outbound journeys even when the only cues defining this point were behind the rat. This indicates that the rat had a "sense" of distance from the origin, which is most likely obtained by path integration. The hypothesis of the role of the hippocampus as a path integrator is supported by the strong association between movements and the theta rhythm as discussed above, and by the fact that the hippocampus becomes silent when the rat is physically restrained.

This dynamic model built by Samsonovich and McNaughton has reproduced many experimental facts about place fields that are discussed above, including doubling of place fields, vanishing and reshaping in distorted environments, directionality in a two-goal shuttling task, rapid formation in a novel environment, and slow rotation after disorientation. Thus, this model appears to be superior to the previously proposed "local view theory" and the static "cognitive map theory" and is consistent with most available
Figure 4.4 Schematic diagram of Samsonovich and McNaughton’s model. Modified after Figure 2 of Samsonovich and McNaughton (1996a).

experimental facts about place cells in the hippocampus.

4.3 Theta phase precession of place specific firing

Early studies on hippocampal single units noted that the CA1 pyramidal cells are also theta modulated (Ranck, 1973). Buzsáki et al. (1983) made an extensive and careful study of the relation of unit activity to theta for pyramidal cells in CA1. Using spike-triggered averaging, theta modulation was examined in relation both to a reference signal recorded in stratum oriens of CA1 and to EEG recorded from the electrode on which the unit was isolated. Most of the recordings were made from animals running for water reward on an activity wheel, but some recordings were also made under urethane anesthesia for comparison. The main finding was that CA1 pyramidal cells fired
preferentially on the negative phase of the EEG waves recorded from CA1 stratum radiatum.

O'Keefe and Recce (1993) found a systematic relationship between phase of the theta cycle and the place-specific firing recorded from rats running back and forth on a linear track. Their observations demonstrated that spikes advanced to earlier phases of the theta cycle as the rat traversed the place field (see Fig. 4.5). These findings were replicated and extended by Skaggs and McNaughton (1996b) in a large number of simultaneously recorded CA1 pyramidal cells using parallel recording techniques. They found that this phase precession effect is also present in granule cells in the dentate gyrus, although the total phase precession appears to be less than that of CA1 pyramidal cells. The phase precession effect is more prominent when the rats are running on a linear track. It is more difficult to assess when the animal is running in random directions in an open field. In addition, preliminary results (Skaggs, 1996a) also indicate that phase precession appears to occur during REM sleep. These findings indicate that phase precession is a very robust effect, distributed across the entire hippocampal population, whenever theta activity is present in the EEG.

The phase precession effect is an extremely interesting phenomenon, and has attracted a number of hypotheses as to its functional roles (O'Keefe and Recce, 1993), and underlying neural mechanisms (O'Keefe and Recce, 1993). O'Keefe has suggested that the phase of place-specific firing codes spatial information in a monotonic fashion. It tells, therefore, whether the rat enters or exits the place field, which cannot be accomplished by rate coding. The question, however, still remains as to how the downstream neurons use this phase information. Skaggs and McNaughton (1996b) have proposed that with the
**Figure 4.5 A-D** Illustration of the phase precession effect in the spike train of a single CA1 pyramidal cell recorded from a young rat running for food reward on a rectangular track.  

A. Spatial pattern of spike activity and firing phase. Each spike is represented by a colored dot at the corresponding location (the small tab on each dot represents the head orientation). The colors correspond to the theta phases indicated in B. The irregular, gray lines represent the rat’s trajectory. Only the corner of the rectangular track containing the place field is shown. Note that, as the rat traversed the place field, the phases of the spikes advanced systematically.  

B. Theta phase of spike discharge versus position on the track. To construct this plot, the rat’s position at each moment was projected onto a one dimensional axis corresponding to the track center. Each point represents a single spike. Phase zero is the preceding peak of the theta cycle during which the spike occurred.  

C. Plot of the theta rhythm and spike train for a single traversal of the place field. The theta rhythm record was digitally filtered in the range of 6-10 Hz. Each short vertical line below the waveform represents a single spike, and the time at which it occurred relative to the theta rhythm is labeled by a dot on the waveform. Below the spike train is a time axis with ticks indicating the time at which the peaks of theta rhythm occurred. The period between two neighboring peaks is one theta cycle. As the rat traversed the place field, the spikes occurred earlier and earlier in the theta cycle.  

D. Histogram of activity versus theta cycle number as the rat passed through the place field over multiple trials. A point was selected on the track, near the center of the cell’s place field and, on each pass through the field, the theta peak which occurred nearest to this point was selected as cycle zero. Near the beginning of the place field (cycle -3), the spikes are distributed towards the end of the cycle. Thereafter the activity distributions shift progressively earlier.
presence of theta phase precession, the information of a sequence of place fields can be compressed within individual theta cycles, and that each portion of the sequence is repeated several times as the rat moves along. This compression and repetition of neural activity sequences may, therefore, make it possible to use long-term potentiation to encode the temporal structure of the rat's experience.

The physiological mechanisms responsible for phase precession remain unclear. O'Keefe and Recce (1993) have suggested that the phase shift is produced by an interaction between extrinsic theta-frequency modulation of pyramidal cells, and an intrinsic tendency of these cells to oscillate at a slightly higher frequency. This model, however, cannot account for the apparently stronger correlation of firing phase with space than with time. Tsodyks et al. (1996) have proposed a model, based on the intrinsic connections of CA3, which is able to account for most of the observed properties of phase precession. This model proposes that cells representing sequential locations along the rat's trajectory are coupled in an asymmetric manner resulting from experience-dependent synaptic modifications. The asymmetry leads the hippocampal spatial representation to propagate spontaneously to the next cells in the sequence, at a rate which is faster than the actual speed of the rat. At the beginning of the theta cycle, it is assumed that a representation of the current location is set up in CA3. During the theta cycle, the asymmetric connections cause the representation to shift progressively to reflect a position ahead of the current location. Build-up of inhibition during the cycle then terminates the activity. At the beginning of the next cycle, the focus of activity reverts back to represent the new current location of the rat (see Fig. 4.6). Note that, in this model, the apparent size of the place field depends on the rate of the asymmetric synaptic propagation during
Figure 4.6 The simulation of population spike activity in a network model during a
simulated run of a rat through a linear apparatus Tsodyks et al. (1996). The hippocampus
was modeled as an interconnected network of 800 excitatory and 200 inhibitory integrate-
and-fire neurons. Each dot represents a spike of an excitatory neuron, which is labeled on
the vertical axis. The time at which a spike discharged is indicated on the horizontal axis.
Vertical lines mark the phase of the theta cycle at which the activity of the network is
minimal. The shallow overall slope is determined by the velocity of the rat; the steep slope
within each theta cycle is determined by the internal dynamics of the network. This
indicates that within one theta cycle, the population activity propagates at a faster rate
compared to the running speed of the rat. At the end of each theta cycle, the population
activity is extinguished because an increase in the inhibition level in the network. At the
beginning of next theta cycle, the population activity reverts to represent the actual spatial
location of the rat as a consequence of input of visual or other sensory cues. The spikes of
one of the neurons are surrounded by circles for purposes of illustration. Note that the
phase of these spikes in relation to the corresponding theta cycle becomes earlier and
earlier, resulting in a phase precession phenomenon. Adapted from Tsodyks et al. (1996).
each theta cycle, which, in turn, is a function of the relative asymmetry of the intrinsic connections. This intrinsic activity propagation also causes a gradual phase shift of the spike within the place field, resulting in the phase precession phenomenon. Thus, this model predicts that the speed of the phase precession and place field size are inversely coupled. A larger place field should have a smaller phase precession. In Tsodyks' model, at the beginning of each theta cycle, the focus of the propagation activity reverts to the current location of the rat presumably by visual cues. This hypothesis, however, is not supported by the finding that robust phase precession is still present during darkness (Weaver et al., 1996). Thus, it is possible that the perceived self-motion information (path integration) may play this role by updating the rat's sense of current position, as proposed by Samsonovich and McNaughton (1996a). The effect of aging on phase precession is examined in Chapter 8, which may also shed light on neural mechanisms underlying this important characteristic of place fields.
CHAPTER 5 THE AGING HIPPOCAMPUS

The amount of information available about the aging hippocampus has increased substantially over the past two decades. The idea that the normal aged brain undergoes massive deterioration has been completely disproven during this time. The anatomical structure and many functions are largely preserved in the old brain. In addition, the aged brain exhibits robust plasticity, and undergoes certain age-related changes which are compensatory in nature. One of the well-established facts about cognitive aging is that there is a large variance between old individuals (see review, Rapp and Amaral, 1992). While some old individuals' memory performance declines significantly compared to young adult individuals, some others can perform at the same level as young adults do. This large individual variance would potentially generate conflicting results between studies, if behavioral variables are not examined, and the proportion of behaviorally impaired old subjects is not known. Thus, it is necessary for studies on neurobiological markers of age-associated memory loss to examine learning behaviors on the same individuals. This functional variability intrinsic to the aged population actually provides an advantage for aging studies to delineate possible factors that are responsible for the age-related memory dysfunction. Another issue that has become increasingly clear over the past decade is that most age-related alterations are continuous quantitative changes rather than abrupt qualitative changes. This requires, therefore, unbiased quantitative techniques to be carefully conducted to study age-related structural and functional changes in the brain. In this chapter, gross neural changes in the human brain will be briefly reviewed first, before examining specific brain regions. Age-related neurobiological changes in the hippocampus will then be reviewed, followed by a discussion of recent progress in the
development of interventions for the treatment of age-associated hippocampal changes and age-related related learning and memory deficits.

5.1 The aging brain

5.1.1 Neuroanatomical changes

One of the best documented brain changes with age is an overall decrease in brain weight and volume (see review, Kemper, 1984). Because brain weight is correlated with body weight and the mean body weight of humans has increased during the past century, some of previous reports of cross-sectional studies may have shown a spurious decline in brain weight with advancing age. After correcting for cohort trends in brain and body size, Miller et al. (1980) found no change in the volume of cerebral hemispheres between ages of 20 and 50 years, followed by a 2% decrease per decade through age 98 for both sexes. This decrease was accompanied by an increasing ratio between gray and white matter, suggesting that the predominant loss was from the white matter. Consistent with these data, a recent magnetic resonance imaging (MRI) study indicated that there is no significant change with age in the total amount of gray matter, however, a significant decline in white matter (Albert, 1993). These findings are consistent with the hypothesis that there is little cell loss in the aged brain, while the connectivity between cells may be reduced at old age.

Morel and Wildi (1952) were the first to conduct a careful study on the volume of the lateral ventricles across age, using 423 brains of patients without mental illness. They noted a progressively increasing volume until about ages of 80-85 for both sexes. Knudsen (1958) measured the volume of the lateral ventricles of 85 males and 98 female brain aged 20 to 90 years and found a similar increase up to the seventh decade. These
observations were confirmed by later computerized tomography (CT) scans (Barron et al., 1976) and MRI studies (Murphy et al., 1992). Additionally, recent studies demonstrate an age-related increase in the subarachnoid space (Stafford et al., 1988). Interestingly, Stafford et al. (1988) found that the increase in fluid within the ventricles and subarachnoid space are highly correlated with deficits in naming and abstract abilities during psychometric testing.

The question of whether there is age-related neuronal loss in the human neocortex has been controversial. This appears to be caused by the differences in cell-counting methods used by different laboratories, or the difference in subject sampling. Most early studies reported significant cell loss in the aged human cortex. The degree of the loss varied between different brain regions (see review, Kemper, 1984). For example, in a serial section study, using nucleolar cell counts of all fields of the hippocampus and the subiculum, Ball (1977) found a 27% loss of cells from the fifth to the ninth decades. In the subiculum, Shefer (1977) noted a 29% neuronal loss when 19-28-year olds were compared to older individuals. Sam (1979) also reported significant neuronal loss in all hippocampal fields within the narrow range of 19% to 25%. Terry and Hansen (1988) noted a decrease with age in the number of large neurons in selected cerebral areas; however, they noted that small neurons in the same brain regions actually increased in number with age. This finding raises the possibility that the total population of neurons may remain stable later in life, but that large neurons may shrink into smaller neuron classes. They also suggested that earlier researchers may have inadvertently included brains from persons with Alzheimer's disease or other dementias known to cause severe cell loss.
It should be noted that most of the previous studies were conducted using biased cell counting techniques. For example, many studies counted the number of neurons by observing parts or profiles of neurons in the brain sections, which, however, depend on the size, shape, and orientation of the object. As shown in Fig. 5.1, if there are two spherical cells embedded in the same reference volume, one of which has twice the diameter of the other, the large cell will be observed 2 times more often than the small one by a series of sections. Recently, an unbiased cell counting method has been developed, using a new stereological approach (West, 1993a). The main principle of this method is the "disector", which involves the use of two sections to count the objects that are present on the second section of a pair but not present on the first, as one proceeds systematically though a series of sections that comprises the region under consideration. The same result can be achieved by counting objects that are observable in the first section of the pair but not observable in the second (fig. 5.1). This counting method, therefore, does not depend on the shape, size or orientation of neurons, but samples objects with a probability proportional to the number of objects. In addition, unbiased sampling strategies to count total numbers of cells in a certain brain region is also emphasized in this method. It appears impractical to go through all the sections of the whole region. Two methods thus have been adopted to reduce the number of sections, that will still reach a reasonable estimate. One is called "the fractioner" method, which involves counting the number of objects in a certain fraction of the all the serial sections. The unbiased estimate of the total number of objects would be the number of objects observed times the reciprocal of the fraction used for analyzing the structure. This has been used by West to count neuronal numbers in the old hippocampus (West, 1993b). Another unbiased sampling strategy is a two-step process. First, the numerical density (i.e., number of objects per unit volume) is obtained using disector techniques for a known volume. Second, an unbiased estimate of
Figure 5.1 A. Schematic diagram showing the previous biased methods which depend on the size of the object. If an object has a diameter twice as large as the diameter of the other one, it will be observed twice as often as the smaller one. B. Schematic diagram showing the stereological disector method. On the left is an object frame containing four objects of different sizes, represented by different patterns. Two dimensional representations of a series of 7 sections are shown in the middle, and only those objects which are observed on the first section of a pair of sections are counted. A total of 4 objects thus would be counted. (modified after West, 1993a).
the entire volume of the region is obtained with point counting techniques. The product of
the two variables is, therefore, an estimate of the total number of objects. This method has
been frequently used in electron microscopic preparations, in which exhaustive sectioning
throughout the entire region is not possible (Geinisman et al., 1992).

Using this new unbiased cell counting technology, West (West, 1993b) examined
the numbers of neurons in the human hippocampal formation of 32 males with ages
ranging from 13 to 85 years old, and found that there is a selective neuronal loss with age
in the subiculum and hilus of the dentate gyrus, but no change in the number of dentate
granule cells or the CA subfields in the hippocampus. This pattern of results has also been
consistently observed in rat and monkey hippocampus (West et al., 1991; West et al.,
1993). Consistent with this study is a recent report using the rhesus monkey, in which no
apparent age-related loss of cortical neurons were found (Peters et al., 1994).
Furthermore, Morris et al. (1996) examined 10 normal, healthy elderly people, and found
no age-related difference in cell numbers between ages 60 and 90 in the entorhinal cortex.
In contrast, a group of age-matched patients with early dementia had lost half the cells in
the entorhinal cortex, and a third group with more advanced Alzheimer's disease was
missing up to 65% of the cells in this brain region. Thus, an emerging picture is that cell
number may actually be preserved in the healthy aging cortex. Previous results regarding
the loss of neurons may arise from the contamination of elderly subjects with those having
early dementia or early signs of Alzheimer's disease, and biased cell counting
methodology. The results obtained by the latter might reflect cell shrinkage rather than cell
loss in the aged brain.

Scheibel and colleagues (Scheibel et al., 1976) described a pattern of progressive
degenerative changes in dendritic process with age in a number of brain regions, including
hippocampus and adjacent cortex, characterized with swelling of the cell body and loss of apical branches on dendritic shafts. Buell and Coleman (1979), in a quantitative study of parahippocampal pyramidal cells, noted an increase in the extent of apical and basal dendrites in normal aging. This late life dendritic growth, however, was absent in senile dementia brains. The discrepancy between the two studies is mainly due to the fact that the former is a qualitative one and the latter is a quantitative one, conducted without knowledge of the age groups from which the tissue was being sampled. Because atrophic cells are present in tissue of all ages, the results from the former study may be caused by a bias in selection of cells.

Amyloid is a complex, glycoprotein-rich material that is rarely observed in tissues of healthy young adults. Infiltration of amyloid into the lining of cerebral blood vessels and free deposits of amyloid within brain tissue are observed with increasing frequency in advanced old age. In one autopsy series of more than 100 geriatric patients, the percentage of subjects with vascular amyloid increased from 8% in the seventh decade to 58% after the ninth decade (Tomonago, 1981). Neuritic plaques are distinctive intercellular structures visible on microscopic examination. A typical mature plaque consists of a central core of amyloid surrounded by glial cells, macrophages, degenerating axons, and occasional dendrites (Kemper, 1984). Neuritic plaques, also called senile plaques, are seen most frequently in neocortical regions of normal aged individuals (Giannakopoulos et al., 1994). They are not seen in the cerebellum or spinal cord and rarely in basal ganglia. Neuritic plaques increase with age from about the fifth decade onward, with two-thirds or more of brains in the ninth decade having neuritic plaques (Jordan, 1971), which are more severe in Alzheimer’s disease. A neurofibrillary tangle is an intraneuronal structure composed of twisted bands or filaments that often displace the
cell nucleus (Kemper, 1984). Neurofibrillary tangles occur as early as the fourth decade and can be detected in the brain of most people over 70 years of age. In normal aging, tangles are either rare or absent in frontal and occipital cortices (Giannakopoulos et al., 1994). The highest concentrations in normal old brains are in the hippocampus and entorhinal cortex and some other regions in the inferior temporal cortex (Vermersch et al., 1995). In Alzheimer’s disease, neurofibrillary tangles are more pronounced in the hippocampus and entorhinal cortex (Vickers et al., 1994), and are more sparse as in the neocortex.

Another well-documented age-related morphological change in the brain is the appearance of lipofuscin, which is a fatty pigment. Lipofuscin occurs typically in postmitotic aging cells of many organs of different species, including humans. It is most likely that lipofuscin is of lysosomal origin and a byproduct of peroxidation processes. It is interesting that age pigment accumulates more rapidly in houseflies that are short-lived and highly active than in those that are long-lived with low activity. This suggests that factors such as metabolic rate as well as chronological age are associated with lipofuscin accumulation. Several quantitative studies examining different brain regions on the rate of lipofuscin accumulation consistently report a linear increase in lipofuscin with increasing age (Mann and Yates, 1974). The effect of lipofuscin on cell function remains unknown. It appears that lipofuscin accumulation does not lead to cell loss, because nuclei with the largest amounts do not show age-related cell death.

5.1.2 Electroencephalographic (EEG) activity

Electroencephalographic (EEG) activity has been examined in young and old adults, typically by recording with multiple scalp electrodes to permit simultaneous
evaluation of the activity of several brain regions. A prevalent view has been that there is a
general slowing of EEG during aging, which is reflected by decreased alpha frequency
and amplitude accompanied by increased amounts of delta and theta slow activity (Busse
and Obrist, 1965). On the other hand, Niedermeyer and Lopes da Silva (Niedermeyer and
Lopes da Silva, 1982) was among the first to suggest that the EEG changes commonly
ascribed to aging might, in fact, result from age-related pathology rather than from normal
aging itself. In agreement with this hypothesis, Katz and Horowitz (1982) reported that
the mean alpha frequency of a group of older subjects who had been carefully screened for
health was similar to that seen in young subjects. Duffy et al. (1984) also found that for a
group of 63 carefully screened male subjects, there was no significant correlation between
age and alpha frequency or amplitude. Moreover, they found that delta and theta slow
activity decreased rather than increased, with advancing age. Likewise, Giaquinto and
Nolfe (1986) reported similar results when comparing middle-aged and elderly cohorts of
healthy subjects. EEG spectral analysis showed no significant difference in slow activity
(delta and theta) between the two groups. In addition, the alpha frequency was
approximately the same for both groups with no significant difference in topographic
distribution. Pollock et al. (1990) also reported no significant correlation between age and
EEG spectral content for an adult sample. A recent study (Duffy et al., 1993) with
optimally healthy older adults confirms that age-related slowing of EEG does not occur in
the absence of medical illness. In fact, there is a trend for decreased slow activity and
increased fast activity with age, with no period of electrophysiological stability in the
decades between 30 and 80 years.

In summary, in the normally aging brain, there appears to be no significant cell
loss in the cortex, hence, no change in the volume of gray matter. The volume of white
matter, however, decreases with age, which is possibly due to a loss of connections. In addition, an enlargement of ventricles is associated with aging. This implies that there may be a cell shrinkage in the old cortex. Previous studies using biased cell counting techniques found cell loss in the old brain, which has been disproven through the use of unbiased stereological methods. Thus the previous biased results may be due to age-related cell shrinkage rather than a real cell loss. The neuritic plague and neurofibrillary tangles, which are the most prominent characteristics of the pathology of Alzheimer's disease, only occur to a limited extent during normal aging, with their occurrence increasing with age. Gross EEG activity is apparently not markedly changed in healthy elderly subjects. These data, therefore, suggest that there is a fundamental qualitative difference between the neural changes associated with normal aging and those associated with Alzheimer's disease. Some age-related declines of larger amplitude observed previously were likely due to the inclusion of a portion of elderly people with early Alzheimer's disease or other mental diseases.

5.2 Hippocampus

5.2.1 Morphology changes

As discussed in the last section, unbiased stereological techniques have revolutionized the field of cell counting for aging studies. Although there were numerous studies on the effects of age on neuronal numbers in the hippocampus (see review, Geinisman, 1995), these data cannot be taken seriously because biased methodology was used. West was the first to apply the unbiased technology to examine cell number in the aged hippocampus of three species: humans (West, 1993b), rats (West et al., 1991) and monkeys (West et al., 1993). It has been consistently observed across the three studies
that cell number is preserved in CA1, CA2-3, and the dentate gyrus of the hippocampus in old mammals. In addition, no differences in the absolute numbers of hippocampal neurons were observed between aged monkeys with poor and good recognition memory performance (West et al., 1993). Thus, there appears to be little cell loss in the aged hippocampus.

It is well established that there is an approximately 30% decrease in the number of synapses in the middle third of the molecular layer of the dentate gyrus of old rats compared with young adults (Bondareff and Geinisman, 1976). There is comparable age-related decrease in the number of synapses involving dendritic shafts and those involving dendritic spines. The width of the molecular layer, as well as the size of the synapses, are essentially the same in young and old rats. Both the number and the size of granule cells are the same between young and old rats, indicating that the loss of synapses in the molecular layer of the dentate gyrus of senescent rats is unrelated to changes in tissue volume, synaptic size or number of granule cells. In addition, the number of synapses in the inner third zone of the molecular layer is also decreased to a similar extent in aged rats, suggesting that the synaptic loss is not only restricted to the perforant pathway, but also to the afferents from commissural fibers and the hilus region. The underlying mechanisms of this partial deafferentation of neurons in the dentate gyrus of the old rat is not clear. It is hypothesized that this deficit is caused by a loss of presynaptic axonal terminals due to a diminished axonal transport of glycoprotein (Geinisman et al., 1992). More recently, loss of synapses has been confirmed using the nonbiased stereological dissector technique (Geinisman et al., 1992). In addition, it was observed that both perforated and nonperforated axospinous synapses were significantly less in both the middle and inner molecular layers of old rats compared to young rats, while the axodendritic junctions do
not undergo any age-related loss. Here the perforated synapses are defined as those with a discontinuous or perforated postsynaptic-density (PSD) profile in at least one serial section and non-perforated synapses as those showing continuous PSD profiles in all consecutive sections. Interestingly, only the number of perforated synapses was significantly correlated with the memory performance of aged rats on the radial-8-arm maze (Geinisman et al., 1986). The rats with memory deficits had fewer perforated synapses compared to the rats with intact memory. In a more recent study, the number of synapses per neuron in the middle molecular layer was estimated using the unbiased stereological method in the dentate gyrus of chronically implanted rats, on which long-term potentiation (LTP) was first induced by high-frequency stimulation of the middle perforant path (Geinisman et al., 1991). LTP is the best candidate for neural plasticity underlying memory formation, which will be discussed in more detail in the next section. The results demonstrate that the induction of LTP in both young and aged animals resulted in a selective increase in the number of only perforated axospinous synapses. These results suggest that perforated synapses may reflect a structural substrate for LTP in the hippocampus, and the decreased number of perforated synapses in the aged hippocampus may reflect a deficit in structural neural plasticity, which may contribute to the memory deficits in old rats.

5.2.2 Electrophysiological Changes

5.2.2.1 Basic biophysical properties

The in vitro hippocampal slice preparation has been used to examine possible changes in the biophysical properties of old pyramidal and granule cells. This preparation has the advantage of absence of anesthetics, which may interact with the age variable. Most studies agree that the basic biophysical properties of hippocampal principle cells,
including resting potential, input resistance, action potential amplitude and duration, EPSP rise time and half width, somatic membrane time constant and rheobase, are well preserved in the old hippocampus (see review, Barnes, 1994). Although Turner and Deupree (1991) present evidence indicating a significant difference in electrotonic length of CA1 pyramidal neurons between aged rats and young rats, another study, using an extracellular analysis of dentate granule cells, did not find a significant difference in electrotonic length (Barnes and McNaughton, 1980b). Recent intracellular studies have also failed to observe altered electrotonic properties of hippocampal CA1 cells (Foster et al., 1991; Barnes et al., 1992). Furthermore, in contrast to the lower excitability in aged CA1 pyramidal cells reported by Turner and Dupree (1991, see also Potier et al., 1992), most studies have reported a lowering of the threshold for action potential generation (i.e., greater excitability) in pyramidal cells of old rats (Landfield et al., 1986).

The results from two groups (Landfield and Pitler, 1984; Deyo et al., 1989) have demonstrated an age-related increase of the slow after-hyperpolarizing potential (AHP) in CA1 pyramidal cells of F344 rats and rabbits. Although another group did not observe significant changes of the AHP in the same region of F344 or Sprague Dawley rats, they did observe a trend in the prolongation of the AHP in aged hippocampal CA1 pyramidal cells (Potier et al., 1993; Potier et al., 1992). This elongation of the AHP appears to be due to a larger influx of Ca²⁺ during depolarization, because the duration of the Ca²⁺ action potential increases significantly in old rats without any alteration in the amplitude (Pitler and Landfield, 1990; Potier et al., 1992), while the inactivation of Ca³⁺ currents is apparently not impaired in aged rats (Pitler and Landfield, 1990). A very recent study (Thibault and Landfield, 1996), using the partially dissociated hippocampal "zipper" slice preparation, suggests that this age-related increase in the voltage-activated Ca²⁺ influx
may be due to an increase in functional L-type Ca^{2+} channels in aged rats. Interestingly, the increase in the channel density was negatively correlated with the rat's performance on the Morris water task. This line of evidence, thus, suggests that an age-related elevation of Ca^{2+} channel density may lead to an enhanced Ca^{2+} influx during depolarization in the aged hippocampus. Since it is well established that elevated Ca^{2+} is neurotoxic (Choi, 1987; Olney, 1994), the "calcium hypothesis of aging" has been supported with these observations. This hypothesis states that persistent challenges by high Ca^{2+} influx could result in gradual deterioration of neuronal structure and function (Khachaturian, 1987; Landfield et al., 1989). In contrast to the findings in CA1, in hippocampal granule cells, Reynolds and Carlen (1989), using single-electrode voltage-clamp methods, found that the voltage-dependent, slowly inactivating L-type Ca^{2+} current is depressed in old compared with cells from young rats. This depression could be reversed by intracellular injection of the Ca^{2+} chelator EGTA, supporting the idea that old cells accumulate excess cytoplasmic Ca^{2+}. This age-related change reflects an advanced stage of the impairment of Ca^{2+} homeostasis in hippocampal neurons. Whether the density of Ca^{2+} channels is also increased in aged dentate gyrus remains an open question. If this does occur in DG too, it would suggest that the increase in the L-type Ca^{2+} channels may eventually result in an elevation of intracellular Ca^{2+} level, which would further impair cellular function.

5.2.2.2 Glutamatergic synaptic transmission.

Combining the in vitro slice preparation and in vivo evoked potential techniques, the results from Barnes' group demonstrates a clear region specificity of age-related changes in glutamate synaptic transmission in the hippocampus. In the dentate gyrus, it was first found that although there is no change in the threshold stimulus intensity necessary to activate the perforant fibers, at stimulus intensities above threshold levels, the
presynaptic fiber potential is smaller in the old rats (Barnes and McNaughton, 1980b). This is consistent with the anatomical data that there is approximately one-third fewer synaptic contacts from medial entorhinal cortex to the middle third of the granule cell dendritic tree in old rats (Bondareff and Geinisman, 1976; Geinisman et al., 1977; Geinisman et al., 1978; Geinisman et al., 1992). For any given stimulus intensity above threshold, the population EPSP as well as intracellular EPSP of dentate granule cells are smaller in old rats compared to young rats (Barnes, 1979; Foster et al., 1991). When the field or intracellular EPSP amplitude is plotted against the size of presynaptic fiber potential elicited at different stimulus intensities, the EPSP response for a given fiber potential amplitude is larger in old rats. This suggests that given the same input from the same number of synapses, the response of granule cells in the old dentate gyrus is greater than that in rats. Thus, it appears that the synapses are stronger in the old rats. Consistent with this, the size of the intracellularly recorded response to stimulation of single afferent fibers, using the method of minimal stimulation (McNaughton et al., 1981), has also been found to be greater in old rats (Foster et al., 1991). Furthermore, quantal analysis of these unitary responses reveals an increase in the quantal size but no change in the quantal content. Although this result does not allow one to conclude whether the change is a pre- or postsynaptic effect, the results described below on the increased sensitivity of old granule cells to the iontophoretic application of AMPA is consistent with increased postsynaptic receptor sensitivity (Barnes et al., 1992). Furthermore, when the population spike amplitude is plotted against EPSP size over different stimulus intensities, old rats exhibit a larger spike for a given EPSP size (Barnes and McNaughton, 1980b). No age-related change was found, however, in action potential threshold in the hippocampal slice preparations. The mechanisms causing the greater ease of discharging an action potential following stimulation of the perforant path fibers is not clear. There is evidence indicating
that older hippocampal cells have more electronic gap junctions than do young hippocampal cells (Barnes et al., 1987b), which may contribute to the increase in excitability in old rats.

The responses of dentate granule cells to iontophoretic application of the specific glutamate agonist, \( \text{D,L-\alpha\,-amino-3-hydroxy-5-methyl-5-isoxalone propionic acid (AMPA)} \), were also examined in hippocampal slices prepared from young and old rats (Barnes et al., 1992). There was no age difference in the response of old granule cells to the AMPA application compared to young. Because there are fewer synapses at which the AMPA could act in the old dentate gyrus, this result also indicates that the response of a single synapse to the application of AMPA is greater in old rats. Thus, the above pattern of results indicates that there is an age-related increase in synaptic strength and excitability in old dentate gyrus, which may functionally compensate for the age-related synaptic loss, conceivably resulting in the same amount of output from granule cells as in young rats.

In contrast to the dentate gyrus, a very different pattern of results was obtained at the Schaffer collateral-CA1 pyramidal cell synapse. At a given stimulus intensity there was a reduction in the size of the Schaffer collateral-pyramidal cell field EPSP response in old rats (Barnes et al., 1992; Deupree et al., 1993; Landfield, 1986). There was, however, no change in the size of the presynaptic fiber potential between age groups for a given stimulus intensity (Barnes et al., 1992; Kerr et al., 1991). Therefore, there was a smaller EPSP response for a given fiber potential amplitude. There was also no change in the size of the intracellularly recorded unitary synaptic response to minimal stimulation between age groups, nor were there changes in any of the quantal parameters. Finally, there was a significant reduction in CA1 pyramidal cell responsiveness to iontophoretically applied AMPA. The most consistent interpretation of this pattern of results is that there is
a reduction in the number of functional synaptic contacts made by individual Schaffer collateral axons onto old CA1 pyramidal cells, but no change in individual synaptic weights. The clear difference in the age-related alterations between CA1 and the dentate gyrus suggests that even very closely related structures in the same brain region undergo distinct age-related alterations. Thus the conclusions obtained from one subregion of the hippocampus should not be generalized to other subregions.

5.2.2.3 Age-related changes in hippocampal plasticity

The hypothesis that memory is stored in the brain by alteration in the efficacy of individual synapses as proposed by Hebb (1949) was supported by the findings of Lømo and his colleagues in the beginning of the 1970s (Bliss and Lømo, 1973). They discovered a long-lasting potentiation of excitatory postsynaptic potentials (EPSPs) at the perforant path-granule cell synapse in the hippocampus of anesthetized rabbits (Bliss and Gardner-Medwin, 1973) and in hippocampal slices (Bliss and Lømo, 1973), which lasted for at least hours. This strengthening of synaptic efficacy after repeated electrical stimulation of hippocampal afferents is now most commonly referred to as long-term potentiation (LTP), which has been observed in all three subregions of the hippocampus, and in other brain areas. LTP is the best candidate for the cellular mechanism of neural plasticity underlying memory formation. It is well established that the NMDA receptor is critically involved in the induction of LTP (Collingridge et al., 1983). A special property of the NMDA receptor is that it requires sufficient depolarization to release it from blockade by Mg$^{2+}$. Once the NMDA receptor is activated by the conjoint pre-synaptic release of glutamate and postsynaptic removal of Mg$^{2+}$ blockade, Ca$^{2+}$ will flux into the postsynaptic cell through NMDA channels, resulting in the activation of a series of events through second messenger systems, thereby, leading to an increase in synaptic strength.
The postsynaptic depolarization can be achieved by several experimental manipulations. The most straightforward method is to stimulate the afferents with trains of high frequency stimulation (greater than 100 Hz, lasting at least 0.5 sec) at intensities that are sufficient to cause cooperativity (McNaughton et al., 1978). Another way to induce LTP is to depolarize the postsynaptic cell directly by intracellular injection of depolarizing currents (Gustafsson et al., 1987). Finally, a reduction in the inhibition level can also lead to an increase in postsynaptic depolarization, resulting in induction of LTP, such as the case of the "primed burst" paradigm (Rose and Dunwiddie, 1986) (see also Chapter 4), or the application of picrotoxin in hippocampal slice preparations.

When supra-threshold stimulus parameters are used, no age difference is observed in the magnitude of LTP. In another words, LTP induction mechanisms are indistinguishable in young and old rats using these protocols (see review, Barnes, 1994). When submaximal stimulation parameters are used (e.g., 4-pulse stimulation and primed burst stimulation), however, an age-related deficit in the induction of LTP can be observed (Deupree et al., 1991). Because the 4-pulse stimulation paradigm does not involve disinhibition, an age-related change in inhibitory processes can not account for all the age-related deficits in LTP induction by the primed burst paradigm. There are two potential factors that could contribute to this age difference. The first is that NMDA receptor mechanisms may be intact in old rats, but insufficient convergence of inputs may occur on the postsynaptic cell because of the loss of functional synapses. Thus, the depolarization may not be sufficient to release NMDA receptor channels from their voltage-dependent Mg\(^{2+}\) block. An alternative hypothesis is that these low levels of stimulation unmask a deficit in NMDA receptor function. The results from a recent experiment have solved this issue. Barnes et al. (1996) reported that when a single presynaptic stimulation is paired
with the same level of postsynaptic depolarization in young and old CA1 pyramidal cells, there was no difference in the LTP induction threshold or amplitude between young and old animals. This indicates that the previously observed LTP induction deficit is not due to a deficit in NMDA receptor function, rather it is most likely caused by the lack of convergence of inputs on the postsynaptic cell to reach the threshold of postsynaptic depolarization required for LTP induction.

Barnes and her colleagues (Barnes, 1979), using chronically implanted rats, found that although the magnitude of LTP induced by high frequency stimulation repeated daily was equal for both young and old rats, LTP of old rats decayed at a rate twice as fast as that of young rats. This result, therefore, indicates an age-related deficit in LTP maintenance in the hippocampus. Furthermore, the decay rate of synaptic enhancement was found to be significantly correlated with spatial memory performance on the Barnes circular platform in both young and aged groups (Barnes and McNaughton, 1980c).

Another way to examine age-related changes in neural plasticity is to examine the speed of kindling. The kindling phenomenon represents a long-lasting alteration in neuronal excitability induced by high frequency stimulation which is repeated daily, until the rats develop full behavioral seizures upon stimulation. It was found that kindling of the hippocampus via perforant path stimulation is significantly slower in old rats compared to young rats (de Toledo-Morrell et al., 1981). Interestingly, the number of trials to reach the kindling criterion was found to be correlated with radial-8-arm maze performance in both young and old rats.

In contrast to LTP or kindling, there is another form of neural plasticity in the brain, called long-term depression (LTD), which is an activity-dependent long-lasting...
decrease in homosynaptic EPSPs. LTD was originally observed in the cerebellum (Ito, 1984), and has been recently elicited in \textit{in vitro} hippocampal slices using low frequency stimulation in immature rats (Dudek and Bear, 1992). LTD is also dependent on the activation of NMDA receptors, and can be completely blocked by NMDA receptor antagonists. Norris et al. (1996) recently reported that LTD is more easily elicited by low frequency stimulation in the CA1 region of hippocampal slices prepared from old rats compared to young rats. In addition, even though the same magnitude of LTP was induced by maximal stimulation in both young and old slices, aged synapses required fewer episodes of low frequency stimulation for the reversal of LTP than young synapses. This result, therefore, suggests that there may be an age-related increase in neural plasticity in the form of LTD in the hippocampus.

It should be noted, however, although LTP can be elicited successfully by artificial stimulation, it has not been experimentally demonstrated that LTP is involved in natural learned behaviors. Thus, it remains an open question whether there is indeed age-related deficits in the LTP-like mechanisms during natural learning and memory performance, resulting in the behavioral changes observed in old rats. The studies described in Chapter 8 and 9 will shed light on this issue.

\textbf{5.2.2.4 Hippocampal theta rhythm and place specific firing}

An important approach to understanding the mechanisms underlying spatial behavioral deficits of old rats is the analysis of the discharge characteristics of single hippocampal cells in freely behaving rats. The first such study (Barnes et al., 1983) reported that place cells in aged rats exhibit decreased spatial selectivity and reliability when recorded on a radial 8-arm maze task. In contrast, using stereotrode recording methods, which allow better cell isolation (McNaughton et al., 1983b), Mizumori et al.
found that the CA1 place fields of old rats were more specific than those of young rats during tests of spatial memory on an 8-arm radial maze (Mizumori et al., 1993). On the other hand, Markus et al. (1994) found no significant difference in information content, spatial selectivity and reliability scores between young and old rats while performing a randomized, forced-choice task on an essentially identical 8-arm radial maze. This discrepancy may be due to a number of factors, including differences in recording methodology or to the fact that the age comparison in the former two studies were conducted by cells, whereas the comparison was made by numbers of rats in the latter study. Statistical comparisons in which sample number is the number of cells rather than the number of rats severely inflates the degrees of freedom in statistical tests and can easily lead to spurious age effects. In Chapter 8, a study of the effect of age on spatial indices of hippocampal place specific firing, uses tetrode recording probes for even greater confidence in cell isolation (McNaughton et al., 1983b). The statistical analysis in the present experiment was conducted by rat rather by the cell.

The effect of age on the theta rhythm has also been examined by several groups. Recording from young and old rats in small rodent cages, Barnes (Barnes, 1979) detected no difference in the average power in the theta frequency range recorded in the hilus of the dentate gyrus. Markowska et al. (1995), however, found that the peak frequency of the theta rhythm in old rats was significantly lower than that of young rats when they were tested on a T-maze spatial alternation task that involved more extensive type I behavior. It has also been reported that the mean frequency of the theta rhythm during spontaneous exploratory behavior is decreased in old rats (Forbes and Macrides, 1984). Because the frequency of theta increases with the velocity at which the animal moves (Arnolds et al., 1979; McFarland et al., 1975; Recce, 1994), and there was no control of velocity in the
latter two experiments, the old rats may have exhibited altered theta due to their slower movements. A study on the effect of age on the relationship between running velocity and theta frequency is described in Chapter 8.

5.2.3 Neurochemical Changes

5.2.3.1 Glutamate Synaptic Transmission

AMPA receptor binding has been found not to be altered in the old hippocampus compared to young rats (Magnusson and Cotman, 1993). The NMDA receptor may, however, be vulnerable to the aging process. The NMDA receptor complex consists essentially of a recognition site for glutamate/aspartate, of a cation channel and a positive allosteric site for glycine. The density of binding at both the glutamate recognition sites and glycine sites of the NMDA complex was decreased in the old hippocampus (Pelleymounter et al., 1990), whereas affinity does not differ between young and old animals (Tamaru et al., 1991). This decline of receptor density appears to be accompanied by similar changes of receptor function. Pittaluga et al. (1993) examined the release of noradrenaline evoked by NMDA in rat hippocampus and found that the maximal effects of NMDA significantly decreased with age. This result was replicated by Gonzales et al. (1991), who also found that the NMDA-induced inhibition of the carbachol-stimulated response is also markedly reduced in an age-dependent manner with losses of 25% and 53% in middle-aged and senescent rats compared to the young rats. Rao et al. (1993) also reported significant decreases in NMDA mediated EPSP in both CA1 and dentate gyrus subfields of the hippocampus in old rats, after the AMPA-receptor mediated EPSP was blocked by CNQX. According to the electrophysiological data reviewed above, it is likely that the age-related decrease in the number of NMDA receptors is caused by the loss of
synapses in the old hippocampus. It is, however, not clear why there is no AMPA receptor loss, assuming that NMDA receptors and AMPA receptors are colocalized at the same synapses. The possible factor contributing to the discrepancy between electrophysiological data and binding studies may arise from the fact that most binding studies were conducted to examine receptor binding in the whole hippocampus, without differentiating between subregions. Interestingly, two groups (Davis et al., 1993) reported that there was a robust correlation between the number of NMDA receptors in the hippocampus and acquisition of the spatial version of the Morris water task. Those animals that showed better learning had a greater number of NMDA receptors in the hippocampus. This suggests that the decrease in the number of NMDA receptors in the hippocampus may contribute to spatial learning deficit in aging. It is also possible that there exists another neural mechanism which affects NMDA density and learning performance in a similar fashion, without both parameters being related.

5.2.3.2 GABAergic Synaptic Transmission

Benzodiazepine drugs have been found to have greater and longer CNS effects in elderly subjects, which cannot be completely counted for by alterations in pharmacokinetics (Greenblatt et al., 1989). Further studies on both human and animal subjects confirm that there is an age-related increase in CNS sensitivity to benzodiazepine, although the underlying mechanism was not exactly known (Nikaido et al., 1990). In contrast to extensive studies on the effect of age on glutamate synaptic transmission, there are few reports on aging-related alterations in GABAergic, inhibitory processes. It appears that there is no age-related changes in the total GABA-A receptor binding or agonist affinity in the hippocampus (Ruano et al., 1991). It has been found, however, that in vivo microiontophoretic application of GABA inhibited pyramidal cell firing to a
greater extent in old when compared to young rats. This effect was not accompanied by any age-related alterations in $^3$H-muscimol binding in the dorsal hippocampus of these same animals (Lippa et al., 1981). Ruano et al. (1991) found that there is an age-related increase in the efficacy of GABA enhancement of benzodiazepine binding in the hippocampus. A subsequent study from this group suggests that it is most likely due to an increase in the density of type I benzodiazepine $\alpha$ subunit (Ruano et al., 1995), hence, an age-associated increase in theallosteric interaction between type I and GABA binding sites in the hippocampus. A patch-clamp study (Griffith and Murchison, 1995) recently revealed that in Fischer-344 rat basal forebrain neurons, whole-cell GABA-A activated currents were greater in old rats in comparison to young rats, and that the benzodiazepine, midazolam, potentiated GABA currents to a greater degree in aged animals. This result is consistent with previous reports of enhanced benzodiazepine activity with age, although more studies are needed before generalizing this result to hippocampus. Potier et al. (1992) found that the fast IPSP, which is mediated by the activation of GABA-A receptors, is unchanged in the old hippocampus in comparison to young rats, while both the amplitude and the duration of slow IPSP, mediated by GABA-B receptor, are reduced in old rats. The response of hippocampal CA1 pyramidal cells to baclofen, a GABA-B receptor agonist, however, was not changed. Their results, therefore, suggest that there may be a decrease in the presynaptic release of GABA, resulting in the age-related decrease in the slow IPSP. The increase in the responsivity of GABA-A receptors to GABA, on the other hand, may have compensated for the reduced presynaptic GABA release in the old rats, resulting in preserved fast IPSP responses. It is known that lesions of the GABAergic striatonigral pathway cause a GABA-A/benzodiazepine receptor supersensitivity in substantia nigra, and an increase in the density of type I benzodiazepine receptor (Ruano et al., 1991). Thus, the above data are consistent with the hypothesis that
the effect of aging on the GABA-enhancement of benzodiazepine binding and the GABA-activated current may be a compensatory age-associated response to an age-related interneuronal degeneration within the hippocampus or GABAergic hippocampal afferents. Recent data suggest that the number of parvalbumin-containing interneurons is preserved in the hippocampus of old rats (Miettinen et al., 1993). The number of calbindin-containing neurons, however, is significantly reduced in both hippocampal CA1 and the dentate gyrus of old rats (Potier et al., 1994). This result is intriguing, because it suggests that there may be a selective loss of certain types of interneurons with aging in the hippocampus. More systematic, quantitative studies using unbiased method are needed to confirm these studies.

5.2.3.3 Cholinergic Synaptic Transmission

Due the fact that one of the most consistent biochemical alterations in Alzheimer's disease (AD) was a dramatic reduction of choline acetyltransferase (ChAT) activity in cortex and hippocampus, and an extensive loss of cholinergic cells in the basal forebrain (Davies and Maloney, 1976), the effect of aging on cholinergic innervation in these brain regions has attracted wide attention. The results for acetylcholine are much more extensive that those on other neuromodulators, such as norepinephrine and serotonin.

In humans, three groups failed to find any cell loss in the midportion of the nucleus basalis of Meynert (nBM) in the basal forebrain of healthy elderly subjects (Whitehouse et al., 1983). McGeer et al. (1984), however, did find a significant decrease in the number of cells that were larger than 35 mm in samples taken from all levels of the nBM in older subjects. Similarly, Mann et al. (1984) also found a loss of neurons greater than 30 mm in the nBM associated with aging. In animal studies, Biegon et al. (1986)
reported that AChE-positive neurons in the medial septum area (MSA) of 31-month-old rats are 10-20% less numerous than in 4-month-old animals. In contrast, Luine et al. (1986) found no age-related difference in AChE-positive cell number in the rat nBM. Consistent with this latter observation, Honberger et al. (1985) looked at AChE-positive neurons in a number of basal forebrain areas including the MSA and nBM in 7-, 15-, and 53-month-old mice, and found no significant effect of age on cell number. They did, however, note a significant decrease in the size of AChE-positive neurons in older animals. If this holds true for humans as well, the results of McGeer et al. (1984) and Mann et al. (1984) can be accounted for by the cell shrinkage in the old basal forebrain. Thus, both human and animal data suggest that there is no extensive cholinergic cell loss in the basal forebrain in normal aging.

ChAT and acetylcholinesterase (AChE) activity have been examined by many investigators, it is generally agreed that their activity in a certain brain region correlates well with the extent of cholinergic innervation in the same region. Disparate results have been reported on the ChAT activity from both human and animal studies using rats, which indicates either a decrease in the aged hippocampus, or no change in comparison to young subjects (see review, Decker, 1987). A similar pattern of results were also obtained from studies on the activity of AChE. Thus, it can be concluded that there is either no or a very subtle decrease in the ChAT or AChE activity occurring in normal aging, which is distinctly different from the Alzheimer's disease. The innervation of cholinergic fibers in the hippocampus may, therefore, be largely intact in the aged hippocampus, which is consistent with the above conclusion that there is no extensive cholinergic cell loss in the basal forebrain.
There is general agreement in the animal literature that basal release of ACh in both cortex and hippocampus is not affected by age, but that stimulation-induced ACh release is much less in old rats (see review, Decker, 1987). For example, Consolo et al. (1986) found that there is no age difference in resting release of ACh in the hippocampus, but K^+-stimulated release of ACh was reduced in the aged hippocampus. This age-related reduction in depolarization-induced ACh release appears not to be due to greater autoreceptor sensitivity in old animals. It has been found that the inhibition of ACh release by the muscarinic agonist oxotremorine is impaired in hippocampal synaptosomes prepared from old rats, indicating that autoregulation may actually be impaired in old rats. These results, therefore, suggest that older animals not only display reduced ACh release but also are less sensitive to autoregulation. In another words, ACh release in vivo may be less tightly regulated by activity in the system. Thus, the age-related decrease in the stimulation-induced release may be caused by either an alteration in the transmitter-releasing process in axonal terminals or a decrease in ACh turnover. The latter is consistent with the observation that there is an age-related decrease in ACh synthesis in hippocampus (Sims et al., 1982).

The effect of aging on the postsynaptic action of ACh has also been examined. Hippocampal neuronal responses to application of cholinergic agonists appear to be consistently reduced in aging. Using in vivo recording, Lippa et al. (1980) found an age by current interaction effect on spike number for iontophoretic application of ACh but not glutamate, suggesting that reduced neuronal responsivity to ACh was not the result of some generalized reduction in neuronal responsivity to excitation. In a subsequent study Lippa et al. (1985) found that the age-related decrease in neuronal responsivity to ACh in the hippocampus did not result from a change in the number of simple spikes but rather
from a reduction in bursting of hippocampal neurons stimulated by ACh application.
Segal (1982) investigated the responses of hippocampal neurons to ACh in hippocampal slice preparations, and also found that the depolarization accompanied by increased input resistance was reduced by age. Finally, Haigler et al. (1985) recorded in vivo from both CA1 and CA3-4, again found similarly diminished responsivity to ACh application in both regions in old rats.

Perhaps the most obvious explanation for reduction in sensitivity to cholinergic agonists would be a reduction in the number of cholinergic receptors in aged rats. Unfortunately the literature is not clear on this point. Some investigators have found a small decrease in the number of receptor binding sites in the hippocampus, but an equal number of others have reported no age-related change (see review, Decker, 1987). At the very least, it can be said that the results for receptor binding studies cannot account for the larger, more reliable effects of aging on receptor-mediated function. Because binding studies do not identify functional receptors, it remains possible that fewer of the muscarinic sites identified in the binding assays are functional in aged animals.

Two groups have studied the effect of age on cholinergic synaptic transmission in the hippocampus by recording the cholinergic slow EPSP in vitro slice preparations (Potier et al., 1992). Both found an age-related decline in the amplitude of the cholinergic slow EPSP in the aged hippocampal CA1 region, although the data in both reports were also open to alternative explanations. Whether this age-related change was, indeed, of cholinergic function, and whether it is a region-specific change, will be addressed in Chapter 6.
In summary, it appears that functional aspects of cholinergic synaptic transmission, such as stimulated presynaptic release, as well as postsynaptic depolarization, are significantly decreased in the aged hippocampus. On the other hand, however, no consistent effect of age has been found in the "static" properties of cholinergic transmission, including ChAT activity, muscarinic receptor binding and spontaneous release of ACh. This stands in contrast to serious degeneration of the cholinergic system in Alzheimer's disease. Data are still lacking, however, regarding the relationship between this age-related alteration in cholinergic function and age-related declines in learning and memory. Correlational studies of cholinergic electrophysiology and behavior will also be described in Chapter 6.

5.2.3.4 Noradrenergic Synaptic Transmission

A consistent finding in human literature has been a severe age-related cell loss in the locus coeruleus (Brody, 1976), which is composed almost entirely of norepinephrine (NA) neurons with projections primarily to cerebellar, cerebral cortex and hippocampus. In a very recent study, however, using unbiased stereological methods, no change was found in pigmented cell number or size in the locus coeruleus of nondemented old persons as compared with that of young individuals (Mouton et al., 1994). These data suggest that the previously reported data may be a function of biased cell counting methods or a contaminated population which may have included individuals with Alzheimer's disease. Consistent with Mouton et al. (1994), no loss of neurons in locus coeruleus is found in aged Fischer 344 rats (Goldman and Coleman, 1981).

Neurochemical studies have indicated a general sparing of noradrenergic indices in the hippocampus. The concentration of NA and its turnover do not change in the old
hippocampus (Gottfries et al., 1983). This is consistent with the hypothesis that the function of noradrenergic afferents to the hippocampus may be preserved in old age.

5.2.3.5 Serotonergic Synaptic Transmission

Serotonergic innervation of the hippocampus originates almost exclusively in the dorsal and medial raphe nuclei. An age-related reduction in cell density as well as axonal fibers of serotonergic neurons has been reported by several studies using biased techniques (Lolova and Davidoff, 1992). The effect of age on neuronal number in the raphe nuclei, therefore, needs to be reexamined using the unbiased stereological methods. The 5-HT content in the aged hippocampus has been found either not to change (Simpkins et al., 1977) or to increase (Timiras et al., 1982) when compared to young rats. There is, however, a general agreement that 5-HT turnover is increased as a result of aging (Simpkins et al., 1977), indicating an increase in 5-HT metabolism. The mechanism underlying this age-related change is not clear. Furthermore, 5-HT receptors are also significantly decreased in the old hippocampus (Marcusson et al., 1984). Consistent with these data, Baskys et al. (1987) found a decrease, in vitro, in postsynaptic actions of 5-HT on hippocampal granule cells.

5.3 Drug treatments for age-associated hippocampal changes and related learning and memory deficits

5.3.1 Calcium channel blocker: Nimodipine

As discussed above, one of the prominent theories regarding the neural substrates that may underlie age-related learning and memory deficits is the "calcium hypothesis of aging" (Kachaturian, 1989). This hypothesis suggests that cells in the aging brain have an
excess of intracellular calcium. This increase in calcium can have deleterious effects on
neurons that may ultimately lead to membrane deterioration and cell dysfunction or even
death in the aging. This hypothesis has been supported by electrophysiological findings
which indicate age-related increases in the duration of AHP (Landfield and Pitler, 1984),
calcium spikes (Pitler and Landfield, 1990), and an increase in the number of L-type
calcium channels in the aged hippocampus (Thibault and Landfield, 1996).

It has been found that in an associative learning task, called eyeblink conditioning,
the hippocampal AHP is reduced after the young rabbit acquires the trace eyeblink
conditioning paradigm (Coulter et al., 1989). This task is known to require an intact
hippocampus because hippocampal lesions disrupt the animal's acquisition of the task
(Moyer et al., 1990). Behavioral deficits in eyeblink conditioning in the trace paradigm
have been observed in the aged rabbit (Deyo et al., 1989), normal aged humans
(Disterhoft et al., 1991) and in Alzheimer's disease patients (Finkbiner and Woodruff-Pak, 1991). Thus, it had been hypothesized that the age-related AHP deficit in the
hippocampus might, at least partly underlie the age-associated behavioral deficit in the
trace eyeblink conditioning task. This leaves open the possibility that pharmacological
intervention, such as reducing the AHP in old hippocampal neurons, could enhance the
learning rate in old animals. Disterhoft and his colleagues first found that intravenous
administration of the L-type calcium-channel antagonist, nimodipine, a dihydropyridine
that crosses the blood-brain barrier, reversed the acquisition deficit of aged rabbits in the
trace eyeblink conditioning task (Deyo et al., 1989). In parallel with this behavioral
change, nimodipine enhances CA1 hippocampal pyramidal neuron excitability in an age-
and concentration-dependent fashion, as demonstrated by a series of electrophysiological
and biophysical studies conducted by Disterhoft and his colleagues. These findings can be
summarized as follows: 1) Intravenous administration of nimodipine caused an age- and concentration-dependent enhancement of the spontaneous firing rate of pyramidal neurons in rabbits. In particular, nimodipine had a greater effect in old than it did in young rabbits (Thompson et al., 1990); 2) In the *in vitro* slice preparation, a moderate dose of nimodipine caused a significant reduction in the AHP in CA1 pyramidal cells of old but not young rabbits (Moyer et al., 1992); 3) Low concentrations of nimodipine also reduced the duration of calcium action potentials in old hippocampal neurons to the level of those young neurons (Moyer and Disterhoft, 1994). Thus, these results suggest that nimodipine may improve the acquisition of the old rabbit on the trace eyeblink conditioning task by blocking calcium channels in the hippocampus, and altering calcium-related physiological processes (e.g., AHP) that lead to cell dysfunction. Furthermore, nimodipine has been found to facilitate learning in a delayed matching-to-sample task in aged primates (Sandin et al., 1990), and in the Morris water task and radial maze in old rats (Schurman and Traber, 1989). An intriguing feature of the effect of nimodipine is that behavioral facilitation has only been observed in old, but not young animals, which is also consistent with neurophysiological data.

### 5.3.2 Cholinergic enhancing drugs

Due to the early findings on the significantly reduced ChAT activity in the brain of Alzheimer’s disease patients, the “Cholinergic Hypothesis of Geriatric Memory Dysfunction” was proposed more than a decade ago (Bartus et al., 1982). This hypothesis has led to numerous studies on the development and testing of potential cholinergic enhancing drugs, including cholinesterase inhibitors, ACh release enhancers, direct muscarinic agonists, and transmitter precursors. These have all been extensively studied and tested both on laboratory animals and clinically in humans. The treatments
using these drugs for Alzheimer's disease has given marginal or negative results (see review, Jenden, 1991). Although it has been suggested that by the time the disease is clinically discernible, it is already too late to rescue cholinergic function by these drugs. This explanation, however, does not appear to be reasonable, considering the fact that in Parkinson's disease a relatively few surviving neurons can account for a therapeutic response to l-DOPA, a precursor to dopamine. After about a decade of extensive research (see also Chapter 6 and 7), the results from the testing of those cholinergic enhancing drugs are consistent with the emerging conclusion that age-related memory deficits and the dementia symptoms in Alzheimer's disease cannot be accounted for by impairment of cholinergic function in the old brain. The contribution of cholinergic deficits to cognitive function is addressed in Chapter 7.

5.3.3 Neurotrophic factor: NGF

Nerve growth factor (NGF), one of the neurotrophic factors, was originally purified from mouse submaxillary gland. It was subsequently found to play an important role in the development of the peripheral nervous system, as well as the development of basal forebrain in the central nervous system. In the adult brain, NGF is present in the hippocampus, cortex and the basal forebrain, whereas the NGF low affinity receptors have been located selectively on cholinergic neurons in the basal forebrain. This distribution of NGF and NGF receptors supports the view that NGF is synthesized by the target areas of cholinergic neurons and retrogradely transported to the cell body of responding neurons in the basal forebrain (see review, Hefti, 1989). Studies on adult animals with partial or complete transections of the fimbria revealed that NGF treatment prevented the lesion-induced loss of cholinergic cell bodies (Hefti et al., 1989), whereas it failed to prevent the loss of GABAergic neurons (Montero and Hefti, 1988). This
suggests that NGF prevents the lesion-induced loss of septal cholinergic neurons by acting on NGF receptors expressed by cholinergic cells. In addition, it has been observed that NGF treatment stimulated the regrowth of cholinergic fibers in the hippocampus, resulting in a pronounced elevation of the density of cholinergic fibers visualized by acetylcholinesterase histochemistry in the denervated hippocampus (Hefti et al., 1989).

Based on the Cholinergic Hypothesis of Geriatric Memory Dysfunction, a series of studies were conducted to examine whether the administration of NGF could alter memory performance of aged animals, as well as cholinergic neuronal function in the basal forebrain. Fischer et al. (1987) found that in the forebrain of animals that were impaired on the spatial version of the Morris water task, the cell body size of cholinergic neurons was smaller than in nonimpaired aged rats. Intracerebroventricular (ICV) infusion of NGF for two weeks was found to counteract the morphological atrophy of cholinergic neurons. Although the NGF administration did not improve the acquisition behavior of impaired aged animals, it did result in an improvement of this group's performance when the rats were tested on the water task 2 weeks later. Thus, the investigators concluded that the NGF administration was able to improve the memory retention, but not acquisition, which may be caused by the prevention of atrophy of cholinergic neurons in the aged rats (Fischer et al., 1987). In a subsequent study (Fischer and Bjorklund, 1991), three age groups (6-10 mo, 18 mo, and 30 mo) were used. Compared to the young group (6-10 mo), 18 mo old rats were already impaired on the Morris water task, although 30 mo old rats were even worse. Meanwhile, there was no significant decrease in the size of cholinergic cells in any of the 18-month-old rats, whereas the size of cholinergic cells in the 30-month-old rats significantly decreased. ICV infusion of NGF improved the spatial memory performance of 18-month-old rats on the Morris water task. It, however, had no
effect on the performance of 30-month-old rats, as assessed by the pathlength measurement, although the atrophy of cholinergic cells in this group of rats was reduced by the NGF treatment. Thus it does not appear that there is a direct relationship between the size of cholinergic neurons and the spatial memory performance on the Morris water task. Furthermore, ICV infusions of other kinds of neurotrophins, neurotrophin 3 (NT-3) or neurotrophin 4/5 (NT-4/5) also improves performance of aged rats on the water task, although their actions are known not to be selective for cholinergic neurons, and NT-4/5 did not reduce the cholinergic neuron atrophy in the basal forebrain (Fischer et al., 1994). These data, therefore, suggest that NGF's action on cholinergic neurons in the basal forebrain may be simply one of the effects of NGF in the brain, which may not be sufficient to cause the improvement in the performance of aged animals.

Recently a neurotoxin, 192 IgG-saporin, has been developed, which recognizes the cholinergic neurons by binding with low-affinity NGF receptors, hence, selectively kills cholinergic cells in the basal forebrain (Wiley, 1992). It has been shown that the selective lesions of cholinergic cells in either the medial septum or the basal forebrain do not cause performance deficits on the spatial version of the Morris water task in the young rats (Berger-Sweeney et al., 1994). Thus, NGF's effect on aged rat behavior on the water task may not be primarily caused by its pharmacological effects on cholinergic neurons, which, nevertheless, may still be involved. The underlying mechanisms for NGF's improvement of aged rats performance on the water task, therefore, need to be investigated further. For example, Williams et al. (1991) found that chronic ICV administration of NGF increased the activity of cholinergic neurons, with the largest effects in the striatum. NGF treatment also increased the effectiveness of neurotransmission between basal forebrain cholinergic neurons and postsynaptic amygdaloid target neurons. In a Y-maze
brightness discrimination task, NGF treatments did not affect the cognitive measure of brightness discrimination, but reduced the number of avoidance attempts, which is a measure of motor function.

5.3.4 Nootropic drugs

The concept for the class of drugs referred to as “nootropic” drugs was originally proposed by Giurgea (1972). It was defined as a class of psychoactive drugs that “selectively improve efficiency of higher telencephalic integrative activities”. Piracetam was the first drug in this class, which is a pyrrolidinone derivative. Animal studies have shown that piracetam enhances learning in the water task, Y-maze and active avoidance task. It also enhances the general resistance of the brain to learning impairments induced by ECS, aging, various agents and trauma (see review, Giurgea, 1977). In human subjects, large doses and long-term administration of piracetam has also been found to be effective in improving the performance of elderly or demented patients on memory and behavioral tests (Smith et al., 1984). Some other pyrrolidinone derivative drugs were subsequently developed, including aniracetam, oxiracetam, and pramiracetam, all of which have similar cognitive enhancing effects as piracetam. Among those, however, Aniracetam is more active, hence, has been more extensively studied and tested. In animal models it also reduces memory and learning impairments caused by age (Kubota et al., 1986) and the effects of cholinergic antagonists, cerebral ischemia and electroconvulsive shock (see review, Lee, 1994). In healthy volunteers, a single oral dose of aniracetam was found to significantly reduce electroencephalographic changes induced by hypoxic hypoxidosis with higher potency than piracetam (Saletu et al., 1980). It also reduced cognitive impairment caused by scopolamine in healthy young volunteers, and also was more active than piracetam in this model (Wesnes et al., 1990). Aniracetam has been
reported to be more effective than placebo in treatment of Alzheimer's disease in several clinical trials (Senin et al., 1991). It has also been shown to reduce the impairment of mental function due to cerebrovascular disease, and to improve the cognitive performance of elderly patients with mild cognitive decline due to various unspecified conditions (see review, Lee, 1994).

The neural mechanisms underlying the aniracetam's effect on learning and memory is not fully understood. Oral administration of aniracetam increases the release of acetylcholine from the rat hippocampus and reverses scopolamine-induced decreases in hippocampal acetylcholine and choline levels (Giovannini et al., 1993). It has also been found that chronic administration of aniracetam elevates levels of various brain biogenic monoamines in both old and young rats (Stancheva et al., 1991). Recent studies, on the other hand, have provided convincing evidence suggesting a significant effect of nootropic drugs on glutamate synaptic transmission. It was first noticed that aniracetam at millimolar concentrations or micromolar concentrations selectively increases AMPA currents by a positive modulating action on glutamate AMPA receptors (Ito et al., 1990), but has no effect on NMDA- (Kaneko et al., 1991) or kainate-receptor-mediated currents (Ozawa et al., 1991). Patch clamp studies suggest that the drug may slow both closing rate and desensitization of the channel (Hestrin, 1992). Aniracetam showed very weak ability to displace $[^{3}H]$-muscimol binding from GABA receptors in mouse synaptic membranes (Martin and Haefely, 1993), and has no affinity for dopaminergic, adrenergic, serotonergic, muscarinic or nicotinic receptors (Martin and Haefely, 1993). Thus, the drug's effect on the cholinergic system, as discussed above appears not to be a direct effect on cholinergic neurons. It has also been found recently that aniracetam potentiates metabotropic glutamate receptor (mGluR)-evoked stimulation of phospholipase C in
primary neuronal cultures (Pizzi et al., 1993). Finally, aniracetam can facilitate the induction of LTP such that the number of afferent bursts needed to elicit a near maximal level of potentiation was reduced by about 50% in the presence of the drug (Arai and Lynch, 1992).

Based on the above evidence, a class of benzoyl-piperidine (BDP) compounds were developed, which freely cross the blood-brain barrier after peripheral administration. These compounds appear to be more potent than aniracetam and lack the labile properties of the latter. These agents also enhance AMPA receptor currents (Arai et al., 1994), and improve the retention of spatial memory on the radial-8-arm maze and Morris water task (Granger et al., 1996b), olfactory learning and memory retention (Staubli et al., 1994), and classical fear conditioning (Shors et al., 1994). The positive modulation of AMPA receptors by BDP compounds also resulted in a facilitation of the field EPSP in the hippocampus in both in vivo and in vitro preparations. This may facilitate LTP induction by improving conditions for cooperativity on the postsynaptic site (McNaughton et al., 1978), and this effect on LTP induction may explain the improved performance of animals on learning and memory tasks. This drug is particularly interesting because there is an age-related deficit in the induction of LTP using submaximal stimulation, which is most likely due to decreased convergence caused by reduced synaptic density in the aged hippocampus. Thus, the BDP compounds could potentially correct this age-related deficit, and a recent report indicates that this drug can reverse an age-associated memory impairment (Granger et al., 1996a).

5.3.5 Environmental enrichment and postnatal handling
The effects of environmental stimulation on behavior in rats were first reported by Hebb (Hebb, 1949) who found that animals reared in "free environments" performed better in learning tasks than rats from "restricted environments". Additional studies provided more evidence for superior learning ability in the Hebb-Williams maze by animals raised in "free environments" as compared to rats reared in "restricted environments" (Bingham and Griffiths, 1952). A similar effect has also been observed in old rats. It has been found that the learning performance in aged rats that had spent a year in an enriched environment (EC) was improved when they were tested at 2 years of age (Doty, 1972). The neural mechanisms underlying this behavioral change is far from well understood. There is evidence suggesting that the enriched environment induces a number of neurochemical, neuroanatomical and behavioral alterations in the brain, including increases in wet and dry weights of the brain, neuron size, dendritic spines, glial proliferation, cortical cholinesterase activity (Bennett et al., 1964). Other data suggest that the brain of aged rats may also undergo similar structural plasticity after exposure to an EC. Diamond et al. (1985) reported aged rats that were exposed to an EC between 766 and 904 days of age developed thicker cerebral cortices than non-enriched animals, especially the frontal and occipital regions. Significant dendritic field changes in the cerebellum have also been observed in EC-housed aged rats (Greenough et al., 1986). Thus, the most significant effect caused by the EC appears to be the increased dendritic branching with an increase in synapses, and this plasticity remained in old age. These neuronal changes, however, occur mostly in primary sensory cortices (e.g., Turner, 1983; Volkmar and Greenough, 1972) and cerebellum, with minimal neuronal changes occurring in hippocampus.
Postnatal handling has a similar effect on behavior. In the handling procedure, the dam is removed from the home cage and the pups are placed in individual containers. After 3-15 min the pups are returned to the mother and the home cage. This procedure is repeated daily until weaning (day 21). The handled (H) rats are compared to the non-handled (NH) pups that are left undisturbed with their mother. During development H rats open their eyes earlier than NH pups, their motor coordination develops faster and they weigh more (Greenough et al., 1972). H rats also perform better in learning tasks, such as avoidance behavior, and they show reduced emotionality in open-field tests and in avoidance learning (see review, Denenberg, 1967). It has also been found that spatial learning in the Morris water task is significantly better in H rats than in NH rats (Meaney et al., 1988).

The mechanism underlying the postnatal handling effect appears to be due to its effect on the adrenocortical system. Physiological studies have demonstrated H animals show an earlier maturation of the adrenocortical response, secrete less corticosterone (Ader and Grotta, 1969) and ACTH (Meaney et al., 1989a) in response to a variety of stressors and show a faster return to basal corticosterone levels following the termination of stress (see review, Meaney et al., 1991). It appears that the young adult H and NH rats do not differ in basal corticosterone levels. The main effects are in their response to stress. It has been reported that aged male rats hypersecrete corticosterone following the termination of stress and under basal conditions (Brett et al., 1983). It is also known that the hippocampus is a major target for glucocorticoids (McEwen et al., 1986) and is a critically important brain region in the regulation of adrenocortical function (Feldman and Conforti, 1980). There are two corticosteroid receptor types: Type I (or mineral-corticotid) receptors bind with high affinity both corticosterone and the mineralocorticoid, aldosterone, but not
the synthetic glucocorticoid dexamethasone. Type II (or glucocorticoid) receptors preferentially bind corticosterone and dexamethasone. In the brain, the type I receptor is highly localized to the septo-hippocampal system, whereas the type II receptor is more diffusely distributed in neurons and glia throughout the brain. The aging hippocampus loses both types of receptors (Meaney et al., 1989b). Thus, it has been suggested that the age-related increase in hypothalamic-pituitary-adrenal (HPA) activity may be related, at least in part, to a loss of receptors for corticosterone in the hippocampus, which in turn leads to dampened sensitivity of the hippocampus to circulating corticosterone, and a decrease in hippocampal inhibition of HPA activity. This may subsequently result in the hypersecretion of corticosterone in old age. This is supported by the fact that the pattern of corticosterone hypersecretion reported in the aged rat is similar to that of young, adult animals with experimentally induced (by chronic exposure to stress or corticosterone) or naturally occurring decreases in hippocampal type II glucocorticoid receptors, all of which show decreased hippocampal type II glucocorticoid receptors, and all hypersecrete corticosterone (Sapolsky et al., 1985). The postnatal handling treatment may improve behavioral performance of old rats by reducing the corticosterone hypersecretion in aging.

The hypothesis that neurons in the hippocampus are vulnerable to glucocorticoid neurotoxicity was proposed nearly 2 decades ago. The idea is that corticosterone hypersecretion in aged rats may cause cell loss in the hippocampus, which might underlie the behavioral deficits of aged rats in learning and memory (Sapolsky, 1992). Although several labs have reported cell loss in the hippocampus (Landfield et al., 1978), which appeared to be reversed by postnatal handling (Meaney et al., 1988), recent unbiased vigorous stereological methods for cell counts reveal no cell loss in the aging hippocampus (West, 1993b), indicating that the hypothesis of hypersecretion of corticosterone causing
cell loss can not be established. It is, however, still possible that the hypersecretion of corticosterone may cause cell shrinkage or cell dysfunction in the aged hippocampus, which may contribute to the learning and memory deficits in old rats.
CHAPTER 6 AGE-RELATED DECREASE IN CHOLINERGIC SYNAPTIC TRANSMISSION IN THREE HIPPOCAMPAL SUBFIELDS

As reviewed in Chapter 2, 3 and 4, an important subcortical input to the hippocampus arises from the medial septum/diagonal band complex, the intact function of which is critical for the normal operation of the hippocampus. This septo-hippocampal projection mainly consists of cholinergic and GABAergic afferents. No age difference was observed in the facilitation of population spikes in the hippocampus by the stimulation of medial septum (Green et al., 1993), suggesting that the GABAergic component of the septohippocampal pathway may not be altered in aging. The current study was conducted to examine the effect of age on function of the septal cholinergic input in the hippocampus, by recording the cholinergic slow EPSP from hippocampal slices (Cole and Nicoll, 1984). At the time this study was being conducted, two groups reported an age-related decrease in the cholinergic slow EPSP in region CA1 of the hippocampus (Potier et al., 1992; Taylor and Griffith, 1993). In the present study, we extend these experiments, employing approaches that strengthen our ability to assess the extent of the functional cholinergic deficit in old rats. First, considering the wide individual variation in behavioral deficits and neurobiological alterations observed in old animals (Barnes, 1979), and the fact that measurements made on multiple cells from one animal are not independent, we have conducted our statistical comparisons between age groups by individual rats. Second, all rats were behaviorally tested in order to demonstrate the presence of expected age-related cognitive changes, and to allow correlation analysis to be conducted. The latter enables a search for possible relationships between acquisition of spatial information and cholinergic function in the hippocampus. Finally, because previous studies have demonstrated that some neuronal alterations in aging exhibit remarkable region-specificity, even within the
hippocampus (Barnes et al., 1991; Barnes and McNaughton, 1980b; Barnes et al., 1992; Foster et al., 1991), each hippocampal subfield was investigated on its own.

6.1 Methods

6.1.1 Subjects

Three age groups of F-344 male rats were used in this experiment. Forty-five young rats (3 wks) were obtained from the Charles River Laboratories, and 45 adult (9 mo) and 47 old (24-28 mo) rats from the National Institute on Aging (NIA) colony at Harlan Sprague-Dawley. Due to the shortage of old retired breeders at Harlan, 21 of the old rats were virgins, and all of the 9-mo rats were retired breeders. None of the rats included in this experiment had cataracts. All rats were housed individually in Plexiglas guinea pig tubs, maintained on full food, and were tested (both behaviorally and electrophysiologically) during the light phase of their 12/12-hour light-dark cycle. Twenty-one 9-mo rats and 27 27-mo rats had participated in a conditioning experiment before they were sliced for the study of CA3 and DG. In this experiment, they were given 2 sessions of scrambled electrical foot shock (1mA) to learn a tone-context association. In order to give a similar number of the young animals this experience, twenty 3-wk rats were also given foot shock after the test on the Morris water task.

6.1.2 Behavioral procedures

All rats were handled at least three times (about 10 min) before behavioral testing. The Morris water task apparatus was a circular pool (120 cm diameter and 36 cm depth) with white walls. The water was maintained at 26-28 °C and was made opaque by adding white, nontoxic, Crayola paint. The pool was in the center of a 2.3 x 2.73 x 2.5 m room
with multiple visual stimuli on the wall as distal cues, and a chair and a metal board against
the wall of the pool as proximal cues. The circular escape platform was 11.5 cm in
diameter. For the spatial version of the water task, the platform was lowered about 1 cm
below the water surface, and maintained at a fixed position; for the cue version of the task,
the visible platform, marked by black tape around its edge, was raised above the water
level approximately 2 cm, and a rectangular wooden board was hung directly above the
platform. The movement of the rat in the pool was tracked by a video camera connected to
a VP114 tracking unit (HVS Image, England) system. The X, Y position coordinates
were acquired by special purpose software (TR, J. Forster). After each block of training
or testing, the rat was kept in an incubator (Ohio Medical Products, Air Reduction Co.
Inc.) at temperatures ranging from 30 - 33°C to prevent hypothermia. Off-line analysis
was accomplished by in-house software (WMAZE, M. Williams), which incorporated
several new water task analysis methods developed by Gallagher et al. (Gallagher et al.,
1993).

On the spatial task, the rat was given 3 blocks of 2 training trials on each day, with
about a 30 min interblock interval. On each trial, the rat was released into the water from
one of seven locations spaced evenly at the side of the pool, which varied randomly from
trial to trial. After the rat found the escape platform or swam for a maximum of 60 sec, it
was allowed to sit on the platform for 30 sec. At the end of the 4th day of training, a
probe test was given, in which the hidden platform was removed and the rat was allowed
to swim in the pool freely for 60 sec. On the 5th day, the rat was given 6 trials for the
visual discrimination version of the water task, with an intertrial interval of approximately
10 minutes. This task involves finding the location of the visible platform when both the
starting location at the side of the pool, and the platform location (in one of the 4
quadrants), were moved randomly after a given trial for all rats. The latency to escape the water, the corrected integrated pathlength (CIPL) and the average distance from the target (AD) were measured to assess the rats' acquisition on both spatial and cue tasks. The latter two measures have been described in detail elsewhere (Gallagher et al., 1993). Briefly, a correction was made so that trial performance was relatively unbiased by differences in distance to the goal from various start locations at the side of the pool. In this correction procedure, the average swimming speed for each training trial (path length/latency) was first calculated. The amount of time required to swim to the goal at that speed from the start location was then removed from the record, prior to computing the corrected integrated pathlength and the average distance from the goal. The average distance, "AD", measures the proximity of the animal's position with respect to the goal and was obtained by sampling the position of the animal in the maze (10 times per second) to provide a record of its distance from the escape platform. In addition, the search time of the rat in each quadrant during the probe trial was also measured.

6.1.3 Electrophysiological Procedures

The cholinergic slow EPSP was examined in each of the three age groups in CA1, CA3, and DG subregions, respectively. One rat was sliced per day. In any given week, usually two animals were sliced from each of the three ages to minimize variability due to changing chamber conditions. All data were collected from three age groups for the CA1 region first, then CA3, and finally DG. Hippocampal slices were prepared essentially as described previously (Barnes et al., 1987b). Briefly, each rat was deeply anesthetized with metofane (Pitman-Moore) and decapitated. Transverse slices (450 μm) were cut using a tissue chopper along the longitudinal axis of the hippocampus, and were then transferred to an interface-type brain slice chamber (Haas et al., 1979) perfused with
artificial cerebral spinal fluid (ACSF) at 1.5-2.0 ml/min. The ACSF consisted of the following in mM: NaCl 124, CaCl₂ 2.5, KCl 4, KH₂PO₄ 1.25, MgSO₄ 1.3, NaHCO₃ 26, dextrose 10 (pH 7.4). The slices were oxygenated with a 95% O₂/5% CO₂ gas mixture, and the bath temperature was maintained at 32 ± 1°C. Eserine (Sigma Chemical Corp.), an antagonist of acetylcholinesterase, was added to the medium (1-2 µM) 1.5 hr after slicing, and recording sessions began 30 min later. For reasons described below, in a separate experiment the general glutamate receptor antagonist kynurenic acid (1 mM, Sigma), and the GABA-A receptor antagonist picrotoxin (50 µM, Sigma), were also added into the medium about 2 hr after slicing.

The cholinergic slow EPSP was recorded intracellularly from either pyramidal cells in CA1 or CA3, or granule cells in DG, using standard bridge-balance current clamp techniques and glass micropipettes (1 mm with filament, World Precision Inst.; 80-120 MΩ) filled with 3 M KAc. The stimulating electrode, made of a pair of teflon-insulated stainless steel wires (diameter, 76 µm), twisted together, was placed in stratum oriens in CA1 or CA3, or in the granular layer in DG, where the cholinergic fibers are relatively rich (Frotscher and Leranth, 1985). Stimulation at these sites produced optimal slow EPSP responses (Cole and Nicoll, 1984). Because the slow EPSP is known to be membrane potential dependent, and is most pronounced at relatively depolarized levels, a direct constant positive current (<0.5 nA) was applied to depolarize the cell membrane close to the threshold of spontaneous action potentials. Not all cells required a depolarizing current to achieve this low level of cell firing. In each hippocampal subregion, biphasic current stimuli (100-200 µs duration, 40-50 Hz) at two intensities (CA1, 400 µA & 700 µA; CA3, 200 µA & 500 µA; DG, 300 µA & 600 µA) were chosen to evoke a moderate and approximately maximal slow depolarization response, respectively. These responses were
Figure 6.1 Example of the cholinergic slow EPSP recorded from the CA1 region of hippocampus, elicited by a 50Hz (0.5 sec) stimulation of stratum oriens in CA1. It was completely abolished by drop application of the muscarinic antagonist atropine (2 mM).
confirmed to be cholinergic, as they could be completely abolished by atropine (Fig. 6.1). Only the cells reaching the minimum health criteria were included for experimentation (input resistance > 15 MΩ; resting membrane potential > -50 mV; action potential > 70 mV). For each cell, stimulation at relatively low and high intensities was alternated every 10-15 min. Each stimulation intensity was repeated 2 or 3 times. Negative 80 μs current pulses were then delivered at 0.1-0.5 nA, 0.33-2 Hz through the recording pipette during the induction of the maximal cholinergic slow EPSP, so that the input resistance of the cell could be measured from the deflections of the membrane potential. The data were acquired in DC mode, amplified 100 times, recorded and analyzed on a PC computer using "Workbench" software (Data Wave Inc.).

6.1.4 Statistical Analysis

The integrated pathlength and average distance data were subjected to a two-way analysis of variance (ANOVA) with repeated measures, which were followed by post hoc Fisher's PLSD tests. The time spent in the target quadrant during the probe test was analyzed using a one-way ANOVA and post hoc Fisher's PLSD tests.

The electrophysiological data were analyzed using one-way ANOVA and post hoc Fisher's PLSD tests for each stimulus intensity. The correlation between electrophysiological and behavioral measurements was determined in each age group using a simple regression analysis.
6.2 Results

6.2.1 Behavior

6.2.1.1 Spatial Acquisition

Three age groups of rats (3 wk, n=31; 9 mo, n=33; 24-27 mo, n=35) were tested on spatial and cue versions of the Morris water task. Because the latency for old rats to reach the hidden platform may be confounded by their slower swimming speed (Gage et al., 1989b), only the data from the corrected integrated pathlength and the proximity to the target are presented here. Old rats exhibited a much longer overall integrated pathlength to reach the platform (F(2, 396)=50.202, p<0.0001) compared to young and adult rats, but there was no significant difference between the latter two age groups (Fig. 6.2A). Post hoc comparisons revealed significant differences between old and younger groups on trial blocks 2, 3 and 4 (p<0.0001), but not on the first day of training. The measurement of the proximity to the target during spatial acquisition also reveals impairments in old rats (Fig. 6.2A). Old rats exhibited a significantly longer overall average distance to the hidden platform than did young and adult rats (F(2, 396)=30.971, P < 0.0001), and adult rats showed significantly longer average distances than did the young rats (P<0.023).

Using a 2-way ANOVA, a significant effect of trial block was found for the integrated pathlength in all three age groups (3 wk: F(3, 123)=28.606, P<0.0001; 9 mo: F(3, 128)=22.943, P<0.0001; 24-27 mo: F(3, 135)=2.835, P<0.0406) and the average distance in young and adult groups (3 wk: F(3, 124)=14.025, P<0.0001; 9 mo: F(3, 128)=7.681, P<0.0001), indicating that though all rats improved their performance over trials, old rats exhibited a much smaller improvement throughout training.
Figure 6.2 A: Mean (± S. E. M.) of the performance scores (corrected integrated pathlength and proximity to target) in the spatial version of the Morris water task for young (open circles), adult (closed circles) and old (closed squares) rats. Each trial block consisted of 6 trials given in one day. On the first block, there was no significant difference in either measurement between age groups; however, on subsequent days, old rats took significantly longer integrated pathlengths to find the hidden platform, and swam further from the target than did young and adult rats (see text for statistical analyses).

B: Mean (± S. E. M.) of the search time in each quadrant of the pool, and the proximity to the target in the probe test for three age groups of rats. In this test the escape platform was removed. While young and adult rats searched significantly longer in the training quadrant, the old rats spent approximately equal amounts of time in each of the 4 quadrants. In addition, old rats swam significantly farther away from the location of the previous platform than did younger rats during the probe test (see text for statistical analyses.)
A

**Spatial Task**

- Integrated Pathlength (cm)
  - Blocks of 6 trials

- Proximity to Target (cm)
  - Blocks of 6 trials

B

**Probe Test**

- Time in Quadrants (sec)
  - Quadrants

- Proximity to Target (cm)
Behavior on the 60-second probe test, given immediately after training, indicates that both young and adult rats spent a significantly longer time in the target quadrant which previously contained the platform (3 wk: $F(3, 124)=63.49$, $P<0.0001$; 9 mo: $F(3,128)=19.22$, $P<0.0001$); however, old rats spent approximately equal amounts of time in each of the 4 quadrants (Fig. 6.2B). The measurement of the proximity to the target reveals that young rats swam closer to the location of the platform than did adult rats ($P<0.0187$), and old rats swam the farthest from the target compared to both other groups ($P<0.0001$; Fig. 6.2B). These data indicate that old rats did not remember the location of the hidden platform as well as did younger rats.

6.2.1.2 Cue Learning

In the cue version of the water task, old rats took a significantly longer overall integrated pathlength to reach the visible platform than did young and adult rats ($F(2, 591)=65.786$, $P<0.0001$; Fig. 6.3A). There was no correlation, however, between the accuracy of performance of old rats in the last block of the spatial task and on the last trial of the cue task (Fig. 6.3B). This suggests that the old rats' impairment in the cue task may not be a major cause of their deficits in spatial acquisition. Furthermore, the steep slope of the acquisition curve of old rats (Fig. 6.3A), implies that old rats improved rapidly throughout trials, and that their performance may have converged with those of younger rats if given more training. For this reason, an additional group of animals (3 wk, $n=8$; 9 mo, $n=8$; 24-28 mo, $n=11$) were tested on the Morris water task using the same experimental protocol, with the exception that 12 trials of training were given in 2 consecutive days in the cue task, instead of 6 trials in one day. These old rats were also spatially impaired; however, their performance in the cue task was not significantly different from that of younger rats after 8 trials of training (Fig. 6.3C). This indicates that
Figure 6.3. A: Mean (± S. E. M.) of the corrected integrated pathlength on the cue version of the Morris water task for young, adult and old rats. Old rats took a significantly longer overall integrated pathlength to find the visible platform compared to young and adult rats. B: There was no significant regression for old rats (P>0.05, dashed line) when performance in the cue task (corrected integrated pathlength of the 6th trial) and the spatial task (corrected integrated pathlength of the last trial block) were compared. C: Mean (± S. E. M.) of the corrected integrated pathlength on the cue version of the Morris water task for an additional batch of young, adult and old rats. In this case 12 trials instead of 6 were given. Old rats eventually reached the performance level of the younger rats after 8 trials of training.
the old rats could, indeed, learn the cue task, though at a slower rate, making it less likely that visual problems were the major factor contributing to the observed age difference.

6.2.2 Electrophysiology

6.2.2.1 Cholinergic slow EPSP

Region CA1. Fifty-one pyramidal cells from 34 rats (3 wk: n=11; 9 mo: n=11; 24-27 mo: n=12) were recorded in CA1. There was no age effect of input resistance, resting membrane potential, amplitude of action potential, or stimulation threshold to evoke single action potentials (see Table 6.1). However, with similar membrane potentials at which the slow EPSP was evoked (membrane potential at test in Table 6.1), there were significant age effects on the amplitudes of the slow EPSP, at both stimulation intensities (400 µA: F (2,24) = 3.615, P<0.042; 700 µA: F(2,32)=16.89, P<0.0001). Post hoc tests revealed that the amplitude of the slow EPSP in old rats was significantly reduced compared to young (400 µA: P<0.0277; 700 µA: P<0.0001) and adult rats (400 µA: P<0.0341; 700 µA: P<0.0037). The 700 µA -evoked response of young rats was also significantly larger than that of adults (P<0.0157; Fig. 6.4). The duration of the response was more variable (see Table 6.2) and did not reveal significant age differences.

Region CA3. Sixty-six cells from 33 rats (n=11 in each age group) were recorded in the CA3 pyramidal cell body layer. As in CA1, there was no effect of age on basic biophysical properties of the cells (Table 6.1). There was a significant effect of age on the size of the cholinergic slow EPSP (200 µA: F(2, 25)=8.458, P<0.0016; 500 µA: F(2,30)=9.92, P<0.0005, Fig. 6.4). There were no age difference, however, in the membrane potential at which the cholinergic slow EPSP was elicited. As revealed by post hoc tests, the amplitude of the slow EPSP of the young group (at 200 µA stimulus
Table 6.1 Basic electrophysiological parameters of cells in three age groups of rats.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>(# of rats)</td>
<td>(MΩ)</td>
<td>(mV)</td>
<td>(mV)</td>
<td>(µA)</td>
</tr>
<tr>
<td>CA1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 wk (11)</td>
<td>16</td>
<td>38.1 ± 4.56</td>
<td>-60.6 ± 1.16</td>
<td>83.7 ± 1.98</td>
<td>182.5 ± 27.1</td>
</tr>
<tr>
<td>9 mo (11)</td>
<td>16</td>
<td>36.8 ± 3.21</td>
<td>-61.3 ± 1.72</td>
<td>85.1 ± 2.60</td>
<td>141.4 ± 30.0</td>
</tr>
<tr>
<td>24 -27mo (12)</td>
<td>19</td>
<td>42.0 ± 4.56</td>
<td>-60.1 ± 0.87</td>
<td>87.5 ± 1.61</td>
<td>131.4 ± 16.7</td>
</tr>
<tr>
<td>CA3:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 wk (11)</td>
<td>26</td>
<td>32.2 ± 1.70</td>
<td>-60.9 ± 0.95</td>
<td>90.4 ± 1.09</td>
<td>51.2 ± 8.43</td>
</tr>
<tr>
<td>9 mo (11)</td>
<td>18</td>
<td>33.8 ± 2.23</td>
<td>-60.5 ± 0.93</td>
<td>89.0 ± 1.44</td>
<td>65.0 ± 9.97</td>
</tr>
<tr>
<td>27 mo (11)</td>
<td>22</td>
<td>29.7 ± 1.62</td>
<td>-63.0 ± 1.44</td>
<td>87.8 ± 1.05</td>
<td>48.9 ± 7.65</td>
</tr>
<tr>
<td>DG:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 wk (9)</td>
<td>24</td>
<td>35.8 ± 2.47</td>
<td>-66.8 ± 1.20</td>
<td>83.7 ± 1.95</td>
<td>91.9 ± 10.6</td>
</tr>
<tr>
<td>9 mo (10)</td>
<td>24</td>
<td>34.3 ± 3.84</td>
<td>-67.5 ± 1.10</td>
<td>78.6 ± 1.55</td>
<td>132.3 ± 16.1</td>
</tr>
<tr>
<td>27 mo (12)</td>
<td>25</td>
<td>34.6 ± 2.31</td>
<td>-65.9 ± 1.07</td>
<td>82.0 ± 1.29</td>
<td>144.4 ± 17.6</td>
</tr>
</tbody>
</table>

All values are mean ± SE. One-way ANOVA for each hippocampal subregion revealed no significant differences between age groups for the above biophysical parameters (P > 0.05). Testing membrane potential: the membrane potential at which the cholinergic slow epsz was induced.
Figure 6.4  Mean (± S. E. M.) amplitude of the slow cholinergic epsp observed in CA1, CA3 and DG for young, adult and old rats, elicited at 2 stimulus intensities. The amplitude of the slow EPSP was severely reduced in the old rats, and the reductions were proportionally similar across three hippocampal subregions. * P<0.05, ** P<0.01, *** P<0.001.

intensity) was significantly greater than those of old rats (P<0.0004). Although the mean amplitude of the slow EPSP recorded from the adult group was larger than that of the old, it did not reach statistical significance (P<0.0518). The duration of the response of old rats, however, was shorter than that of the adults (P<0.0086). There was no significant difference between young and adult rats in both the amplitude and duration of the slow
EPSP. At the 500 μA stimulation level, the amplitude of the slow EPSP in old rats was significantly lower than that of young (P<0.0002) and adult rats (P<0.0065), whereas there was no significant difference between the latter two age groups. No age effect was found on the duration of the response.

**Region DG.** Seventy-three cells from 30 rats (3 wk: n=9; 9 mo: n=10; 27 mo: n=11) were recorded in DG granule layer. As in CA1 and CA3, there was no significant difference in the basic electrophysiological parameters of the cells between each age group (Table 6.1). For both stimulation intensities, there were significant age effects on both the amplitude (300 μA: F(2, 28)=19.174, P<0.0001; 600 μA: F(2,28)=30.759, P=0.0001; Fig. 6.4) and the duration (300 μA: F(2, 28)=5.743, P<0.0081; 600 μA: F(2,28)=9.007, P=0.0009; Table 6.2) of the cholinergic slow EPSP. Post hoc tests revealed that the amplitude of the slow EPSP in old rats was significantly reduced compared to young (300 μA: P<0.0001; 600 μA: P<0.0001) and adult rats (300 μA: P=0.0031; 600 μA: P=0.0001). The duration of the slow EPSP in old rats was also significantly smaller than young (300 μA: P=0.0191; 600 μA: P=0.0009) and adult rats (300 μA: P=0.0037; 600 μA: P=0.0015). There was no significant difference, however, between the latter two age groups in either the amplitude or the duration.

Overall, the size of the cholinergic slow EPSP was compromised in all three hippocampal subregions in old rats. Compared to the average of young and adult rats, the amplitude of the cholinergic slow EPSP of old rats was reduced approximately 59% in CA1, 55% in CA3, and 56% in DG. Comparisons across the three subregions revealed no significant difference in the amplitude of the slow EPSP induced by the stimulation at low intensity for any of the three age groups. At higher stimulation intensity, the amplitude of the slow EPSP in CA3 was significantly larger than in CA1 and DG in both
young (P<0.0069) and adult (P<0.0134) groups; however, there was no region difference
in the old rats (P>0.05).

6.2.2.2 Input Resistance Change During the Cholinergic Slow EPSP

Because input resistance was measured at the end of the recording session for each
cell, some cells were lost before it could be obtained. The input resistance change was
calculated as the percent change in the amplitude of the membrane deflections, induced by
direct negative current pulses, during the slow EPSP compared to that during baseline
(Fig. 6.5A). In CA1, the increase of input resistance during the slow EPSP, obtained
from twenty-seven cells of 23 rats (3 wk: n=6; 9 mo: n=7; 24-27 mo: n=10), was overall
significantly less in old rats (F(2,20)=9.629, P<0.0012; Fig. 6.6B). In CA3, the change
of input resistance during the slow EPSP obtained from thirty cells of 24 rats (3 wk,
n=10; 9 mo, n=7; 27 mo, n=7) reveals no significant difference between young and adult
groups; however, the increase in input resistance was significantly less in old rats when
compared with young rats (P<0.05; Fig. 6.5B). A significant correlation was found
between the amplitude of the slow EPSP and the increase in input resistance recorded from
the cells of young rats (R²=0.396, P<0.009, data not shown), but not in adult and old rats
(possibly due to the small number of cells). In DG, the input resistance change during the
slow EPSP was obtained from thirty-four cells of 21 animals (3 weeks: n=8; 9 months:
n=6; 27 months: n=7). Although the mean increase of input resistance was smaller in old
rats than in adult rats, and largest in youngest rats, none of these differences reached
statistical significance (3 wk and 27 mo: P<0.075; 9 mo and 27 mo: P<0.23, Fig. 6.5B).
There were, however, significant correlations between the amplitude of the slow EPSP
Figure 6.5 The age-related decrease in the percent change of input resistance during the slow cholinergic epsp. A: A representative waveform of the cholinergic slow EPSP, accompanied by an increase in input resistance (left). The change in input resistance can be more easily observed in the increased amplitude of the membrane potential deflection (magnified at right) during constant negative current pulses delivered through the recording pipette. The response to hyperpolarizing current shown in 1 is before the tetanic stimulation, in 2 is at the peak of the slow EPSP, and in 3 is after the membrane potential returned to baseline. B: Mean (± S. E. M.) of the percent increase of input resistance at the peak of the cholinergic slow EPSP recorded in CA1, CA3 and DG for young, adult and old rats. The change in input resistance was reduced in all subregions for the old rats, but did not reach statistical significance for DG (* P < 0.05; ** P < 0.01). C: Example of the correlations between the changes in input resistance and the amplitude of the cholinergic slow EPSP in DG of old rats (P < 0.0001).
Change of Input Resistance During Cholinergic Slow Epsp (%)

Amplitude of Cholinergic Slow Epsp (mV)

Input Resistance Change (%)
and the change in input resistance in adult ($R^2=0.641$, $P<0.0004$, data not shown) and old rats ($R^2=0.742$, $P<0.0001$; Fig. 6.5C).

6.2.2.3 Post-tetanic Hyperpolarization (PTH)

When stimulating stratum oriens in CA1 or CA3 with high frequency, a series of responses were elicited: 1) a postsynaptic excitatory response, 2) followed by a hyperpolarizing potential, and finally 3) the cholinergic slow EPSP (Fig. 7.1). The hyperpolarization response will be referred to here as post-tetanic hyperpolarization (PTH). The nature of the PTH is not completely clear. It may contain a feedback IPSP component (Cole and Nicoll, 1984) and a hyperpolarization mediated by a $\text{Ca}^{2+}$-dependent $K^+$ current (Alger et al., 1990), both of which are proportionally related to the size of the preceding excitatory response. In DG, the PTH was seldom observed, possibly due to the stimulating electrode location in the cell body layer, which might activate fewer excitatory synapses than the placements in CA1 and CA3. The area, maximal amplitude, and duration of the PTH were measured on all the responses included for the cholinergic slow EPSP comparison (Table 6.2). The amplitude and the area of the PTH were quite variable (Table 6.2), and there were no differences between age groups. As can be seen in Fig. 6.6A, the duration of the PTH recorded from old rats, however, was significantly longer than younger rats in both CA1 (400 μA: $F(2,25)=4.768$, $P<0.0176$; 700 μA: $F(2,31)=7.296$, $P<0.0025$) and CA3 (200 μA: $F(2,23)=5.309$, $P<0.0127$; 500 μA: $F(2,32)=5.55$, $P<0.0085$; Table 6.2). There was no significant difference in this measure between young and adult rats (Fig. 6.6A).

The prolongation of the PTH in old rats raised the serious possibility that the
Figure 6.6 A: Mean (± S. E. M.) of the duration of the post-tetanic hyperpolarization (PTH), observed in CA1 and CA3 for young, adult and old rats. The PTH was significantly prolonged in old rats (* P < 0.05; ** P < 0.01). B: The duration of the PTH recorded from the CA1 region of slices treated with the medium containing kynurenic acid (1mM) and picrotoxin (10 mM) was significantly suppressed, and not different in amplitude between age groups. C: The amplitude of the cholinergic slow EPSP (Means ± S. E. M.), is shown for each age group in the presence of kynurenic acid. In spite of the absence of a difference in the PTH under these conditions, the cholinergic slow EPSP was still significantly reduced in old rats following this treatment (** P<0.01).
reduced cholinergic slow EPSP observed in old rats could be due to the prolonged PTH, which might consequently dampen the cholinergic depolarization. This raised questions about the interpretations of a smaller slow EPSP in old rats both in the present experiment and in previous studies in CA1 (Potier et al., 1992). In order to test this hypothesis, the effect of age on the cholinergic slow EPSP was studied again in hippocampal slices which were incubated in a medium containing kynurenic acid (1 mM) and picrotoxin (50 μM). These agents block glutamatergic and fast GABAergic synaptic transmission, respectively, leading to a suppression of the PTH. The slices were prepared from another group of rats (3 wk, n=8; 9 mo, n=8; 24-28 mo, n=11), in which the old rats were severely impaired in the spatial version of the Morris water task, as measured by the corrected integrated pathlength ($F(2,69)=10.032, p<0.0007$) and the proximity to the target ($F(2,69)=11.466, p<0.0004$); however, there were no significant age differences in the cue version of the task after 12 trials of training ($F(2, 24) = 0.749, P<0.48$). Part of these behavioral results were reported in the Cue Learning part of the Results section, and are shown in Fig. 6.3C.

A total of 70 pyramidal cells (3 wk, n=18; 9 mo, n=24; 24-28 mo, n=28) were recorded in the CA1 region. Consistent with our previous data, there was no age effect on the basic biophysical parameters of these cells. The duration of the PTH induced by the 0.5 sec 50Hz stimulation on stratum oriens was greatly reduced, though not completely abolished by kynurenic acid and picrotoxin. Nevertheless, under this condition there were no statistically significant differences in the duration of the PTH between age groups ($P>0.05$, Fig. 6.6B). The amplitude of the cholinergic slow EPSP, however, was still significantly reduced in the aged rats at both stimulation intensities that were delivered (200 μA: $F(2, 24)=9.501, P<0.0009$; 500 μA: $F(2,24)=10.421, P<0.0010$; Fig. 6.6B), whereas there was no significant difference in the duration of the slow EPSP between
Table 6.2 Cholinergic slow epsp and post-tetanic hyperpolarization (PTH) in three age groups of rats (mean ± S.E.M.)

<table>
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<tr>
<th>Parameters</th>
<th>3 wk</th>
<th>9 mo</th>
<th>24-27 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stimulation (μA)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CA1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 slow epsp amplitude(mV)</td>
<td>4.92 ± 0.68</td>
<td>4.72 ± 1.26</td>
<td>2.05 ± 0.48*</td>
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<tr>
<td>slow epsp duration (sec)</td>
<td>34.1 ± 6.00</td>
<td>24.4 ± 4.37</td>
<td>26.5 ± 5.21</td>
</tr>
<tr>
<td>PTH area (arb. units)</td>
<td>3.58 ± 1.49</td>
<td>15.5 ± 7.19</td>
<td>19.1 ± 5.87</td>
</tr>
<tr>
<td>PTH amplitude (mV)</td>
<td>1.42 ± 0.55</td>
<td>4.63 ± 1.20*</td>
<td>5.26 ± 0.82**</td>
</tr>
<tr>
<td>PTH duration (sec)</td>
<td>2.42 ± 0.90</td>
<td>4.69 ± 1.47</td>
<td>8.31 ± 1.46*</td>
</tr>
<tr>
<td>600-800 slow epsp amplitude(mV)</td>
<td>8.37 ± 0.89</td>
<td>5.80 ± 0.60*</td>
<td>2.78 ± 0.57**</td>
</tr>
<tr>
<td>slow epsp duration (sec)</td>
<td>35.9 ± 8.41</td>
<td>33.3 ± 8.02</td>
<td>37.8 ± 9.00</td>
</tr>
<tr>
<td>PTH area (arb. units)</td>
<td>7.67 ± 2.84</td>
<td>21.3 ± 5.87*</td>
<td>25.3 ± 4.33**</td>
</tr>
<tr>
<td>PTH amplitude (mV)</td>
<td>2.07 ± 0.63</td>
<td>4.68 ± 1.36*</td>
<td>4.8 ± 0.60*</td>
</tr>
<tr>
<td>PTH duration (sec)</td>
<td>3.63 ± 1.20</td>
<td>5.10 ± 1.05</td>
<td>10.2 ± 1.51**</td>
</tr>
<tr>
<td><strong>CA3</strong></td>
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</tr>
<tr>
<td>200 slow epsp amplitude(mV)</td>
<td>6.50 ± 0.75</td>
<td>4.73 ± 0.64</td>
<td>2.76 ± 0.57***</td>
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<tr>
<td>slow epsp duration (sec)</td>
<td>28.4 ± 5.54</td>
<td>40.7 ± 2.59</td>
<td>19.1 ± 5.35**</td>
</tr>
<tr>
<td>PTH area (arb. units)</td>
<td>15.2 ± 3.74</td>
<td>24.9 ± 7.62</td>
<td>31.0 ± 4.69</td>
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<tr>
<td>PTH amplitude (mV)</td>
<td>5.76 ± 1.27</td>
<td>9.44 ± 2.25</td>
<td>7.63 ± 0.78</td>
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<td>PTH duration (sec)</td>
<td>3.36 ± 0.67</td>
<td>4.04 ± 0.83</td>
<td>7.05 ± 1.08**</td>
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<td>500 slow epsp amplitude(mV)</td>
<td>9.76 ± 1.25</td>
<td>8.10 ± 0.84</td>
<td>4.06 ± 0.80**</td>
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<td>slow epsp duration (sec)</td>
<td>39.1 ± 5.68</td>
<td>41.3 ± 7.51</td>
<td>37.1 ± 6.66</td>
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<tr>
<td>PTH area (arb. units)</td>
<td>24.1 ± 5.69</td>
<td>32.2 ± 7.79</td>
<td>50.3 ± 9.06*</td>
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<td>PTH amplitude (mV)</td>
<td>6.87 ± 1.14</td>
<td>8.12 ± 1.77</td>
<td>9.48 ± 1.52</td>
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<tr>
<td>PTH duration (sec)</td>
<td>4.56 ± 0.80</td>
<td>5.28 ± 1.01</td>
<td>9.20 ± 1.29**</td>
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<td><strong>DG</strong></td>
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<tr>
<td>300 slow epsp amplitude(mV)</td>
<td>5.09 ± 0.42</td>
<td>4.17 ± 0.44</td>
<td>2.02 ± 0.28**</td>
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<tr>
<td>slow epsp duration (sec)</td>
<td>44.5 ± 4.59</td>
<td>49.8 ± 6.73</td>
<td>27.6 ± 3.81**</td>
</tr>
<tr>
<td>600 slow epsp amplitude(mV)</td>
<td>6.26 ± 0.20</td>
<td>5.35 ± 0.39</td>
<td>2.55 ± 0.41***</td>
</tr>
<tr>
<td>slow epsp duration (sec)</td>
<td>60.1 ± 6.74</td>
<td>58.6 ± 4.78</td>
<td>32.6 ± 4.40***</td>
</tr>
</tbody>
</table>

Statistical comparison using 1-way ANOVA for each hippocampal subregion between young (3 wk) and adult (9 mo) rats: * P<0.05; and between younger (3 wk & 9 mo) and aged (24-27 mo) rats: * P<0.05, ** <0.01, *** P<0.001. arb. units = arbitrary units.
three age groups (P>0.05). Because these data are consistent with those obtained previously in CA1, without the blockade of glutamatergic and GABAergic synaptic transmission, the reduced cholinergic slow EPSP in old rats compared to younger rats cannot be attributed to the longer post-tetanic hyperpolarization.

6.2.3 Correlations Between Electrophysiology and Behavior

Initially four behavioral measures and one electrophysiological measure were chosen for the correlation analysis: the corrected integrated pathlength (CIPL 19-24), the proximity to the target of last day of training (AD 19-24), and search time in the target quadrant (TQ1) in the probe test for spatial memory; corrected integrated pathlength (CIPL trial 6) for cue acquisition; and finally, the amplitude of the cholinergic slow EPSP at the high intensity. Only one out of 36 possible correlations reached statistical significance with the above behavioral measures (Table 6.3). In CA1, the amplitude of the cholinergic slow EPSP in old rats was negatively correlated with the integrated pathlength (R=-0.665, P<0.0223). In addition, the amplitude of the slow EPSP in CA1 in both young and old rats were marginally significantly correlated with the search time in the target quadrant in the probe test (Table 6.3). The relationships between the duration of the PTH in CA1 and above behavioral measures in each age group have also been examined and no significant correlations were found (P>0.05).

Although the behavioral measures used above have been used to test for brain-behavior relationships in the past, we noted that there was significant variability in some rats' performance even in the last block of spatial training. Power estimates were therefore conducted to determine whether the n in the present study was sufficient to detect group differences, if they existed (Keppel, 1991). For CA1 of the old rats, the power estimate
Table 6.3 Correlations between each behavioral measure and the cholinergic slow EPSP Amplitude of Slow Epsp (mV)  

<table>
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<tr>
<th></th>
<th>CIPL 19-24</th>
<th>AD 19-24</th>
<th>Probe TQ</th>
<th>Cue CIPL6</th>
<th>Slope</th>
</tr>
</thead>
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<td></td>
</tr>
<tr>
<td>3 wk (11)</td>
<td>-0.212</td>
<td>-0.467</td>
<td>0.586*</td>
<td>-0.192</td>
<td>-0.096</td>
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<tr>
<td>9 mo (11)</td>
<td>0.173</td>
<td>0.082</td>
<td>-0.240</td>
<td>0.486</td>
<td>-0.083</td>
</tr>
<tr>
<td>24-27 mo (12)</td>
<td>-0.665</td>
<td>-0.044</td>
<td>0.568*</td>
<td>-0.109</td>
<td>-0.405</td>
</tr>
<tr>
<td><strong>CA3:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 wk (11)</td>
<td>0.186</td>
<td>0.113</td>
<td>-0.151</td>
<td>-0.151</td>
<td>-0.204</td>
</tr>
<tr>
<td>9 mo (11)</td>
<td>0.131</td>
<td>0.097</td>
<td>0.049</td>
<td>-0.140</td>
<td>0.670</td>
</tr>
<tr>
<td>27 mo (11)</td>
<td>0.193</td>
<td>0.144</td>
<td>0.296</td>
<td>0.154</td>
<td>-0.172</td>
</tr>
<tr>
<td><strong>DG:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 wk (9)</td>
<td>0.254</td>
<td>0.337</td>
<td>-0.294</td>
<td>0.469</td>
<td>-0.266</td>
</tr>
<tr>
<td>9 mo (10)</td>
<td>-0.029</td>
<td>-0.004</td>
<td>-0.317</td>
<td>-0.170</td>
<td>0.026</td>
</tr>
<tr>
<td>27 mo (12)</td>
<td>-0.177</td>
<td>0.048</td>
<td>-0.246</td>
<td>0.009</td>
<td>-0.343</td>
</tr>
</tbody>
</table>

*(1): p<0.0584, *(2): p<0.0539; Values underlined are statistically significant at < 0.05.

Abbreviations:

CIPL 19-24, average corrected integrated pathlength of trial 19-24;

AD 19-24, average proximity to the target of trial 19-24;

Probe TQ, search time in the target quadrant in the probe test;

Cue CIPL, the corrected integrated pathlength of trial 6 in the cue version of the water task;

Slope, the slope of the regression line between the natural log of trial CIPL and trial number for each rat.
suggests that the data are reliable (0.96); however, for the rats contributing to the data for CA3 and DG, the power was less than 0.3. This indicates that the variance of the last 6 training trials in those groups of rats was, indeed, so large that more rats would be required to detect the correlation if it exists (up to 108 according to the Pearson-Hartley power chart in Keppel, 1991). Because over 100 rats is not a realistic sample size for these kinds of electrophysiological experiments, we sought another representation of the behavioral data that resulted in less variability: the slope of the natural log of the integrated pathlength over all acquisition trials, for each rat. The assumption in this measure is that each rat's improvement over 24 trials on the spatial task can be described by an exponential function. To accomplish this, the natural log of the integrated pathlength of each trial for each rat was first calculated. A simple regression analysis was then performed, with the trial number as independent variable and the LN (integrated pathlength) as dependent variable. The ratio (F) between the residual variance with respect to the regression line and the variance in the data itself is 0.797±0.024 (to be significantly different at the 0.05 level, F has to be larger than 2.03), indicating an excellent fit of the regression lines to the data (Fig. 6.7A). As illustrated in Fig. 6.7B, the aged rats had a significantly smaller slope compared with adult and young rats (F(2,91) = 11.206, P<0.0001). This supports the conclusion that old rats did not improve over the 24 training trials as well as did the younger rats. Using this slope measure, no significant correlations were found between spatial behavioral performance and the cholinergic slow EPSP, except for the adult CA3 group (Table 6.3). This positive correlation, however, implies that a larger slow EPSP may be associated with poorer behavioral performance. Because the large number of correlation analyses performed, it is likely that this result is spurious.
Figure 6.7 A. Examples of simple regression analyses on natural logs of the integrated pathlength vs. training trial numbers in the spatial version of the water task for one young, one adult and one old rat.

B. Mean (± S. E. M.) of the slope of regression lines for three age groups of rats. The slope for old rats is significantly smaller than those of young and adult rats (*** P<0.001).
Taken together, the pattern of these data do not support the idea that the alterations in the cholinergic system alone are responsible for the spatial behavioral deficits observed.

6.3 Conclusions

The present data demonstrate that the amplitude of the cholinergic slow EPSP induced in the CA1 region of the hippocampus is severely reduced in aged rats when compared to young and adult rats, which is consistent with previous results (Potier et al., 1992). In addition, an age-related decrease in the slow EPSP was also apparent following the blockade of glutamatergic and GABAergic transmission. This rules out the possibility that the previously described age difference in CA1 was an artifactual result arising from the interaction of high-frequency stimulation-related events (e.g., postsynaptic excitatory and inhibitory responses) with the expression of the slow EPSP. The present data also demonstrate a similar proportional decrease (about 50%) in functional cholinergic transmission in hippocampal regions CA3 and DG. Because the basic biophysical properties of cells in all hippocampal subfields of the in vitro slice preparation were equivalent between the three age groups, the specific alteration in the cholinergic response is not likely to be due to differential cell quality between groups. The slow EPSP is mediated by the blockade of a type of leak K+ channel (Madison et al., 1987), which results in an increase in cell input resistance. This increase in input resistance was also reduced in the old rats, along with the smaller amplitude of the slow EPSP. Taken together, these data suggest that there is a region-independent deterioration of cholinergic synaptic transmission in the senescent hippocampus. Furthermore, at higher stimulation intensities, the slow EPSP in the youngest rats tended to be even larger than in the adults, implying that cholinergic synaptic transmission may decline slowly over the lifespan.
The contributions made by age-related changes in pre- and post-synaptic mechanisms to the deficit in the cholinergic slow EPSP remain unclear. Responsiveness of CA1 neurons to ACh or muscarinic agonists is decreased at old ages either in *in vitro* (Lippa et al., 1981; Lippa et al., 1985; Lippa et al., 1980; Potier et al., 1992; Segal, 1982; Taylor and Griffith, 1993) or *in vivo* anesthetized preparations (Lippa et al., 1981). Because muscarinic receptor density undergoes little or no change in old animals, it has been difficult to argue that receptor number accounts for age-related decreases in neuronal sensitivity to ACh (Decker, 1987; Lippa et al., 1981; Lippa et al., 1985); however, it remains possible that structural changes in the receptor molecule may alter the functional response (Lippa et al., 1985). M3 and M1 subtypes of muscarinic receptors contribute to the cholinergic slow EPSP (Muller and Misgeld, 1986), and are known to be coupled to the IP3 second messenger system. However, no clear age-related changes in IP3 turnover in aged hippocampus have been detected to date (Andson et al., 1992; Burnett et al., 1990; Surichamorn et al., 1989). Furthermore, presynaptic cholinergic terminals in the hippocampus undergo negligible changes as indicated by ChAT and AChE assays (for review, (Decker, 1987), and basal release of ACh is not changed (Damsma et al., 1987; Meyer et al., 1984). On the other hand, depolarization-induced ACh release, either by electrical stimulation (Pedata et al., 1985; Sastry et al., 1983), or high K+ (Meyer et al., 1984; Takei et al., 1989), does decrease in the aged cortex and hippocampus. Thus, the question of pre- versus post-synaptic locus of the age-related deficit in the cholinergic slow EPSP remains open. It is possible that tetanic stimulation at a given intensity may activate fewer cholinergic fibers in the hippocampus of old rats compared to young rats, which may have caused the age-related decrease in the slow EPSP observed in the present study. Although this possibility cannot be completely ruled out, the available evidence does not support the idea that this is the major factor contributing to the age-related deficit
in the slow EPSP. First, the age-related decrease in the amplitude of the slow EPSP occurred not only when moderate stimulation intensities were used, but also when maximal stimulation intensities were delivered. In the latter case, all the fibers were presumably activated. Second, pharmacological applications of muscarinic agonists have consistently induced smaller post-synaptic responses in the aged hippocampus (Lippa et al., 1981; Lippa et al., 1985; Lippa et al., 1980; Potier et al., 1992; Segal, 1982; Taylor and Griffith, 1993). This indicates that the age-related decrease in cholinergic synaptic transmission is, at least partially, due to a post-synaptic deficit. Furthermore, presynaptic release of ACh elicited by high K⁺ has also been found to be significantly reduced in the old hippocampus (Meyer et al., 1984; Takei et al., 1989). The high K⁺ solution should lead to an equal depolarization of cholinergic fibers in both young and old tissues. Finally, it has been observed that there is no age difference in the amplitude of the presynaptic fiber potential in hippocampal CA1 during tetanic stimulation at 200 Hz (personal communication, E. Rosenzweig). This indicates that, at least for glutamatergic afferents, the same number of axonal fibers are activated during high frequency stimulation in both young and old rats.

The spatial learning deficits observed in the present study are consistent with many other experiments that have shown changes in the ability of aged rats to utilize or retain spatial information (Gage et al., 1984a). On the cue version of the water task, however, many of the aged rats in this study took a significantly longer pathlength to find the visible platform in the first 6 trials of training, compared to younger rats. Although the steepness of the learning curve suggests that they were improving, another possible explanation is that the old rats have a visual deficit, which might also contribute to their performance impairments in the spatial task. The main argument against this possibility is that the old
rats did eventually reach equivalent performance levels compared to young and adult rats, when given 6 additional trials on the cue task. Furthermore, there was not a significant correlation between the old rats' performance on the spatial and the cue versions of the task. Such a correlation would be expected if the common factor primarily responsible for these deficits was vision. Although none of the old rats in the present study had cataracts, some contribution to the deficit from visual defects cannot be ruled out. Other possibilities for the slowness of old rats to learn the cue task have also been suggested, including adoption of ineffective strategies for task solution, such as swimming against the side wall (Decker et al., 1988; Rapp et al., 1987).

Our correlation analyses did not reveal consistent significant relationships between the amplitude of the slow EPSP and performance on the spatial or cue version of the water task. This suggests that an age-related deficit of cholinergic function in the hippocampus does not, at least in isolation, account for the behavioral impairments of old rats on the Morris water task. The exact role of the cholinergic system in learning, memory and dementia is a current topic of debate (see review: Fibiger, 1991 #127), at least in part because it has only recently been possible to destroy cholinergic cells selectively. The hypothesis most consistent with recent data suggests that the cholinergic system may not be necessary for learning and memory per se. Rather, it may be involved in attention and arousal, which profoundly modulate cognition (for reviews see: Dunnett et al., 1991). In fact, selective destruction of cholinergic cells in the medial septum alone has not been found to affect spatial acquisition in the Morris water task (Baxter et al., 1995). An important observation, however, is the fact that while partial depletion of brain serotonin or ACh individually may not affect spatial behavior in the water task, the combined treatments do (Nilsson et al., 1988; Richter-Levin and Segal, 1989). One parsimonious
explanation that arises from these data is that an interplay between different subcortical neurochemical systems may exist for the normal operation of the hippocampus and cortex. In young rats, when the cholinergic septal input is damaged, other subcortical inputs may act in a compensatory way to maintain normal behaviors. In old animals, age-related deficits occur in multiple subcortical systems. This may result in reduced flexibility and loss of the capacity of one system to compensate for another. Consistent with this idea, serotonergic deficits have been found in the aged brain (Baskys et al., 1987; Marcusso et al., 1984), and may, in conjunction with the functional cholinergic change, contribute to observed age-related behavioral deficits (Levkovitz et al., 1994; Richter-Levin and Segal, 1993). This hypothesis of a necessary conjunction of neurotransmitter defects before cognitive changes arise may also explain why it is sometimes difficult to detect correlations between strictly cholinergic measures and behavior.
CHAPTER 7 DIFFERENTIAL EFFECTS OF SELECTIVE IMMUNOTOXIC LESIONS OF MEDIAL SEPTAL CHOLINERGIC CELLS ON SPATIAL WORKING AND REFERENCE MEMORY

The projection from the medial septum is one of the major inputs to the hippocampus, and damage to the medial septum can mimic the behavioral effects observed following damage to the hippocampus. For example, rats with medial septal damage are impaired on T-maze alternation (Rawlins and Olton, 1982), performance on the spatial version of the Morris swim task (Hagan et al., 1988), and reference and working memory measures of performance in the radial eight-arm maze (Crutcher et al., 1983). Thus, it appears that the integrity of the septo-hippocampal projection is necessary for the normal operation of the hippocampus. It is well known that a substantial component of this projection is cholinergic. Until recently, it had been thought likely that these lesion effects were due to the disruption of cholinergic cells in the medial septum. Alternate interpretations are also possible, however, due to the lack of specificity of older lesion methods, and the fact that at least some of the deficits observed may be caused by damage to GABAergic cells in the medial septal area, which project intrinsically as well as to the hippocampus (Freund and Antal, 1988).

A neurotoxin 192 IgG-saporin targeting at low-affinity NGF receptor has been recently developed (Wiley, 1992), which selectively kills the cholinergic cells in the basal forebrain but spares other types of neurons in the same region, because only cholinergic neurons bear low-affinity NGF receptors (see Chapter 3). This is indeed a useful tool to examine explicitly the septal cholinergic projection to the hippocampus, and to assess its effect on spatial learning and on the modulation of hippocampal neural activity. The
present study, therefore, was designed to explore the effects of selective deprivation of cholinergic input to the hippocampus, using this neurotoxic lesion, on forms of memory that can be independently assessed on the radial maze (i.e., spatial working and reference memory).

7.1 Methods

7.1.1 Subjects

13 male Fischer-344 rats (9 to 14-month-old retired breeders) were obtained from Charles River Laboratories. Following 1 week of adaptation to the laboratory, the rats were weighed and handled daily for 3 days. Access to food was then restricted, and, for the remainder of the experiment, the rats were maintained at about 80% of their ad libitum body weights. Water was readily available.

7.1.2 Behavioral testing apparatus

A semi-automated black Plexiglas 8-arm radial maze (Olton & Samuelson, 1976) was used to test the behavioral performance of the rats. Eight alleys, or arms (58 x 5.5 cm), radiated from a round central platform (19.5 cm diameter) that was elevated 79 cm above the floor. The arms were bordered by edges raised 4 mm above the surface. Aluminum food cups were located at the distal ends of the arms. Each arm was hinged such that the proximal half could either be raised so that it was flush with the central platform or lowered to restrict access to that arm from the center. Presentation of individual arms was accomplished by remote control. The maze was placed in the center of a 12 x 12 ft room illuminated evenly by 4 dim lights on the ceiling. Two white
curtains, 4 posters, and several wooden behavioral apparati leaning against the wall could serve as distal spatial cues.

7.1.3 Behavioral testing procedures

7.1.3.1 Working memory task.

The training procedure consisted of two stages. In the first stage, the rat was placed on the central platform of the maze without access to any of the arms at the beginning of each trial. Four randomly chosen arms were simultaneously presented. Immediately after the food (chocolate milk) was retrieved from each of the four arms, all 8 arms were presented. The rat was allowed to complete the trial by selecting those arms not entered before. Ten trials were given to each rat on each day. After the rat's performance reached a level with less than 1 error/trial on average, the second training stage began. On some trials, a delay of 1 or 2 min duration was imposed between the choice of the 4th arm and access to all 8 arms. During the delay period, the rat was confined to the center platform without access to any arms. Each rat was given 12 trials on each day, 4 trials with delays of 0, 1 or 2 min, respectively. The procedure for the trial with 0-min delay was the same as that in the first stage of training. Delays during the 12 trials were delivered randomly. When the rats' performance reached an asymptotic level, they were tested for 12 consecutive days further. Surgery was then performed. After a 10-day postoperative recovery, the rats were tested using the same procedures and delays as those given before the surgery for another 2 weeks. Entry into an arm that the rat had visited before was counted as an error. Pre-delay and post-delay errors were analyzed separately.
7.1.3.2 Reference memory task.

After all the rats completed testing on the first problem, training began using a new procedure. The apparatus remained the same as in the working memory task. At the beginning of each trial, the foodcups on all arms except 2 and 5 were filled with chocolate milk, and the rat was placed on the central stage of the maze without access to any of the arms. All 8 arms were then simultaneously presented. Arms 2 and 5 remained at the same location. The trial was ended after the rat visited all baited arms. Fifteen trials were given to each rat on each of 7 consecutive training days.

7.1.4 Surgery

Each rat was randomly assigned to either control (n=5) or lesion (n=8) groups. Lesion and control surgeries were identical in every respect, except that sterile phosphate-buffered saline (0.5 µl) was microinjected into control rats, whereas 192 IgG-saporin was microinjected into the lesion rats at a concentration of 0.2 µg/µl of sterile phosphate-buffered saline (0.7 µl). All surgical procedures were conducted under pentobarbital (Nembutal, 33 mg/kg, i.p.) anesthesia following N. I. H. guidelines for rats. Supplements of 0.04 ml were administered as necessary. The anesthetized rat was placed in a stereotaxic apparatus, and an incision was made to expose the skull. One hole was drilled in the skull at stereotaxic coordinates A-P 0.7 mm anterior to bregma, L 0.0 mm. The injection was made at a depth of DV = -5.5 from brain surface. A 28-gauge Hamilton syringe filled with either sterile phosphate-buffered saline or 192 IgG-saporin (gift of Dr. Ronald G. Wiley, Vanderbilt University, Tennessee) was then lowered into the desired location. Saline or immunotoxin was then slowly injected over a 3-minute period. The syringe was left in place for another 3 minutes to limit diffusion up the needle track. After
the surgery, the incision was cleaned and closed with silk surgical suture. Ten ml of sterile isotonic saline was injected subcutaneously to prevent dehydration during recovery, and bicillin (0.5 ml, 300,000U/ml) was injected intramuscularly to prevent infection. Each rat was also given diluted Tylenol (50 mg/kg cherry flavored) to drink for 24 hr after surgery to lessen postoperative pain.

7.1.5 Neurochemistry

After behavioral testing was completed, each rat was anesthetized with metofane and sacrificed by decapitation. The brain was removed and rapidly dissected on ice. The entire hippocampus was removed bilaterally and stored at -70°C. Tissue samples from the frontolateral sensorimotor and parietal-occipital cortices of 6 rats were also taken bilaterally and stored at -70°C.

Choline acetyltransferase (ChAT) activity was measured by the formation of [¹⁴C]-acetylcholine from [¹⁴C]-acetyl-coenzyme-A and choline, according to the method of Fonnum (1969). Protein content of the homogenate was determined by the method of Lowry et al. (1951), with bovine serum albumin as the protein standard.

7.2 Results

7.2.1 ChAT activity

Five of the rats that were injected with immunotoxin (n=8) had significantly decreased hippocampal ChAT activity (nmol/hr/mg protein) compared to the rats that were injected with sterile phosphate saline (n=5, 32.1% ± 2.9 of control; F(1,8) = 12.379, P < 0.0078). The ChAT activity of the other 3 rats was in the range of that of control rats (97% ± 8.6 of control; F(1,6) = 0.172, P < 0.69). For the ChAT activity in frontolateral
sensorimotor and parietal-occipital cortices, there were no apparent differences between rats injected with immunotoxin and those injected with sterile phosphate saline. Hence, the 5 rats with significant decreases in hippocampal ChAT activity were counted as the lesion group for subsequent behavioral analysis. The other 3 rats were grouped with 5 control rats for the control group. There was no significant difference in any of the behavioral measurements between the 3 rats that were added to the control group and the original 5 control rats (P > 0.35).

7.2.2 Behavioral Testing

7.2.2.1 Working memory task

The number of working memory errors during the first 4 choices (pre-delay phase) were averaged over blocks of 3 consecutive testing days, which included 12 trials per day. There were 4 blocks before and after the surgery, respectively. Before surgery, there was neither an effect of treatment, F(1,44) = 0.859, P < 0.3589, nor of block, F(3,44) = 1.275, P < 0.8050. After the surgery, however, there was a significant effect of treatment on the rats' performance, F(1,44) = 8.428, P < 0.0058, but no effect of block, F(3,44) = 1.147, P < 0.3408. This indicates a significant difference in pre-delay working memory errors between the control and lesion groups after the surgery, as shown in Fig. 7.1A.

For the analysis of post-delay working memory errors, the average number of errors per trial was computed as a function of delay. A three-way ANOVA, with variables of delay (0, 1, 2 min), treatment (control or lesion) and block (1-4; 1 block per 3 days), was performed on the data before and after surgery, respectively. Before surgery, there was only a significant effect of delay, F(2,132) = 337.437, P < 0.0001, but no effect of either treatment group or block (Fig. 7.1B). Post hoc Fisher's PLSD analysis revealed
Figure 7.1 Effect of selective immunotoxic cholinergic lesions in medial septum on the performance of the rats in a working memory task on the radial 8-arm maze. 

A: Errors averaged from 4 blocks (1 block per 3 consecutive testing days) before and 4 blocks after surgery show that lesioned rats made significantly more working memory errors compared to the control rats on the first 4 arm choices.

B: Mean errors of rats (averaged over blocks) in both treatment groups before surgery following 0, 1 or 2 minutes delays. There was a delay-dependent effect on performance, and no difference between treatment groups.

C: After surgery, lesioned rats made more errors at all delays, and the deficit was not delay dependent. Error bars are one standard error of the mean.
significant performance difference between all delay conditions (all \( P < 0.0001 \)), confirming that performance of this task is delay-dependent. After the surgery, there was a significant effect of delay, \( F(2,132) = 143.129, P < 0.0001 \), and treatment, \( F(1, 132) = 32.565, P < 0.0001 \), but no effect of blocks. The interaction between treatment and delay, however, was not significant (\( F(2,132) = 0.640, P < 0.5287 \)), suggesting that the lesion effect on the task is not delay-dependent. Additional one-way ANOVA analysis conducted on each delay, with 4 blocks of data used as repeated measures, revealed a significant effect of lesion at zero delay (\( F(1,33) = 9.393, P < 0.0108 \)). At delays of 1-min and 2-min, the lesion groups tended to make more errors compared to the control group, although the difference did not reach statistical significance (1-min-delay, \( F(1,33) = 3.942, P < 0.0726 \); 2-min-delay, \( F(1,33) = 3.619, P < 0.0836 \)). The data averaged from 4 blocks for each group are shown in Fig. 7.1C.

### 7.2.2.2 Reference memory task

Entry into the nonbaited arms (2 or 5) was counted as an error. The number of errors the rat made during 15 trials on each day of 7 testing days was used for a one-way ANOVA with 7 repeated measures. There was no significant difference between treatment groups (\( F(1,66) = 1.369, P < 0.2667 \)). As shown in Fig. 7.2A, the two groups of rats appear to learn to avoid arms 2 and 5 equally well over 7 days of training.

The performance of the rats within each training day was also examined. As shown in Fig. 7.2B, both control and lesion groups made more errors in the first 5 trials on a given day, than on the last 10 trials, but there were no difference between groups.
Figure 7.2 Performance of control and cholinergic lesioned rats in the radial-8-arm maze reference memory task in which the same two arms were always unbaited. A: Data are the mean of total errors made on the unbaited arms in 15 trials; error bars are one standard error of the mean. B: Data are normalized errors (ratio between the error of each trial, and the total error of that day) averaged over 7 days. The rats made the most errors on the first 5 trials of the day (1-5), and fewer errors on subsequent trials (6-10, 11-15). Note that the pattern of errors is similar between treatment groups.

7.3 Conclusions

The present study examined the effects of selective removal of cholinergic input to the hippocampus on two forms of spatial learning and memory which were assessed on the same radial 8-arm maze apparatus. The immunolesion significantly impaired performance on the working memory version of the task. The lesioned rats made more errors during the pre-delay choices among 4 arms, and on post-delay choices among all 8
arms. This deficit, however, was not delay-dependent (Fig. 7.1C), i.e., the relative
deficit did not become worse with increasing delay. This is consistent with the conclusion
that the deficit is one of acquisition rather than retention. In contrast, the same lesions did
not affect performance on the reference memory task in which two arms were never baited
(Fig. 7.2A). This result is consistent with those of previous studies in which selective
cholinergic lesions have not produced spatial reference memory problems in the Morris
swim task (Berger-Sweeney et al., 1994; Baxter et al., 1995). The data from this
reference memory task also suggest that it is unlikely that the deficit observed in the
working memory version of the radial maze problem in the present study arose because of
general performance deficits.

Taken together, these results indicate that the cholinergic input from the medial
septum plays a role during acquisition of trial-specific information in the spatial working
memory situation, but is not necessary for encoding relevant spatial information that is
unchanging from trial to trial. This distinction has some similarity to that which has been
drawn between fact and event memory in humans (e.g., Squire, 1992), both of which are
impaired by damage to the medial temporal lobe. A number of recent reports have
suggested that lesions of either the cholinergic inputs to the neocortex or to the
hippocampus may result in deficits that are linked more to attentional processes than to
learning and memory (Baxter et al., 1995). The present data are consistent with this
general idea, if one assumes that attention affects the ability to enter items into working
memory. It is inconsistent with the attentional explanation, however, if by attention
deficit we mean susceptibility to distraction-induced forgetting once an item has been
initially stored in working memory. This would have predicted a relative decrease in
performance as a function of delay, which was not observed. A resolution to this apparent
discrepancy may reside in the idea that only specific types of attentional mechanisms are affected by removal of the cholinergic system (Chiba et al., 1995). On the other hand, a sufficient characterization of the present results is that the cholinergic septo-hippocampal projection can facilitate the temporary acquisition of location information, but does not play a role in longer lasting retention of place-reward associations.

Although various markers for cholinergic function are known to be altered in old animals (e.g., Decker et al., 1988), it remains controversial how this dysfunction relates to cognitive deficits (Gallagher et al., 1994). The results in Chapter 6, however, indicated that age-related declines in electrophysiologically-measured functional cholinergic transmission (in CA1, CA3 and fascia dentata), did not correlate with spatial behavioral deficits in old rats as measured on the Morris swim task (Shen & Barnes, 1996). These data are, in fact, consistent with the demonstration that selective lesions of cholinergic cells in medial septum do not result in behavioral deficits in the Morris pool (Berger-Sweeney et al., 1994; Baxter et al., 1995), or on the reference memory version of the 8-arm maze.

The present results may also be consistent with the observations that old animals exhibit delay-independent working memory impairments on the radial 8-arm maze (Chrobak et al., 1995).

Given the relatively mild effect of the septo-hippocampal cholinergic system damage on spatial working memory task, it will be of interest to understand why nonspecific lesions of the medial septum lead to robust spatial working and reference memory deficits. In this regard, it will be important to understand the role played by GABAergic projection cells from the medial septum on the computational operations of the hippocampus.
CHAPTER 8 THE EFFECT OF AGING ON PHASE PRECESSION AND PLASTICITY OF HIPPOCAMPAL PLACE CELLS

As reviewed in Chapter 4, there are three prominent aspects of hippocampal physiology, which have attracted interest with respect to their possible roles in spatial learning, and whose change with age could contribute to age-associated memory impairment: place-specific firing (O'Keefe and Dostrovsky, 1971) of single neurons ("place cells"); the 7-12 Hz theta rhythm in the EEG (Green and Arduini, 1954), which is tightly related to "spatial" behaviors (e.g. walking, rearing) (Vanderwolf et al., 1975), and the long-lasting change in synaptic efficacy that can be induced by patterned activation of hippocampal afferents (i.e., long-term potentiation [LTP]) (Bliss and Lømo, 1973). O'Keefe and Recce (1993) recently found that single cell firing advances gradually in phase as the rat passes through the place field (see Fig. 4.5). Thus, the effect of age on the theta rhythm, place specific firing, as well as the phase precession are the focus of the present study. Furthermore, Mehta et al. (1996) have demonstrated a rapid, experience-dependent expansion of place fields in young rats during performance of a repeated route following task. This is a phenomenon likely to depend on an LTP-like mechanism. Thus, it was also of interest to determine whether this process is altered by age, which may provide more evidence on age-related alterations in LTP-like mechanisms in natural behaviors.
8.1 Methods

8.1.1 Animals and behavioral training

Six pairs of young (11-12 mo) and old (25-31 mo) male Fischer 344 rats, obtained from the National Institute on Aging (NIA) colony at Harlan Sprague-Dawley, were used in this experiment (see Table 8.1). Rats were delivered in pairs, housed individually in Plexiglas guinea pig tubs, and maintained on a 12/12-hour light-dark cycle. They were handled for a minimum of 5 min per day for 3 days before entering behavioral testing. All rats underwent testing of their spatial and visual discrimination abilities on the Morris water task (details as in Shen and Barnes, 1996). Thereafter, they were food-deprived and maintained at approximately 85% of their ad libitum body weights. Subsequent behavioral training consisted of several steps. For the first 2-3 days, 1 young and 1 old rat were trained to forage for randomly scattered chocolate cake sprinkles in a 62 x 70 cm box for 60 min per day. They were then trained on a linear track (122 x 14 cm). For the first 3 days on the track, rats were allowed to forage for scattered chocolate sprinkles for about 90 min. During the subsequent week, chocolate food reward was only offered alternatively at the two ends of the track. Finally, the rats were first placed into a "nest" in the training room to rest quietly or sleep for 1 hr, then were transferred onto the track and allowed to obtain food reward at the two ends for 30 min. This was followed by another session in the nest in which they slept for another hour. Implantation of electrodes occurred at the end of this phase of training.

8.1.2 Surgery and construction of electrodes

The construction of the electrode assembly ("hyperdrive") has been described in detail elsewhere (Gothard et al., 1996b). Briefly, the electrode array consisted of 14
Table 8.1 Age of rats during recording sessions, and total number of sessions conducted and included in the present study.

<table>
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<th>Sessions</th>
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<td>1b</td>
<td>12</td>
<td>2</td>
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</tr>
<tr>
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<td>Young</td>
<td>2b</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
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<td>Old</td>
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<td>29.5</td>
<td>3</td>
</tr>
<tr>
<td>5397</td>
<td>Young</td>
<td>3b</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>5408</td>
<td>Old</td>
<td>4a</td>
<td>30.5</td>
<td>3</td>
</tr>
<tr>
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<td>Young</td>
<td>4b</td>
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<td>6</td>
</tr>
<tr>
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<td>5a</td>
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<td>4</td>
</tr>
<tr>
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<td>Young</td>
<td>5b</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>5697</td>
<td>Old</td>
<td>6a</td>
<td>26.5</td>
<td>6</td>
</tr>
<tr>
<td>5672</td>
<td>Young</td>
<td>6b</td>
<td>11.5</td>
<td>4</td>
</tr>
</tbody>
</table>

* Two sessions were actually conducted, but the data from one session were corrupted due to a computer malfunction.
independently movable "tetrodes", twelve of which were used for unit recording, one as reference for differential recordings, and the other specifically for recording EEG. Each tetrode consisted of four twisted polyimide-coated nichrome wires (13 μm). The tips were gold-plated, resulting in impedances at 1 kHz in the range of 300-600 kΩ. Each tetrode was inserted into two nested plastic cannula (78 and 110 μm in diameter respectively), which were themselves inserted into a 30-gauge stainless steel cannula. The stainless steel cannula was bent at a 30 ° angle near the end, so that the tips of all the tetrodes were placed within an area less than 2 mm across, while the other ends fanned out into a cone. Each tetrode was attached to a metal screw, one turn of which equaled approximately 320 mm in depth. Each tetrode wire was attached to a contact point on a multiple-pin connector plug.

NIH guidelines were followed for all surgical procedures used for electrode implantation. Briefly, rats were deeply anesthetized with Nembutal (sodium pentobarbital), and placed in a stereotaxic apparatus. The skull was cleared of skin, and 7-8 holes were drilled to accommodate jeweler’s screws to anchor the implant. A circular hole was drilled over the dorsal hippocampus on the right side of the brain, at coordinates of approximately 3.8 mm posterior to bregma and 2.0 mm lateral to the midline, into which the independently movable, 14 tetrode electrode array (the “hyperdrive”) was positioned and cemented in place with dental acrylic. Immediately after the surgery, the 12 tetrodes used for recording single units were advanced one full turn (320 μm), and the tetrode used for recording EEG and the reference tetrode were advanced further into the brain towards their final target areas.
8.1.3 Recording techniques and procedures

The connector plug on the hyperdrive device was attached to a custom-built headstage containing two microchips (Multichannel Concepts, Inc., Gaithersburg, MD), each with 25 unity-gain FET amplifiers, for impedance reduction. The signals from the headstage were carried by a multi-wire cable to a set of seven, rack-mounted, eight-channel, software-controlled amplifiers (Neurolynx Corp., Tucson, AZ), and then to a group of seven, 80486-based microcomputers. Signals were amplified 3000 - 10,000 times, depending on signal size, and were filtered with a bandpass of 600 - 6000 Hz before being sent to A/D cards (Data Translation, Inc., Marlboro, MA) on the microcomputers. Data were acquired using Discovery software (Data Wave Inc., Broomfield, CO). Whenever the amplitude of the spike signal exceeded a predetermined threshold, each tetrode channel acquired a one-millisecond sample of data at a rate of 32 KHz. These spike samples were time-stamped and stored on hard disk. Additionally, signals from one channel of each tetrode were sent to another eight-channel amplifier, amplified by a factor of 2000 and filtered with a bandpass of 1 - 100 Hz. These additional channels were used to record EEG continuously at 200 Hz, for storage on hard disk.

For tracking the movement of the animals on the behavioral apparatus, two small arrays of infrared LEDs were attached to the headstage, one extending to the front, the other to the back of the rat. Position data were acquired by a tracking device (Dragon Tracker model SA-2, Boulder, CO), which extracted and stored the coordinates of the front and back diode arrays at a resolution of 256 x 256 pixels (2.3 pixels/cm for the current experiment). This information was sent to the master microcomputer at 20 Hz and recorded on disk. The data were later reprocessed off-line, with custom-written software on a Sun workstation to correct or delete misidentified points.
At the end of the daily recording session, the data were transferred from the seven microcomputers to a Sun workstation for further processing steps, including unit identification and isolation. The principle of tetrode recording technique was proposed by McNaughton et al. (1983b) as an extension of the stereotrode recording method, and has subsequently been described in detail (Gothard et al., 1996b). Briefly, the tips of 4 micro-wires of a tetrode are so closely spaced that most cells recorded by any of the wires will simultaneously be recorded by one or more of the other wires, typically at a different amplitude. The units, therefore, were isolated by displaying all orthogonal 2-D projections of the 4-channel relative amplitude data, and applying boundaries to each apparent unit cluster by the use of a custom interactive program running on a Sun workstation.

After the rat had recovered from surgery, the reference electrode was lowered into the corpus callosum (about 1400 - 1600 µm under the dura), and the EEG electrode into the vicinity of the hippocampal fissure to optimize recording of the theta rhythm. The other twelve tetrodes, however, were gradually lowered, over the course of a number of days, into the CA1 cell body layer of the dorsal hippocampus. During this recording optimization period, the rat was trained to run in a single direction (clockwise) on a rectangular track (94 cm x 40 cm; 6 cm in width) in the recording room for at least 20 laps. The arrival of each tetrode into the hippocampus was recognized by several criteria, including the presence of 100-300 Hz "ripples" in the EEG (Buzsáki et al., 1992), the polarity of "sharp waves" in the EEG (Buzsáki et al., 1986; Suzuki and Smith, 1987) and the sudden appearance, at a depth of about 2 mm below the dura, of large numbers of simultaneously recorded cells with complex spike discharges as rats were in a quiet waking state. Each rat underwent 2-6 recording sessions. The fewer cells recorded per
session, the more sessions were conducted, in order to get approximately equal cell sampling from each rat. Each session consisted of three phases. First, the rat was placed in a "nest" on top of the rectangular track, and was allowed to sleep for about 30 - 90 min, during which recording was conducted to determine overall cell numbers. Because most cells are active during sleep, whereas many cells are silent when the rat is active in a given environment (Thompson and Best, 1989), the proportion of detectable cells with place fields on the maze could be determined using this procedure. In the second phase of the session, the rat was placed onto the rectangular track and allowed to run in the clockwise direction for 20-35 laps. Food reward was provided at 2 corners of the track. In the final phase, the rat was placed back into the nest to sleep again for another 30 - 90 min, during which time the activity of single units was also recorded.

8.1.4 Data analysis

8.1.4.1 Identification of units

Pyramidal cells in the CA1 region of the hippocampus were distinguished from theta cells using standard criteria (Buzsáki et al., 1983). The classification criteria for inclusion into the pyramidal cell category was that the cell must fire at least a small number of complex spike bursts during the recording session, be recorded simultaneously with other complex spike cells (in the stratum pyramidale), have a spike width (peak to valley) of at least 300 μsec, and an overall mean rate below 5 Hz during the behavioral recording session. To be included into the theta cell category, on the other hand, the cell would be required to fire no complex spike bursts, have a spike width of less than 300 μsec and a mean firing rate above 5 Hz during the behavioral recording session.
8.1.4.2 Quantification of theta rhythm

The EEG data were digitally filtered into the 6 - 10 Hz band. For behavioral episodes, all EEG was analyzed for periods when the animal’s running velocity exceeded a predetermined threshold (6 cm/sec), which ensured a reliable separation of theta and non-theta EEG. For REM sleep, the presence of theta was determined by inspection (see Results). In practice, there was no difficulty in separating theta from non-theta EEG and there was high inter-observer reliability. For the analysis of age effects on theta rhythm, the instantaneous velocity, theta frequency and theta amplitude were determined every 0.2 seconds. The instantaneous frequency was defined as the inverse of the time between successive peaks of the filtered EEG. The instantaneous amplitude was computed as the height of the initial peak of each cycle to the baseline. For the calculation of velocity, the distance between the 2nd preceding position point, and the 2nd following position point (sampled at 20 Hz), was divided by the intervening time.

8.1.4.3 Spatial firing measures

Field size, field rate integral and spatial information per second were used to assess spatial firing (Skaggs et al., 1993). For purposes of data analysis, the rectangular maze was "linearized" by projecting each point on the rat’s trajectory onto the nearest point on a line traveling down the center of each arm of the track. This line was divided into 64 equal-length bins; the total occupancy time and total number of spikes were computed for each bin. The spatial information per second was computed according to:

\[
\text{Information/sec} = \sum_{i=1}^{N} p_i \lambda_i \log_2 \frac{\lambda_i}{\lambda}
\]  

(1)
Where the environment is divided into non-overlapping spatial bins $i = 1, \ldots, N$, $p_i$ is the occupancy probability of bin $i$, $\lambda_i$ is the mean firing rate for bin $i$, and $\lambda$ is the overall mean firing rate of the cell. This equation was derived by considering the cell as a communication channel whose input is the rat’s location and whose output is the cell’s spike train. Information per second is a measure of the robustness of spatial firing (Skaggs et al., 1993).

8.1.4.4 Phase precession analysis

A "place field" was first defined according to the criteria of Muller et al. (1987): a group of adjoining bins (sharing at least one side) with the average firing rate of each bin exceeding a specified threshold. For this study the minimal number of adjacent bins was set at 10, the analysis was restricted to cells with at least 100 spikes in the recording session, and the threshold was set at a constant rate of 1 Hz. The value of 1 Hz, was chosen because the average mean rate of all the complex spike cells firing on the maze was around 1 Hz. In one analysis, thresholds of 0.5 and 2 Hz were also used for comparison. The size of a place field was defined unidimensionally, i.e., firing locations with respect to the principle track axis were analyzed.

Each spike, that occurred in the presence of theta rhythm was assigned a nominal phase, according to the fraction of the time between the theta peaks at which it occurred. Precisely, the phase assigned to a spike at time $t$ was $(t-t_0)/(t_1-t_0)$, where $t_0$ and $t_1$ are the times of the preceding and following peaks of the filtered EEG signal. Note that the phase is always a number between 0 and 1, which correspond to $0^\circ$ and $360^\circ$ respectively. The theta phase of the spike was then plotted against the location of the animal on the track at which the spike occurred (e.g., Fig. 8.1A). Because phase is periodic and the entry
Figure 8.1 Illustration of the regression analysis for phase precession from one representative cell. A. Firing phase versus position as described in Fig. 4.5B. B. As described in Methods, the data points above the discontinuity in the plot in A were replotted at the corresponding phase one cycle earlier and a regression line was fitted using least-squares minimization. As shown by Skaggs et al. (1996), firing phase typically changes non-linearly with position, with an accelerated advance near the end of the place field. C. Non-linear transform, log(1-phase), was applied in order to improve the accuracy of the numerical estimates of the rate of phase change and the initial phase on field entry. As can be seen in the example, this transformation tended to reduce the correlation of the residuals of the linear fit.
phase of the place fields was usually somewhat after the peak of the theta cycle, the
distribution of points in this plot was typically discontinuous. In order to examine the
correlation between the phase and location, the point of discontinuity was estimated by
inspection and 360° was subtracted from the points above the discontinuity, resulting in a
continuous distribution points in the phase vs. location plot (Fig. 8.1B). As suggested by
Skaggs and McNaughton (1996), the relationship between firing phase and location is not
linear, in that the phase precession usually accelerates when the animal traverses the place
field (Fig. 8.1B). The data were therefore linearized using a log(1-phase) transformation
(this is sometimes referred to as g(phase) in the following). Regression analysis was then
performed using a conventional least-squares minimization method (Fig. 8.1C), to
examine the relationship between log(1-phase) vs. location, as well as that between phase
vs. location. The entry phase and exit phase of the place field were the phases predicated
by the regression line for the position of the first and the last spike of the place field,
respectively. The slope, coefficient of determination (r^2) of the regression lines, phase
change (difference between the entry phase and the exit phase) as well as field size were
averaged for each rat. The comparison between young and old rats was then conducted by
animal, using one-way ANOVA.

The mean angle of either the entry phase or the exit phase, which are circular (i.e.,
periodic) variables was calculated for each rat using:

\[ x = \frac{\cos\phi_1 + \cos\phi_2 + \ldots + \cos\phi_n}{n} \]  \hspace{1cm} (2)

\[ y = \frac{\sin\phi_1 + \sin\phi_2 + \ldots + \sin\phi_n}{n} \]  \hspace{1cm} (3)

mean angle = \text{arctan}(y/x) if \( x > 0 \); 180° + \text{arctan}(y/x) if \( x < 0 \) \hspace{1cm} (4)
where \( n \) is the number of place fields recorded.

## 8.2 Results

### 8.2.1 Effect of age on spatial and visual discrimination behaviors

*Morris water task.* Repeated measures ANOVA were conducted on the spatial and visual discrimination data. The old rats were significantly poorer at learning the location of the hidden platform in the water pool. Although both age groups showed improvement over trials (\( F(1,23) = 2.97, p < 0.0001 \)), the old rats swam longer, less direct routes to it (\( F(1,10) = 4.99, p < 0.05 \)). Both young and old rats showed improvement over trials in the visual discrimination version of the task (\( F(1,11) = 1.96, p < 0.04 \)), with no effect of age on learning performance (\( F(1,9) = 0.36, p < 0.56 \)). Thus old rats were selectively impaired, compared to young rats, on the spatial version of this task.

### 8.2.2 Effect of age on theta rhythm during REM sleep

The mean frequency and amplitude of theta rhythm during REM sleep were first compared between young and old rats. Because the rat is motionless during REM sleep, any effect of movement velocity on the theta rhythm can be ruled out in this state. For data analysis, only those robust REM sessions lasting more than 30 sec were included, totaling 41 REM episodes from young rats (mean duration: 113 ± 7 S.E.M., 54 S.D. sec) and 60 REM episodes from old rats (mean duration: 129 ± 14 S.E.M., 87 S.D. sec). The mean theta frequency and mean theta amplitude of each episode were then averaged for each animal by taking the instantaneous frequency of REM calculated in blocks of 0.2 sec. These frequency blocks were averaged over the entire session to obtain the mean frequency for each session. There was no significant difference in either the theta
frequency \( (F(1,10) = 1.09, P < 0.32; \) Fig. 8.2C) or the theta amplitude \( (F(1,10) = 0.02, P < 0.89; \) Fig. 8.3C) between young and old rats. Note that the amplitude of the theta rhythm was quite variable between individual rats of both age groups, presumably due to the fact that the amplitude is sensitive to the exact location of the EEG electrode.

### 8.2.3 Effect of age on theta rhythm during behavior on the rectangular track

There were two food reward locations on the rectangular track, at which the EEG changed from predominantly theta to large irregular activity (LIA), when the rats stopped to eat (Vanderwolf et al., 1975). Therefore, the EEG at stationary or low velocities was excluded from the data analysis, to prevent the contamination of theta rhythm by LIA. Two methods were used to achieve this: one was to eliminate measurement when the velocity was less than 6 cm/sec (absolute velocity cutoff); the other was to eliminate the data collected in the lowest 15% of the velocity range (relative velocity cutoff). Due to the generally low running speed of old rats (see below) and their consequent longer time periods on the track, the relative velocity cutoff led to significantly larger data sample in old rats in comparison to young rats \( (P < 0.01) \). Only the absolute velocity cutoff data are therefore shown here, although similar results were obtained from both kinds of analysis.

For each recording session, regression and correlation analyses were used to examine the relationship between the instantaneous velocity and the frequency of theta rhythm recorded when the rat was traversing the rectangular track. Consistent with previous results (Recce, 1994), there was, in general, a small linear relationship between the velocity and frequency, (i.e., the faster the rat ran, the higher the frequency of theta rhythm was.) (Fig. 8.2A). All 26 sessions recorded from young rats and 17 out of 19
Figure 8.2 The effect of age on the frequency of theta rhythm. A. Two representative examples, one from a young rat, the other from an old rat, for the regression analysis of the relation between instantaneous running velocity and theta frequency. Low velocity (< 6 cm/sec) data were excluded to prevent contamination of results with non-theta mode EEG (LIA). The overall age comparison of the intercept and slope are summarized in the bottom panel. Two lines were drawn by taking the mean values of the intercept and slope for each age group. The shaded regions were drawn to reflect the standard errors of both the intercepts and slopes. B. The means (± S.E.M.) of the regression slopes and the correlation coefficients for theta frequency versus running velocity. Although the correlation coefficient was significantly lower in old rats, a significant difference in the slopes was not detected. C. The means (± S.E.M.) of the intercept of the regression lines, and the theta frequency during REM sleep. The intercept was slightly but significantly smaller in old rats, whereas there was no difference in the REM theta frequency between the two age groups. D. The mean (± S.E.M.) of the ratio between the intercept value of theta frequency and the mean REM theta frequency for a given rat. The ratio was approximately 1.0 in young rats and slightly, but significantly, smaller in old rats. (* P < 0.05; ** P < 0.01.)
sessions recorded from old rats exhibited statistically significant correlations between the velocity and theta frequency (P<0.05). The intercept and slope of the regression line for each session, as well as the correlation coefficient, were averaged for each rat, followed by one-way ANOVA analysis between young and old rats. As shown in Fig. 8.2C, the intercept of the regression line in old rats was significantly smaller compared to young rats (F(1,10) = 17.232, P < 0.002). Although the slope of the regression line in old rats also tended to be smaller than that of young rats, the difference was not significant (P < 0.30; Fig. 8.2B). The Pearson correlation coefficient, however, was significantly lower in old rats than in young (F(1,10) = 6.96, P < 0.025; Fig. 8.2B), possibly because of the reduced range of velocity in the old rats. These results are also summarized in the lowest panel in Fig. 8.2A, which shows that the frequency of theta rhythm in old rats was lower than that of young rats at all velocities measured. It was noted that the intercept value of the theta frequency of young rats is at the same level as the mean theta frequency during REM sleep (REM: 7.30 ± 0.04 Hz; Maze: 7.34 ± 0.06 Hz), suggesting that this intercept value indeed reflects theta without the influence of any movement. In old rats, however, the intercept of theta frequency was slightly lower than the mean REM theta frequency (REM: 7.24 ± 0.04 Hz; Maze: 6.959 ± 0.07). Therefore the ratio between the intercept frequency and mean frequency during REM was calculated for each animal, and compared between age groups. As shown in Fig. 8.2D, the ratio was about 1 in young rats and slightly smaller than 1 in old rats. This ratio was slightly, but significantly different between the two age groups (after log transformation, F(1,10) = 13.348, P < 0.0044).

The relationship between the amplitude of the theta rhythm and running velocity of the rats was also examined, using the same EEG data and regression analysis. Twenty-two out of 26 sessions recorded from young rats and 17 out of 19 sessions recorded from old rats exhibited significant correlations between running velocity and theta amplitude (P
Figure 8.3  The effect of age on the amplitude of theta rhythm.  A. Two examples, one from a young rat, the other from an old rat, for the regression analysis of the relation between instantaneous running velocity and theta amplitude. The bottom panel shows the summarized data in the same way as in Fig. 3A, except that the shaded regions representing the S.E.M. were only drawn on one side of each line to avoid overlap.  B. There was no significant difference between young and old rats in either the slope or the correlation coefficient.  C. The means (± S.E.M.) of the intercept of the regression lines, and theta amplitude during REM sleep. There was no significant age effect on either measurement; however, as shown in D, the ratio of mean theta amplitude during REM and the amplitude intercept values during maze behavior, while close to 1.0 in young rats, was significantly reduced with age. (** P < 0.01)
Amplitude of Theta Rhythm (µV)

Ratio of Theta Amplitude

Theta Amplitude (µV)

Intercept (µV)

Regression Coefficient

Slope (µV/num/sec)
The intercept of regression lines was quite variable, and there was no significant difference between the two groups ($F(1,10) = 0.625, P < 0.45$; Fig. 8.3C), although the mean intercept of old rats was smaller than that of young rats. Furthermore, there was no significant difference in the slope of regression lines between age groups ($F(1,10) = 0.226, P < 0.64$; Fig. 8.3B). In addition, there was no difference in the Pearson correlation coefficient between young and old rats ($F(1,10) = 0.00077, P < 0.98$; Fig. 8.3B). Because the amplitude of the theta rhythm depends on the location of EEG electrodes, the REM theta was used as the reference for each animal. The ratio between the intercept value of theta amplitude on the maze and the mean amplitude of REM theta was calculated for each rat. As shown in Fig. 8.3C and 8.3D, the intercept value of the amplitude of movement related theta rhythm was close to the theta amplitude during REM sleep in the young rats (i.e., the ratio is close to 1). The ratio, however, was less than 1.0 in the old rats. One-way ANOVA revealed a significant difference in the ratio between the two age groups (after log transformation, $F(1,10) = 10.915, P < 0.0080$; Fig. 8.3D).

8.2.4 Selection of complex spike cells

Of the complex spike cells (young: $n = 847$; old: $n = 590$) recorded when the animals were sleeping, 57.4% of cells from young rats and 49.7% of cells from old rats had place fields on the maze (see Methods), while the others became virtually silent. One hundred and ninety cells from young rats (18.1%) and 84 from old rats (12.4%) were identified as theta cells, and all of them fired during both sleep and maze sessions. A Chi square test revealed a significant difference in the observed proportions of theta cells between the two age groups ($P < 0.0014$). Twelve cells recorded from young rats and 9 cells from old rats could not be placed into either category, and, therefore, were not used.
Table 8.2 Number of cells recorded from young (n=6) and old (n=6) rats, while asleep and during behavior on the rectangular track.

I. Cell types

<table>
<thead>
<tr>
<th>Age</th>
<th>Complex Spike Cells</th>
<th>Theta Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of cells firing</td>
<td># of cells on track</td>
</tr>
<tr>
<td></td>
<td>during sleep (%)</td>
<td>(% active)</td>
</tr>
<tr>
<td>Young</td>
<td>847</td>
<td>486 (57.4)</td>
</tr>
<tr>
<td>Old</td>
<td>590</td>
<td>293 (49.7)</td>
</tr>
</tbody>
</table>

II. Number of cells with field locations at nonfood locations of the rectangular track vs. at food locations on the track

<table>
<thead>
<tr>
<th>Age</th>
<th># of cells firing on track (%) total active</th>
<th># of cells firing at food locations (%) total active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>354 (72.8)</td>
<td>132 (27.7)</td>
</tr>
<tr>
<td>Old</td>
<td>248 (84.6)</td>
<td>45 (15.4)</td>
</tr>
</tbody>
</table>
for data analysis. Because the EEG recorded at reward locations was mostly LIA, for which theta phase is undefined, only those cells with independent place fields on the part of the rectangular track other than reward locations were included for further analysis. Note that those cells with multiple place fields which included both reward and non-reward locations were included. The number of pyramidal cells included for analysis were 354 for young rats and 248 for old rats, as shown in Table 8.2. The amplitude and width of the spike waveforms of those cells with place fields on the track were similar between young and old rats (Table 8.3), indicating that there was no difference in the quality of unit isolation between the two age groups.

8.2.5 The effect of age on general firing characteristics

There was no difference between groups in the mean firing rate during either slow-wave sleep (SWS) or REM sleep before the behavior on the rectangular track (Table 8.3). The mean rate during behavior on the track, however, was significantly lower in old rats than in young rats (Table 8.3). Old rats exhibited smaller place fields than young rats ($F(1,10) = 8.519, P < 0.015$, Fig. 8.4A and 8.4B), and smaller field rate integrals ($F(1,10) = 5.395, P < 0.04$; Table 8.3). This age difference can not be attributed to the threshold value (1.0 Hz) for determining the field size, because, using either a lower (0.5 Hz) or higher (2.0 Hz) threshold, the field sizes of old rats were also significantly smaller than those of young rats (0.5 Hz, $F(1,10) = 9.809, P < 0.011$; 2.0 Hz, $F(1,10) = 8.494, P < 0.015$; Fig. 8.4B). The differences between the means of each group were similar for a given threshold cutoff value. Furthermore, in both age groups, the field size increased as the threshold value decreased, which is consistent with the previous finding (Muller et al., 1987), in which a linear relationship between field size and the logarithm of the
Figure 8.4 Effects of age on place field characteristics. **A.** Representative examples from young and old rats of data from cells with single, double and triple place fields on the rectangular track. The individual spikes are color-coded according to firing phase as in Fig. 1. **B.** Age comparison of place field sizes (computed for the entire recording session) using different cutoff thresholds (0.5 Hz, 1.0 Hz, and 2.0 Hz). At all three thresholds, the field size of old rats was significantly smaller than that of young rats to a similar extent. *P < 0.05. **C.** Frequency distribution (in percent) for the number of discrete place fields exhibited per cell in each age group. A place field was defined as a contiguous region of at least 10 bins with firing rates above 1 Hz. The distributions were approximately exponential, with no significant difference between ages. Note, the percentages are for cells with at least one field. Cells with no field are not included.
A

- Young (6)
- Old (6)

B

- Field Size (cm)
- Threshold (Hz)

C

- Percent (%) of cells
- Number of Fields
threshold cutoff value was found. Interestingly, the ratio of field rate integral to field size was almost identical, suggesting a general broadening, rather than a mere rate increase.

The maximum rate and information per second in cells of old rats also tended to be lower than those of young rats, although none of these differences were statistically significant (P > 0.05, Table 8.3).

It is clear that some cells have more than one place field in a given environment. Therefore, the effect of age on the number of fields on the track was examined. Only 48.6% of old cells and 40.7% of young cells had single place fields, and a substantial proportion of cells had two or more "place fields" as defined here. There was, however, no difference in the relative proportions of cells with a given number of fields between young and old rats (NP Kolmogorov-Smirnov test: Chi square = 5.716, P < 0.11; Fig. 8.4C). The average number of place fields per cell was 1.9 ± 0.2 in old rats, and 2.1 ± 0.09 in young rats (F(1,10) = 0.653, P < 0.44). Nor were there differences between ages in the proportion of cells with no place field at all on the recording apparatus.

8.2.6 Effect of age on phase precession

The relationship between the firing phase and position within the field was examined as described in the Methods section. The phase precession of the majority of cells had a total phase change within 360° within a given place field, although many cells had more than one field (see Fig. 8.4C). A few cells (6 young and 6 old), however, exhibited apparent double (i.e., bimodal) fields which overlapped spatially and in which phase precession extended across 2 theta cycles. These cells will be described in more detail elsewhere. In these cases, the precession slope was taken as the average of the
Table 8.3  Firing characteristics of complex spike cells recorded from young \((n = 6)\) and old \((n = 6)\) rats. Only cells with place fields on the track are included \((n_{\text{young}} = 354, n_{\text{old}} = 248)\).

<table>
<thead>
<tr>
<th>Cell Firing Property</th>
<th>Young (S.E.M.)</th>
<th>Old (S.E.M.)</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (mV)</td>
<td>214.8 (14.1)</td>
<td>229.0 (30.3)</td>
<td>0.179</td>
<td>0.68</td>
</tr>
<tr>
<td>Width (msec)</td>
<td>0.34 (0.011)</td>
<td>0.34 (0.013)</td>
<td>0.031</td>
<td>0.86</td>
</tr>
<tr>
<td>mean rate in SWS (Hz)</td>
<td>0.62 (0.017)</td>
<td>0.62 (0.098)</td>
<td>0.002</td>
<td>0.97</td>
</tr>
<tr>
<td>mean rate in REM (Hz)</td>
<td>0.55 (0.045)</td>
<td>0.71 (0.108)</td>
<td>1.867</td>
<td>0.20</td>
</tr>
<tr>
<td>mean rate on maze (Hz)</td>
<td>1.11 (0.083)</td>
<td>0.81 (0.103)</td>
<td>5.007</td>
<td>0.05*</td>
</tr>
<tr>
<td>info/sec</td>
<td>1.49 (0.150)</td>
<td>1.03 (0.168)</td>
<td>4.251</td>
<td>0.07</td>
</tr>
<tr>
<td>maximum rate</td>
<td>11.93 (0.61)</td>
<td>9.05 (1.32)</td>
<td>3.930</td>
<td>0.08</td>
</tr>
<tr>
<td>field size (cm)</td>
<td>30.96 (1.34)</td>
<td>23.84 (2.04)</td>
<td>8.519</td>
<td>0.02*</td>
</tr>
<tr>
<td>field rate integral (cm x Hz)</td>
<td>179.1 (12.1)</td>
<td>134.7 (14.8)</td>
<td>5.395</td>
<td>0.04*</td>
</tr>
<tr>
<td># of fields per cell</td>
<td>2.1 (0.1)</td>
<td>1.9 (0.2)</td>
<td>0.653</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Figure 8.5 The effect of age on phase precession and place field size. A. Phase precession plots for two representative CA1 complex spike cells, one from a young rat (blue dots) and the other from an old rat (red dots). The scatter plots and regression lines for raw and transformed \([g(\text{phase}) = \log(1-\text{phase})]\) data are shown on the left and right respectively. For illustration, the two fields have been aligned at the position where the first spike occurred. The X-axis, therefore, represents the normalized position of spike occurrence relative to that of the first spike within the field. Both fields start and end at approximately the same phase. The phase advance of the spikes recorded from the old rat, however, progresses more quickly in the old rat, and the field size is correspondingly smaller (see Fig. 8.4B). B. The slope magnitudes for phase and \(g(\text{phase})\) vs. location were significantly larger in old rats, indicating a more rapid phase precession with distance (* \(P < 0.05\)). C. There was no difference in the total phase change of place fields between young and old rats when using either of the regression analyses. Total phase change was taken as the difference in estimated phases at the beginning and end of the place field. The total phase change obtained from the \(g(\text{phase})\) transformation was slightly greater than for the raw phase data as expected due to the linearizing effect of the transformation. There were no differences in the coefficients of determination for the regression lines \((r^2)\) of young and old rats.
slopes within the two cycles. The mean total phase change within place fields was first averaged within rats, and then within age groups. The slopes of the regressions of phase vs. location and g(phase) vs. location (see methods) were both significantly greater in old rats than in young (F(1,10) = 6.654, P < 0.027 and F(1,10) = 10.41, P < 0.009, respectively, Fig. 8.5B). There was no difference in the total phase change over the place field between young and old rats (F(1,10) = 0.092, P < 0.76 and F(1,10) = 0.014, P < 0.91, respectively; Fig. 8.5C). It should be noted that both the field entry phase and exit phase are values derived from the regression lines. Because of scatter about the regression line, the total phase change calculated from the difference between exit phase and entry phase is substantially smaller than 360°, although the range of actual spike phase values typically approached 360°. The total phase change computed from the g(phase) transformation was somewhat larger than that obtained from the raw phase data. This is consistent with the apparent nonlinearity of the raw phase data (e.g., Figs. 8.1 & 8.5A). The coefficients of determination of the two regression functions were also compared between young and old rats. A two-way ANOVA revealed no significant effect of age (F(1,10) = 0.595, P < 0.46), no significant effect of analysis (phase vs. g(phase), F(1,10) = 3.301, P < 0.083), nor a significant interaction between analysis and age (F(1,10) = 1.049, P < 0.33). It is surprising that the log transformation overall did not lead to a significantly higher r², although it can be seen visually that the log transformation has linearized the data. This discrepancy may be due to the fact that, although the log transformation linearized the data at the end of the place field, it increased the variance of the data at the beginning of the place field. This effect can be seen in Fig. 8.1 and Fig. 8.5A. There was no significant effect of age on either the entry phase (young: 230° ± 8.7;
old $227.0^\circ \pm 4.7; U^2 = 0.116, P>0.20$) or the exit phase (young: $12.7^\circ \pm 20.4; \text{old:} 5.27^\circ \pm 16.1; U^2 = 0.06 P>0.05$).

8.2.6 Effect of age on the expansion of place fields

It has been shown previously that place fields enlarge within a few traverses of a route even in a familiar environment (Mehta et al., 1996). The effect of age on this field expansion was also examined. The field sizes, as measured by the distance between the first spike and last spike in the cell's place field, on lap 1, lap 5, lap 10, and lap 15 were averaged for each animal (these points were selected on the basis of Mehta et al.'s finding that the changes in place fields saturate within 2-3 laps). One old animal ran less than 15 laps, thus data on lap 15 were lacking. Two-way, repeated measures ANOVA revealed a significant effect of lap ($F(3,27) = 9.247, P < 0.0002$), a significant age effect ($F(1,9) = 6.065, P < 0.036$), and a significant interaction between age and lap ($F(3,27) = 7.591, P < 0.0008$). As shown in Fig. 8.6A, for the young group, the field size increased on lap 5, lap 10, lap 15, in comparison to lap 1. In the old group, however, the field size remained approximately the same across laps. For lap 1, there was no significant difference in the field size between the two age groups ($F(1,10) = 0.296, P < 0.60$). On the other hand, there were significant or marginally significant differences in the field size on the other laps between young and old rats (lap 5: $F(1,10) = 4.861, P < 0.052$; lap 10: $F(1,10) = 4.792, P < 0.053$; lap 15: $(1,9) = 9.79, P < 0.012$). The mean value of the phases of the first and last spikes of the place field for each lap was calculated for each rat using circular statistical methods (Equations 2-4, section 8.1.4.4). The difference between the mean entry and mean exit phase for each lap was then calculated for each rat. A 2-way ANOVA analysis revealed no effect of age ($F(1,39) = 0.587, P < 0.45$), no effect of lap ($F(3,39) = 0.378, P < 0.77$), and no significant interaction between age and
**Figure 8.6** The effect of age on experience-dependent place field expansion as the animal traversed the rectangular track. 

**A.** The mean (± S.E.M.) place field sizes for laps 1, 5, 10, and 15 of young (n = 6) and old (n = 6) rats. While the place fields of young rats expanded significantly from lap 1 to lap 5, and remained at the higher level on laps 10 and 15, those of old rats did not undergo any lap-related change. 

**B.** The mean (± S.E.M.) of the slope of the phase precession as a function of lap for the two age groups. The slope was calculated by taking the mean difference of entry and exit phases for laps 1, 5, 10, 15 for each animal, divided by the corresponding mean field sizes. There was a significant effect of age on the slope for laps 5-15 (*P < 0.05), but no effect for lap 1. 

**C.** The mean (± S.E.M.) of the velocity through the place field for each lap for the two age groups. There was no effect of lap on the mean velocity. The velocity of old rats, however, was significantly smaller than that of young rats (*P < 0.0001). 

**D.** Velocity tuning curves of firing rate for young and old rats. This was quantified by dividing the total number of spikes at each velocity (in 5 cm/sec bins) by the total time spent at that velocity. The overall velocity range of old rats was smaller than that of young rats. Although, for both age groups, there was a tendency for firing rate to increase with running velocity, the firing rate for old rats reached a lower plateau level more quickly than for young rats. The arrows indicate the mean velocities for the two age groups.
A

Field Size (cm)

- young (6)
- Old (6)

Laps

B

Slope

- Young (6)
- Old (6)

Laps

C

Velocity (cm/sec)

- Young (6)
- Old (6)

Laps

D

Mean Rate (Hz)

- Young (6)
- Old (6)

Velocity (cm/sec)
lap (F(3,39) = 0.672, P < 0.57) on the phase change within the place field. In young rats, the lack of a significant effect of lap on the total phase change, combined with a significant effect of lap on place field size, suggests that the rate of change of phase with distance decreased across laps in young animals. Due to the small number of spikes per trial, however, it was not possible to obtain reliable slope functions for single cells on a trial by trial basis. Therefore, the slope was estimated by taking the mean difference of entry and exit phases for trials 1, 5, 10, and 15 for each animal, divided by the corresponding mean field sizes. The results were compared between age groups (Fig. 8.6B). There was a significant effect of age on the slope for laps 5-15 (F(1,33) = 5.513, P < 0.025) but no effect for lap 1 (F(1,10) = 0.063, P < 0.81). The mean running velocity for each lap was also determined. A 2-way ANOVA revealed a significant effect of age on mean velocity (F(1,39) = 35.776, P < 0.0001), but no effect of laps (F(3,39) = 0.320, P < 0.81), nor significant interaction between age and lap (F(3,39) = 0.245, P < 0.86; Fig. 8.6C). Thus, the change in field size with lap number in the young rats was not due to any change in their running speed. There was, however, an overall effect of running speed on firing rate, as has been shown previously (McNaughton et al., 1983a). This was quantified by dividing the total number of spikes at each velocity (in 5 cm/sec bins) by the total time spent at that velocity (Fig. 8.6D). For both age groups, there was a significant tendency for firing rate to increase with running speed (F (4,40) = 72.86, P < 0.0001); however, the relative increase was significantly less in the old animals (F(4, 40) = 20.34, P <0.0001). Thus, the difference in mean firing rate between young and old rats cannot be accounted for by a difference in mean running velocity. The main effect of aging on place fields appears to be a failure of place field expansion during the first few passes through the field on a given day, leading to a smaller mean place field size and firing rate in old rats.
8.3 Discussion

8.3.1 Effect of age on experience-dependent place field plasticity

The main effect which emerges from the present study is a loss of experience-dependent plasticity in the spatial firing properties of aged hippocampal pyramidal cells. Old rats failed to exhibit the expansion of place fields that normally occurs in young rats during the first few traversals of a route (even a "familiar" one) on a given day (Mehta et al., 1996). There was no difference between ages, however, in the initial size of the place fields, and no significant difference in firing rate during either REM or slow-wave sleep. The failure of this experience-dependent expansion provides an explanation for the earlier reports that place fields, averaged over numerous trials, are smaller in old rats (Mizumori et al., 1993). The mechanism of the place field expansion in young animals is currently unknown. On track-mazes it is asymmetric, in the sense that the expansion is predominantly in the direction opposite to the direction of motion of the rat (Mehta et al., 1996). This characteristic was predicted by Blum and Abbott (1996) on the basis of the temporal asymmetry of LTP induction mechanisms (Gustafsson et al., 1987). In their model, when the animal traverses a sequence of locations, asymmetric LTP causes cells at a given location to activate subsequent cells in the sequence, before the rat actually reaches their original firing locations, thus causing the fields to enlarge in the direction opposite to the animal's motion. It appears plausible, therefore, that the age deficit in place field expansion may be a consequence of a failure of an LTP-like process. The LTP deficit explanation is made more plausible by recent in vitro findings on age-related deficits of LTP induction (Deupree et al., 1991), and new evidence suggesting that such an LTP induction failure in old rats may result from a reduction in the net synaptic input in old pyramidal cells (Barnes et al., 1996). The latter effect appears to be a consequence of a
reduced number of functional synaptic contacts made by a given CA3 pyramidal cell axon (Barnes, 1994). Additionally, an age-related deficit in LTP maintenance has also been found, which is significantly correlated with a loss of spatial memory capacity (Barnes and McNaughton, 1980c). Nevertheless, other explanations remain possible and should be considered. At least one of these, however, can be excluded. It is known that the transition from quiesence to activity is accompanied by changes in brain temperature, and that this change is associated with alterations in hippocampal evoked potential waveforms (Moser et al., 1993). Three observations rule this temperature effect out as a possible source of the place field plasticity. First, Mehta et al. (1996) showed that the expansion effect occurs robustly in rats which have been running continuously for 20 minutes or more when the rat is placed on a novel track, even in cells which had expanded fields on the familiar track. Second, the expansion is directionally asymmetric, which cannot be explained by a mere excitability change. Finally, Erickson et al. (1991) studied carefully the effects of age on the behavior- (temperature) dependent change in evoked potentials. There was no difference between young and old rats.

Temporally asymmetric LTP was also invoked by Tsodyks et al. (1996) to explain the theta phase precession effect. In this model, the asymmetric modification of intrinsic connections (as in the model of Blum and Abbott, 1996), leads to a dynamic error in the internal representation of the animal’s actual position. At the beginning of each theta cycle, the rat’s position is initialized by the external input. The asymmetry, however, causes cells whose true place fields lie ahead of the rat to begin to fire before the rat reaches the original field (thus making the time averaged field appear larger and shifted backward, as in the Blum & Abbott model). Tsodyks et al. (1996) suggested that one consequence of the theta rhythm was a periodic extinguishing of the activity of the network, which would enable the position representation to be reset by external input at
**Figure 8.7** Schematic representation of the behavior of the phase precession model of Tsodyks et al. (1996). The model is based on the assumption that each place cell (presumably in CA3) receives both spatially selective external connections and intrinsic connections from cells with nearby place fields, and that the latter are strengthened asymmetrically during unidirectional running on a track (see text). The cells are arranged schematically along the Y axes in the plots according to the locations at which they receive their maximum spatially selective input. In each plot, therefore, the heavy line represents both the position of the rat as a function of time, as well as the location of the “correct” representation of the animal’s position along the corresponding axis of its “cognitive map”. At the beginning of each theta cycle, the extrinsic input causes the cells representing the actual location to become active; however, as a consequence of the experience dependent, asymmetric plasticity of the intrinsic connections, the cells representing locations further along the route become active before the rat actually reaches the location. Thus, the internal representation of location moves forward faster than the rat, at a rate which depends on the strength and asymmetry of the intrinsic connections. The model assumes that the activity is essentially terminated at the end of each theta cycle, thus allowing the position representation to be corrected by the extrinsic input. Three consequences of this dynamic are that, following the plasticity, place fields are bigger than they would be if only the extrinsic connections influenced place cell firing; the increase in size is in the direction opposite to the direction of motion (see also Abbott and Blum, 1996); and the firing phase begins late in the theta cycle and precesses to progressively earlier phases as the field is traversed. The plots illustrate the general behavior of the model under conditions of: **B** reduced plasticity (i.e., weaker asymmetry) of intrinsic connections; **C** Reduced frequency of the theta rhythm; and **D**, slower movement of the animal, compared to the control condition (A). According to the model, neither the reduction of theta frequency nor the slower running velocity account for the smaller place fields in old rats, whereas a failure in experience-dependent plasticity (i.e., **B**) accounts for both the smaller fields and the more rapid phase precession.
A. Control

B. Weaker asymmetry of intrinsic connections

C. Slower Theta Rhythm

D. Slower Movement

- Place field
- Internal activity propagations
- Duration of firing
- Rat position vs. time
- Theta cycles
- Theta phases (0 - 1) of cell's firing vs. theta cycle
the beginning of the next cycle. Thus, the internal representation would periodically move ahead of the rat during each theta cycle and then jump back to its current position at the beginning of each theta cycle. Cells representing the current location would thus fire early in the cycle, while those representing positions ahead of the rat would fire later. The firing would thus precess from later to earlier phases as the rat ran through the place field. This model predicts that an asymmetric increase in the strength of connections between cells with adjacent place fields would result in a reduced rate of change of phase with position, with no change in the total phase shift. This effect was observed in young animals following the field expansion. According to the model, the firing phase is a function of position and not of time. Hence firing phase versus position would not be affected by running velocity *per se*, which is slower in old rats. Another prediction of the Tsodyks et al. (1996) model, is that place field size should be inversely related to theta rhythm frequency, assuming that firing rates remained the same (see Fig. 8.7); however, the elevation in theta frequency that occurs with increased running velocity is accompanied by an increased firing rate, so these effects may cancel each other out with respect to their influence on place field size.

What would be the expected functional consequence of the loss of place field expansion during experience? The information transmitted by an ensemble of neurons can be substantially improved if each neuron is broadly tuned in the sense of an increase in the integral of its probability density function (e.g., Lehky and Sejnowski, 1990) as appears to be the case in the young animals (field rate integral, Table 8.3). The lack of field broadening might thus be expected to lead to a loss of precision of the spatial code in old rats. Secondly, simulation studies (Shen and McNaughton, 1996) suggest that LTP of intrinsic connections among cells with overlapping place fields during spatial exploration could lead to selective reactivation of representations of recently visited places in the
complete absence of the corresponding external input, for example, during sleep. Such reactivation is known to occur in hippocampus of young rats (Skaggs and McNaughton, 1996), but recent studies indicate that this phenomenon is much reduced in old rats (Gerrard et al., 1996). It seems likely that the loss of experience-dependent place field plasticity in old rats and the loss of memory trace reactivation during sleep are closely related and reflect a general decline in experience-dependent synaptic plasticity. As discussed by Shen and McNaughton (1996), however, non-synaptic, non-associative explanations of these phenomena remain possible, given the appropriate, pre-existing synaptic matrix.

8.3.2 Age-related changes in hippocampal theta rhythm

Theta rhythm frequency was slightly reduced in old rats, for any given running speed. There was no age effect, however, on either the frequency or the amplitude of the theta rhythm in REM sleep. In young rats, the zero velocity intercept for both frequency and amplitude matched the corresponding values during REM sleep. In contrast, in old rats, the zero velocity intercepts for movement-related theta differed from REM theta in both frequency (5%) and amplitude (20%). Interestingly, while the correlation between the theta frequency and running velocity was significantly decreased in the old rats, there was no age difference in the correlation between the theta amplitude and velocity. Thus, theta frequency and amplitude undergo distinct age-related changes.

What could account for the differential effects of age on theta frequency and amplitude during REM sleep and movement? Serotonergic raphé neurons and noradrenergic locus-coeruleus neurons exhibit maximal activity during active waking, and become virtually silent during REM sleep (Trulson and Jacobs, 1979). Although NE does not appear to be essential for theta generation (Sainsbury and Partlo, 1993), the
serotonergic projections to the medial septum and/or hippocampus have an effect of desynchronizing hippocampal EEG and reducing EEG amplitude (Assaf and Miller, 1978). 5-HT synthesis, accumulation rate, and turnover are increased in aged rats (Venero, et al., 1993) and aberrant serotonergic fibers appear in the molecular layer of the dentate gyrus and stratum lacunosum molecular of CA1 (van Luijtelhaar et al., 1992) in old rats. Thus, age effects observed during waking may be caused by dysfunctional serotonergic projections in the aged brain. Selective lesions of cholinergic septal neurons decrease the theta amplitude but not theta frequency (Lee et al., 1994). Thus, cholinergic alterations which are known to occur in old hippocampus (Potier et al., 1992) may contribute to the theta amplitude effect observed during waking; however, because the cholinergic input to the hippocampus is active in both REM sleep and movement, it is unlikely that cholinergic changes can account for the differential effect of age on theta amplitude during these states.

8.3.3 Effect of age on the number of hippocampal CA1 interneurons

There was a significant (~35%) reduction in the observed proportion of theta cells recorded in the old rats. This is consistent with a recent report that the number of interneurons containing calbindin is significantly decreased in hippocampal CA1 and dentate gyrus of old rats (Potier et al., 1994), whereas the number of interneurons containing parvalbumin is preserved (Miettinen et al., 1993) (Potier et al., 1994). Unfortunately, at present it is not possible to assign hippocampal interneurons recorded extracellularly during behavior to corresponding anatomical categories. Potier et al. (1992) also reported an age-related decrease in the synaptic GABA-B response (slow IPSP) with no change in the postsynaptic response to GABA-B agonist baclofen. Finally, the efficacy of GABA enhancement of benzodiazepine binding is enhanced in the aged
hippocampus (Ruano et al., 1991), and microiontophoretic application of GABA inhibits pyramidal cell firing to a greater extent in old rats (Lippa et al., 1981). Lesion of the GABAergic striatonigral pathway causes a GABA-A/benzodiazepine receptor supersensitivity in substantia nigra (see Ruano et al., 1991), and thus, the comparable effects seen in hippocampus are consistent with interneuron loss.

8.3.4 Summary and Conclusions

Place field size at the beginning of a repeated, stereotyped behavioral task is not different between age groups, but the expansion of place field size that occurs in young rats after a few traversals does not occur in the old animals. This difference is unlikely to be related either to changes in the theta rhythm between age groups (which were very modest, or nonexistent), or to alterations in the phase of theta at which single cells fired during entry and exit of their place fields. Regardless of the exact mechanism of the attenuated place field expansion in old rats, the present results contribute to a growing body of evidence suggesting that a deficit of functional plasticity of information transmission within the hippocampus could be a major factor in age-related memory impairment.
CHAPTER 9 COGNITIVE MAP MULTISTABILITY IN AGED RAT HIPPOCAMPUS

It has been reported that the maintenance of LTP of hippocampal synapses is reduced in old rats (Barnes, 1979). Additionally, in vitro studies (Deupree et al., 1991) have provided evidence indicating a reduced probability or magnitude of LTP induction when using submaximal stimulation, which is most likely due to a loss of synaptic connectivity among hippocampal pyramidal cells in aged rats (Barnes et al., 1992). Unfortunately, it has proven exceedingly difficult to observe LTP in hippocampal synapses during natural behaviors (Barnes, 1995). It is, therefore, not possible yet to determine directly how and whether the age-related changes observed under artificial conditions are translated into functional changes in spatial memory capacity. As suggested in Chapter 8, however, indirect evidence can be obtained by examining the dynamic changes of place specific firing of populations of hippocampal neurons.

The present study was conducted to examine the effect of age on the reliability of place field firing in a familiar environment, after an intervening period during which the rat was exposed to a novel environment. As reviewed in Chapter 4, an emerging view of hippocampal place cell activity (McNaughton et al., 1996) is that the place field is the consequence of pre-configured synaptic interactions among neurons with neighboring place fields in an environment, and that self-motion signals and the head direction reference, rather than relationships among landmarks per se, may be critical for determining relative position on a place field map (Gothard et al., 1996b). Landmark information, however, becomes bound to map locations through associative learning, and can thus serve to correct for the inevitable drift-error in the head direction system (Knierim et al., 1995). According to this view of hippocampal dynamics, the initial
formation of place field does not depend on external sensory stimuli, but becomes coupled to them through an experience-dependent, LTP-like mechanism. This coupling of internal spatial representations to environmental cues can not only provide correction for drift error, but can also enable the selection of the correct place field map on each entry into the same environment. Thus, it can be hypothesized that if an LTP-like deficit occurs in the aged hippocampus, old rats could exhibit a deficit in the coupling between landmarks and the internal maps. This could potentially lead to an impairment in the selection of the right place field map. This hypothesis is tested in the present experiment.

9.1 Methods

The subjects of the present experiment were the same 6 pairs of young and old rats described in Chapter 8. The rectangular track used in Chapter 8 formed one half of a full rectangular figure-8-maze. The present experiment was conducted in the days following the experiment described in Chapter 8. The major manipulation in the present experiment was exposure of the rats to the "novel" portion of the figure-8 maze. This was accomplished by raising the partition on the figure-8 maze, allowing the rat to enter the unfamiliar half part of the maze. This novel rectangle shared a common side with the familiar region. After this exposure, the rat was again recorded on the familiar portion of the maze. For each recording session, well-isolated, hippocampal CA1 pyramidal cells were monitored through 5 recording phases: Sleep 1: data were recorded (about 30 min) as the rat sat quietly and/or slept in a small "nest" placed over the maze; Maze 1: the nest was removed and the rat was allowed to make multiple traversals of the track (10-15) laps, always running in the clockwise direction; Novel Treatment: the rat was transferred to the novel portion of the track, and was allowed to traverse the track for the same number of laps as in Maze 1, however, running in the counter-clockwise direction; Maze 2: the rat
was returned to the track in its original configuration; *Sleep 2*: the rat was returned to the “nest”. The stability of cells throughout the data collection period was determined by verifying that spike waveform distributions were consistent from *Sleep 1* to *Sleep 2*. Two sessions were recorded for most of the rats. For two old rats, the *Maze 2* phase was not completed in one of the two sessions, which, therefore, were not included for data analysis.

To determine the reliability of the spatial firing patterns of the cells following the novel exposure, the rectangular track on the familiar side of the figure-8 maze was divided into bins (bin size 1.7 x 1.7 cm). A firing rate map was constructed for each cell for the *Maze 1 & Maze 2* sessions by calculating the mean firing rate within each bin, and the spatial correlation of these distributions was computed. When the spatial firing distributions are completely correlated between *Maze 1* and *Maze 2*, the coefficient should be close to 1. On the other hand, if the place fields change between *Maze 1* and *Maze 2*, the coefficient should be around 0.

### 9.2 Results

A total of 474 complex spike cells were recorded from young rats during *Sleep 1* (9-57 for sessions), and 403 for old rats (28-59 for sessions). Among these cells, 313 cells (66% of cells fired during *Sleep 1*) recorded from young rats and 273 from old rats (68%) had place fields on the *Maze 1* portion of the experiment.

As shown in Fig.9.1A, the place specific firing of most young cells was highly correlated between *Maze 1* and *Maze 2* with the spatial correlation coefficient mostly around 0.8. The spatial correlation coefficient in old rats, however, was distributed in a
Figure 9.1 A. Frequency distributions of place correlation coefficients between Maze 1 and Maze 2 phases ($r(m1,m2)$). For young animals, the distribution was unimodal, reflecting a high degree of consistency of the place field map between the two recording phases within a session. In old animals, the distribution was bimodal, with one peak near that of the young animals, and another peak around 0.2. B. Frequency distributions of ensemble average place field correlations between Maze 1 and Maze 2. The young rats exhibited high correlations of place fields maps for all the recording sessions. In old rats, however, place field maps were highly correlated between phases on 80% of trials, and almost completely uncorrelated between phases on the other 20% of trials.
Figure 9.2 A. Frequency distributions of place correlation coefficients between the halves of Maze 2 phase ($r(m_2,m_2)$). For both young and old animals, the distribution was unimodal, reflecting a high degree of consistency of the place field map between the two recording phases within a session. B. Frequency distributions of ensemble average place field correlations between the halves of Maze 2 phase. Both young and old rats exhibited high correlations of place fields maps for all the recording sessions.
Table 9.1 Mean spatial correlation coefficient of place specific firing for young and old rats.

<table>
<thead>
<tr>
<th>Maze</th>
<th>Young (n = 12) Mean ± S.E.M.</th>
<th>Old (n = 10) Mean ± S.E.M.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>m(1,1)</td>
<td>0.786 ± 0.012</td>
<td>0.746 ± 0.033</td>
<td>1.493</td>
<td>0.24</td>
</tr>
<tr>
<td>m(1,2)</td>
<td>0.698 ± 0.024</td>
<td>0.550 ± 0.062</td>
<td>5.638</td>
<td>0.027*</td>
</tr>
<tr>
<td>m(2,2)</td>
<td>0.750 ± 0.026</td>
<td>0.747 ± 0.025</td>
<td>0.009</td>
<td>0.92</td>
</tr>
</tbody>
</table>

bimodal fashion (Fig. 9.1A). While a substantial number of cells had higher correlation coefficients around 0.8 as in young rats, another group of cells had coefficients around 0.2, indicating that this group of cells had quite different place fields in Maze 1 and Maze 2. A Kolmogorov-Smirnov Test revealed a significant difference in this distribution between the two age groups (Chi Square = 38.69, P < 0.0001). These spatial correlation coefficients were also averaged across all simultaneously recorded cells to provide an ensemble estimate of the similarity of the spatial distributions of cell firing between the Maze 1 and Maze 2 phases. As shown in Fig. 9.1B, in the young rats, the place field firing distributions were highly correlated between the first and second maze episodes in all 12 recording sessions. The old rats, however, exhibited virtually complete rearrangements of the place field distributions in 2 out of 10 sessions. In these cases, the
Figure 9.3 Representative place field distributions from 1 young and 1 old rat recorded on two consecutive episodes of running on the familiar rectangular track of a figure-8 shaped maze (Young, Old). In each plot, the gray lines indicate the trajectories of the rat, superimposed over multiple traverses of the maze. The locations at which spikes from individual pyramidal cells were emitted are represented as colored dots, with a different color for each neuron. The color scheme is consistent between episodes (Maze 1, Maze 2), for each rat. For clarity, only a representative subset of the simultaneously recorded cells from a single recording session is illustrated. Place fields of the young rat were highly correlated between the Maze 1 and Maze 2 on a given day. In the old rat, however, the place fields recorded during Maze 2 was completely uncorrelated with that of Maze 1.
mean spatial correlations dropped to near zero, indicating no relationship between the place field distributions in the *Maze 1* and *Maze 2* sessions (see also Fig. 9.3).

Within each maze phase, the spatial correlation coefficient between the first and the other half of the phase was also computed for each cell, and compared between the two age groups. Most of the cells of young and old rats showed high stability within one maze session, and there was no significant difference between young and old rats (Kolmogorov-Smirnov Test, *Maze 1*: Chi square = 7.105, $P < 0.06$; *Maze 2*: Chi-square = 0.212, $P < 0.13$, Fig 9.2A). For the averaged correlation for each session, all the sessions of young rats and all the sessions of old rats had high spatial coefficient (Table 9.1), indicating that the place fields of old rats were at least as stable as those of young rats within each maze session (Fig. 9.2B).

### 9.3 Conclusions

The above results indicate that although both young and old rats had a similarly extensive experience in a familiar environment, after a brief period of experiencing a novel environment, the place field maps of the old rats were more likely to be disturbed when they came back to the familiar environment, while the place field distributions in young rats did not undergo rearrangements. It should be noted that the multistability of place fields in the old rats appears to be an all-or-none phenomenon, i.e., on 80% of the sessions the place field map in old rats was as stable as young rats, on the other 20% of the sessions, however, the place cells underwent an almost complete remapping. According to the hypothesis stated at the beginning of this Chapter, these results suggest that there may be an age-related deficit in the LTP-like mechanism, which could lead to an inability of the old animals to effectively bind landmarks to their internal spatial representation. After a period of absence in the environment, old rats tend to select the
wrong map. This place field multistability phenomenon has also been observed when the rats had a much more extensive exposure (2 hours) to a number of novel environments (Suster et al., 1996). This age-related tendency to choose the wrong cognitive map may be due to a LTP-mechanism deficit, and may contribute to spatial memory deficits in old rats. This hypothesis can be further tested if the animal’s spatial memory performance is also tested in the familiar environment before and after the "novel" exposure, so that the relationship between the rat’s memory performance and the stability of place field maps can be examined.
CHAPTER 10 FINAL CONCLUSIONS AND DISCUSSION

In conclusion, the studies in this dissertation have focused on the effect of aging on two functional aspects of the hippocampus: cholinergic function and electrophysiological activity during spatial behaviors, including hippocampal theta rhythm and place specific firing. The results indicate that (1) cholinergic function is severely compromised in the aged hippocampus; (2) hippocampal theta rhythm undergoes only mild age-related changes; (3) there are age-related changes in place field plasticity and place field map stability, which are likely consequences of a deficit in LTP-like mechanisms in old animals.

In the study of the age effects on hippocampal cholinergic function, all three subfields of the hippocampus were examined. In addition, spatial learning and memory were tested on the same individuals on the spatial version of the Morris water task, in order to investigate whether there is a relationship between cholinergic function and memory impairments in old rats. In contrast to the known region specific changes of glutamate synaptic transmission in the hippocampus, the results of this study reveal a general deterioration of cholinergic synaptic transmission across all three subregions of the hippocampus. Few significant correlations, however, were found between the age-related decrease in cholinergic function and the magnitude of the spatial learning deficit in old rats on the Morris water task. This indicates that cholinergic dysfunction in the hippocampus may not be primarily responsible for the behavioral deficit of old rats in this task. This conclusion is supported by the subsequent lesion study, which demonstrates that selective lesions of cholinergic septo-hippocampal input in young adult rats do not have any observable behavioral effect on performance of a reference memory task on the radial-8-arm maze. The lesion did result in a modest impairment in performance of a working
memory task, however, this impairment was not delay dependent. This suggests that the hippocampal cholinergic input may play a modulatory role on the acquisition of information for short-term memory, but may not be necessary for the acquisition of reference memory nor for memory retention processes. Thus, the age-related decrease in hippocampal cholinergic function may directly contribute to the behavioral deficits of aged rats observed in working memory tasks (Wallace et al., 1980), but not in reference memory tasks such as the Morris water task. It should be emphasized, however, that the aging process is not equal to experimental lesions. While the latter can selectively affect one aspect of brain function, the former consists of a constellation of changes, which are relatively mild compared to experimental lesions. Although our conclusion is that the cholinergic dysfunction in the aged hippocampus per se may not be sufficient to cause any observable behavioral deficits in acquisition of reference memory, such as in the Morris water task, it remains possible that its concurrence with other neural deficits may lead to a loss of compensatory capacity of the system, thereby, resulting in an observable behavioral deficit. For example, Richter-Levin and Segal (Richter-Levin and Segal, 1989) found that partial lesions of either serotonergic or septal input to the hippocampus do not affect the behavior of rats on the spatial version of the Morris water task. The combination of both lesions, however, leads to a significant impairment in the same task.

One of the difficulties for the investigation of neural mechanisms underlying age-related memory impairment is that our knowledge about how information is processed and stored in the brain during normal memory operation is still very far from complete. Since Hebb stated the famous Hebb's rule in his book "The Organization of Behavior" (Hebb, 1949), it is generally proposed by both experimentalists and theoreticians that some persistent form of synaptic modification is the basic substrate of memory in the mammalian brain. This is supported by the discovery of long-term potentiation (LTP) in
the hippocampus (Bliss and Lømo, 1973). Thereby, LTP has been investigated intensively and is widely regarded as the cellular mechanism of learning and memory. This LTP phenomenon, however, is observed only when patterns of artificially electrical stimulation are administered to the afferent fibers of the hippocampus. There has not been convincing, direct evidence indicating the existence of LTP in natural behaviors (Barnes, 1995). Recent developments in theoretical frameworks as well as experimental studies provide an opportunity to study the LTP-like process in freely behaving animals in an indirect way. The observation of place field expansion on the first few trials when the animal traverses the route in a familiar environment is an example of this approach (Mehta et al., 1996). This place field plasticity is not caused by behavioral changes, but is likely caused by an asymmetric strengthening of synapses through a LTP-like mechanism. Our studies described in Chapter 7 provide evidence indicating that there is an age-related deficit in this place field plasticity, which, therefore, may be a consequence of an age-related deficit in underlying LTP-like mechanisms. This conclusion is also supported by the finding described in Chapter 9 that old rats tend to choose the wrong place field maps when re-entering a familiar environment after a short period of interference. According the theoretical frameworks proposed by Samsonovich and McNaughton (1996a, 1996b), the binding of distal cues in the environment to the correct place field map is dependent on LTP-like processes. Thus, the multistability of place field maps in old rats may also be a consequence of a deficit of LTP-like mechanisms in the aged hippocampus. The results from Chapter 8 and Chapter 9, therefore, are consistent with the LTP studies using patterned electric stimulation. As reviewed in Chapter 5, there is an age-related deficit in LTP maintenance (Barnes and McNaughton, 1980a) as well as in LTP induction elicited by submaximal electrical stimulation (Deupree et al., 1991). The latter age-related change is most likely due to a loss of functional synapses in hippocampal CA1 region of old rats,
thereby, less synaptic convergence on CA1 pyramidal cells under submaximal stimulation of Schaffer collaterals (Barnes et al., 1991). Because the LTP-like phenomenon is the best candidate for the synaptic basis of memory formation, the results in this dissertation strongly suggest that there is an age-related deficit in LTP mechanisms in the hippocampus in natural learning situations, which may directly contribute to the memory impairment in old animals.

The aging of the brain is a multi-variate process. As indicated in this dissertation, more than one aspect of hippocampal function are compromised in old age. As our knowledge on age-related alterations in the brain is increased, it will also become more challenging to understand whether and how these alterations lead to behavioral deficits. Although correlational studies are useful, they can never prove causal relationships. To fully appreciate the dynamic changes underlying the memory impairment in normal aging, computational approaches would be another useful way in which to integrate experimental data, and to explore the possible relationships between a variety of age-related neural alterations, which may lead to a better understanding of how information processing is changed in old age. This is a relatively new approach, used by Barnes et al. (1994). As reviewed in Chapter 5, there has been a constellation of electrophysiological evidence suggesting an age-related loss of functional synapses in hippocampal CA1 (Barnes et al., 1991). When this experimental finding is placed into a computational network, the result of the simulation predicts that either a given memory, or experimentally-induced LTP will decay faster in a system with fewer synapses. Interestingly, there are electrophysiological data that suggest that LTP at the perforant path-granule cell synapse decays over days much faster in old rats, which is correlated with faster forgetting of old rats in a spatial memory task (Barnes and McNaughton, 1985). Thus, this computational approach implies that the two experimental findings, the loss of synapses and the reduced LTP
maintenance, may actually be related phenomena which may subsequently result in the faster forgetting of old animals. Thus, the computational approach is a promising new tool for the research on neurobiology of aging, and should advance our understanding of how complex patterns of age-related neurobiological changes are interrelated and impact the observed behavioral changes.

Finally neurobiological research on aging is not an independent field, rather, it highly depends on our understanding of the mechanisms underlying the normal operation of memory. With the fast pace of progress in both fields, once the neurobiological basis of learning and memory is understood, the treatment of the age-related memory deficits may also be attainable in the foreseeable future.
REFERENCES


Alvarz-leefmans FJ, & Gardner-Medwin AR (1975) Influences of the septum on the hippocampal dentate area which are unaccompanied by field potentials. J. Physiol. (London) 249:14P-16P.


Nikaido AM, Ellwood EHJ, Heatherly DG, & Gupta SK (1990) Age-related increase in CNS sensitivity to benzodiazepines as assessed by task difficulty. *Psychopharmacol. (Berlin)* 100:90-97.


EG & Peters A (Eds.), *Cerebral Cortex*, (Vol. 6, pp. 345-456). New York: Plenum Press.


