I. INTRODUCTION

Aging is a natural process that must be studied intensively, for it remains one of the most agonizing problems in all biology. Not only must gerontologists continue to concentrate on performing the research necessary to understand aging, but they must assume an increasing role in the application of their knowledge for betterment of the status of the aged.


One way to underscore the urgency for research in the area of aging is to appreciate the dramatic demographic shift in the United States and in other countries of the world. According to the Administration on Aging, only 2% of the United States population was age 65 and older just 100 years ago, whereas today over 12% of the population is in that age category. By 2030 the projected percentage of people over the age of 65 is 20%. Accompanying this demographic shift is an increase in the incidence of disease associated with advancing age, for example, stroke, heart disease, and Alzheimer’s disease. Research aimed at understanding the aging process, including how learning and memory processes change as a function of age, is therefore needed to provide the basis for the development of better preventative strategies.
and treatment strategies to enable an increasingly older population to age successfully.

In the general field of aging research, substantial effort has been focused on pathological aging that may be associated with diseases such as Alzheimer's disease. While this disease (and many others) is certainly devastating in terms of its impact on quality of life and learning and memory function, many of us will not suffer from a dementing condition as we age. Instead, most of us will develop mild memory deficits known as *age-associated memory impairment* (AAMI; Crook et al., 1986; Crook and Ferris, 1992; Ferris and Kluger, 1996). The memory deficits associated with AAMI are relatively subtle in comparison to those associated with a dementing disease, but nevertheless those who experience such changes in their memory function may find this troublesome. Because we will all experience some change in our memory function as we get older, the need for research aimed at identifying normal nonpathological aging is of great importance.

This chapter highlights selected domains of cognition that can be studied across mammalian species and that have known age-related neurobiological underpinnings. Studies conducted on both human and nonhuman species will be considered, although data from rodent studies will make up the bulk of the following discussion.

## II. METHODS MATTER

*How old would you be if you didn't know how old you was?*

_Satchel Paige_

A large literature supports the idea that memory and other cognitive processes do decline or change with advancing age. Important methodological and other considerations must, however, be taken into account before interpreting such changes as negative consequences of brain aging. At least three primary issues require consideration: (1) What does it mean to be “old”? (2) How can aging be studied? (3) What variable are you studying, and how are you measuring it?

Aging is difficult to define, in either biological or cognitive terms. The simplest solution to the question of “how old” an individual is to assign a number to that person based on the number of years he or she has lived. This assessment of aging, however, has a number of shortcomings (Bourliere, 1970; Ingram, 1983). To take an extreme example, some older individuals, for example, centenarians, may be more cognitively intact than someone 30 years younger than themselves (see Perls, 2004). This highlights a key concept: Individuals appear to age at different rates in chronological time, and therefore biological age does not always match the number of years we have been alive.
In terms of mnemonic function, our cognitive abilities change throughout the course of our development (from birth to death); but for each of us, this change will take its own individualistic course. Although such issues certainly complicate the study of aging, they also provide an opportunity to discover the processes that allow some of us to age successfully, in the absence of debilitating learning and memory decline. Nevertheless, chronological age is still the most widely used predictor of the functional or biological age of the organism being studied (Costa and McCrae, 1980). For humans, "old" is typically considered to begin at 65 years, and for many laboratory animals, particularly rats, ages past the point of a 50% mortality rate for the particular organism or strain being studied are considered "old."

Experimental design is another critical issue to consider in aging research. Many human studies in the aging field employ either a cross-sectional design or a longitudinal design, and both methods of data collection offer important advantages and disadvantages in the study of aging. Typical cross-sectional studies compare a group of young adults in their late teens or early 20s (young adults are typically recruited from university introductory psychology classes) to a group of aged adults, yielding data on age differences. This study design is probably the most efficient and cost-effective means of conducting an aging study. Cross-sectional studies, however, are subject to confounding cohort differences and according to some (e.g., Hofer and Sliwinski, 2001; Salthouse and Nesselroade, 2002) tend to exaggerate age differences.

On the other hand, longitudinal studies, which follow the same individuals over time, are expensive and logistically difficult to conduct, but essentially they eliminate many confounds introduced as a result of cohort effects. These types of studies yield data on age changes. Moreover, longitudinal studies address the issue discussed earlier, that individuals can age at different rates. However, longitudinal studies also have disadvantages, which include underestimation of age differences because of selective subject attrition (i.e., as a result of illness) and practice effects.

Most animal studies employ a cross-sectional strategy, for many of the same reasons as discussed earlier, and therefore many of the same issues apply. Cohort effects in animal studies may be due to differences in housing conditions (i.e., individual or group housing) or to other life history differences of the animals being used. Some of the difficulties inherent in simple cross-sectional and

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1Cohort differences are group differences that arise as a result of factors such as socioeconomic status, cultural differences, and educational status in groups of individuals who are born at different time periods.

2Currently, there are several large-scale longitudinal studies under way. One example is the Seattle Longitudinal study, which began in 1956. Every seven years, all people who have previously participated are retested. A new group of participants is also added at seven-year intervals. In total, over 6,000 people have participated in this particular longitudinal study (Schaie, 1996).
longitudinal studies can be circumvented using a cross-sequential design, in which groups of individuals of different ages are tested repeatedly over several years. For example, two groups of subjects, one in their 20s and one in their 60s, can be tested to determine age differences in performance on a memory task and then later retested to yield data concerning age changes on the same task.

A third issue to consider is the type of learning or memory under investigation (e.g., long-term memory versus short-term memory) and how performance is assessed (e.g., number of correct responses versus reaction time). Some studies may be focused on long-term memory changes, for example, how vividly autobiographical memories are recalled in an aged population. Other studies may focus on short-term memory changes, for example, working memory. This is a good place to point out that the outlook for the healthy aging individual is not as grim as many suppose. While many age-associated cognitive changes do occur, there are also many things that remain stable as we get older, and thus the aggressively negative stereotype of the elderly is largely unfounded. For example, although working memory, episodic memory, and declarative memory abilities may be affected as one ages, other types of memory, such as autobiographical memory, and semantic knowledge tend to remain stable over the adult life span (for review see Hedden and Gabrieli, 2004). Paradoxically, if vocabulary is tested in isolation, older adults would be found to outperform younger adults (Schaie, 1996; Park et al., 2002); while if tests of “executive function” are given (such as the Wisconsin card-sorting task), then even those individuals in their 40s will likely show age-related decline (for a recent review and meta-analysis see Rhodes, 2004).

One variable that influences the outcome of any study is how performance is measured. For example, if performance on a memory task is based on measures of cued recall or recognition, older individuals often perform as well as their younger counterparts; whereas if the task is one of recall, aged individuals tend to have more difficulty than do younger subjects (e.g., Schonfield and Robertson, 1966; Harwood and Naylor, 1969; Craik and McDowd, 1987). In addition, older individuals tend to be less able to learn new information quickly when compared to younger adults, but this age difference can be reduced by allowing the aged individual to have more practice time or to determine his or her own pace of learning (e.g., Canestrani, 1963; Monge and Hultsch; 1971). Thus, laboratory studies of learning and memory that incorporate timed tasks may not be the best measures of learning and memory abilities in aged individuals. This also holds true for studies using rats (or other animal species) since the aged animals used for any study may be frail or less physically capable as compared to their younger counterparts. If older animals are tested in terms of “how fast” they perform (such as how fast they reach a goal on a maze), large deficits can be observed; but these deficits can be reduced if accuracy measures that are independent of speed are used (such as the distance that is
traveled to reach the goal). Designing studies that minimize these issues or take them into account is therefore equally important in studies using human or nonhuman subjects.

III. LEARNING AND MEMORY CHANGES ASSOCIATED WITH AGING

There is a large literature that has examined the question of whether learning and memory function is altered during the aging process. Tasks in which age-related impairments have been found include classical conditioning, such as eyeblink and heart rate conditioning, conditioned taste aversion, fear conditioning, operant tasks such delayed matching-to-sample and delayed nonmatching-to-sample tasks, and instrumental tasks such as active avoidance, passive avoidance, and maze learning tasks (for review see Barnes, 1991; Ingram, 2001; Rosenzweig and Barnes, 2003). The performance of older animals has been shown, in some instances, to be markedly different from that of younger animals, whereas performance on other tasks is unaffected or only marginally affected in aged subjects. Instead of providing a comprehensive review of the literature regarding age-related cognitive changes, the following discussion focuses on one particular behavior that consistently changes with age, that of spatial learning and memory. This form of learning and memory involves the ability of the organism to acquire and retain information that is critical for successful navigation through space.

The rationale for choosing this particular behavior is based on the notion that the study of aging, using nonhuman animal models, is most effective when the behavior under investigation has some analog to human behavior. If the behavior under investigation changes in an age-dependent manner in both the nonhuman animal model and the human case, then the task of making inferences from the nonhuman subject is greatly simplified. Moreover, a great deal of accumulated evidence indicates that in all mammals tested, spatial abilities are altered during aging. Spatial learning and memory has been the subject of laboratory studies for decades, does not require language to test, and therefore represents an excellent example of a behavior that can be readily tested in both human and nonhuman subjects. This is the focus of the following section.

A. Spatial Learning and Memory: Rodent Models

Maze-learning tasks are very useful methods for investigating age-related changes in rodents, in part because rodents are naturally excellent foragers and therefore tend to learn these sorts of tasks exceptionally well. In such tasks, the animal begins at a specific start position in the maze and then must navigate
to a goal position to receive food or water reward (positive reinforcement) or to avoid foot shock, bright light, or cool water (negative reinforcement). Simple mazes require the animal to learn a limited number of discriminations to reach the goal target, and delays can be imposed to complicate the task. Complex mazes, on the other hand, require the animal to learn and remember a greater number of discriminations in order to reach the target goal (see Fig. 15-1).

Behavioral investigation of age-associated memory changes using mazes dates back at least to the seminal work of Stone (1929a, 1929b), who used a battery of tests to investigate age-associated learning and memory changes in rats up to 24 months of age. Although the results reported by Stone did not reveal significant differences between groups of rats when the data are considered on a per-animal basis, a small proportion of the oldest rats tested by Stone did show deficits. One possible explanation for the weak aging effect reported by Stone is that he used a long-lived strain of rat with an average life span of 36 months. Thus, he may have been collecting data from "biologically younger" animals or rats in late maturity as opposed to old age. In fact, subsequent studies have consistently found age-related deficits using multiple T-mazes (similar to one the Stone himself used) and shorter-lived rat strains (e.g., Goodrick, 1968; Klein and Michel, 1977; Skalicky et al., 1984; Ingram, 1985; Goldman et al., 1987; Lohninger et al., 2001). For example, an early study by Verzar-McDougall (1957) reported that with advancing age, rats have greater difficulty with both the acquisition and the retention of the complex T-maze task (Fig. 15-1A). Moreover, after 20 months of age, individual variability increases significantly, with the oldest rats performing the worst. These studies highlight the importance of considering the issue of whether the subjects tested are truly "aged."

Many other tasks have been used to assess spatial memory in rats of different ages. One such task is the circular platform task (also known as the Barnes maze), which was developed specifically for age comparisons of spatial memory (Fig. 15-1B). This task is well suited for testing aged animals because it does not require food or water deprivation for motivation, and performance is not affected by age-related changes in speed or stamina. For this task, the rat is placed onto a large, open, brightly lit platform and must discover which of 18 holes, located on the perimeter of the platform, leads to a dark escape chamber. Rats prefer dark enclosures to brightly lit open spaces and are therefore motivated to complete the task based on this preference. The escape hole is always placed in the same location of the room with respect to distal cues in the environment. Thus, the most effective strategy for solving this task over multiple trials is to remember the location of the escape box in relation to the distal cues in the room (i.e., spatial strategy). Compared to young rats, aged rats tend to have difficulty learning the location of the escape tunnel and retaining the memory of the tunnel's location from day to day (e.g., Barnes,
FIGURE 15-1  (A) Diagram of the 14-unit T-maze, or Stone maze. The start box is indicated by an S, and the arrows show the direction in which the animals must travel to find the goal box (G) correctly. Guillotine doors are used to divide the maze into five segments (indicated by lines at choice points) so that the animals are not allowed to go backward through the maze. Motivation for performance can either be food or water reward at the goal or foot shock. (After Ingram, 1988). (B) Photograph of the Barnes maze, or circular platform task, illustrating the rat making an “error” by looking into a hole that is not over the dark escape chamber. The arrow points to the correct location of the hole over the goal, which the rat must find on the basis of the features of the environment distal to the platform. (C) Photograph of a rat swimming in the spatial version of the Morris water maze. The water is made opaque so that the submerged platform is not visible to the animal. The rat must use distal cues in the room to locate the escape platform. The arrow indicates the location of the hidden platform. (D) Photograph of a radial eight-arm maze, with a rat prepared for and attached to electrophysiological recording equipment. Food reward is available at the ends of each of the arms in cups, and on any given trial the cup is baited only once (Gallagher and Bostock, 1985).

1979; Barnes et al., 1980; Barnes and McNaughton, 1985; Barnes et al., 1989; Markowska et al., 1989; McLay et al., 1999; Greferath et al., 2000). These findings have been replicated by Bach et al. (1999) in the aged mouse using a modified version of the circular platform task that included a control version for determining how well older animals could see and use distal cues.

Perhaps the most widely used test of spatial learning and memory in rodents is the Morris water maze (Morris, 1981). In its most basic form, a large circular tub of water is filled with opaque water, and it is the rat's job to locate a hidden escape platform submerged just beneath the surface of the water (Fig.
15-1C). On each trial, the rat is released into the water at different predetermined start locations and allowed to swim until either the escape platform is located or a certain amount of time has elapsed. Initially, rats will swim around the pool randomly. But after having found the platform over repeated trials, most young animals learn to swim directly to the escape platform. The two dependent variables that are typically measured are the latency to find the hidden platform and the length of the path taken to reach the platform, although other, more complex analyses can be used, such as Gallagher's “corrected integrated path length” or “learning index” (Gallagher et al., 1993). After a certain number of training trials (typically 20–24), a probe trial can be administered in which the platform is removed from the tank. Those animals that have learned the task (i.e., the location of the platform) will spend a considerable amount of time swimming in the area where the platform was previously located, whereas those animals that have not successfully learned the task will continue to swim randomly around the tank.

Numerous laboratories have demonstrated that aged rats are consistently slower at learning the Morris water maze task than are adult rats and show poorer retention of the hidden platform location from test day to test day than do younger animals (e.g., Gage et al., 1984; Biegon et al., 1986; Pelleymounter et al., 1987; Rapp et al., 1987; Markowska et al., 1989; Frick et al., 1995; Barnes et al., 1997b; Shen et al., 1997; Bizon et al., 2004; Nicholson et al., 2004; Tombaugh et al., 2005). When the performance of individual rats is assessed for each trial, both young and aged rats show bimodal performance on early learning trials (Barnes et al., 1997b). This means that on some trials, both age groups sometimes take a short path to the platform, but on others take a long path. Over the course of training, however, the performance of young rats is increasingly unimodal, since most young rats will learn consistently to take the most direct path to the platform. In contrast, the aged rats continue to display relatively bimodal performance, even on the final days of training. This indicates that aged rats are not completely forgetting or not learning the location of the hidden platform; in fact, aged rats do show moderate improvements in performance over the course of training. Rather, aged rats have more difficulty than young rats in consistently retrieving the correct memory for the platform's location.

The Morris water maze can be modified to become a visual discrimination task instead of a spatial task, with the platform made visible above the waterline. A large beacon can also be placed over the platform, making it easier to find. When this nonspatial version of the task is used, aged rats without visual deficits have little difficulty solving the task (e.g., Rapp et al., 1987; Rosenzweig et al., 1997; Shen et al., 1997). Additional studies have demonstrated that aged rats can perform well on maze tasks when alternative strategies for solving the task can be used. For example, Barnes et al. (1980) investigated the kind of strategies aged and adult rats use to solve a T-maze
task, altering the task so that it could be performed using (1) a local-cue strategy in which the rat learns to associate a particular arm of the maze with a rubber mat on the track surface; (2) a response strategy in which the rat learns to turn left or right, depending on the sequence of behaviors already performed on the maze; (3) a place strategy in which the rat learns to enter the arm located in a particular area of the room, using extra-maze cues as a coordinate system to make this decision. This study revealed that aged rats were more likely than adult rats to use a response strategy to solve the task, whereas the adult rats were more likely to use a place strategy. This finding, along with those of other laboratories, suggests that aged rats may not use effective spatial strategies to solve these problems (e.g., Barnes et al., 1980; Rapp et al., 1987; Gallagher and Pelleymounter, 1988).

Finally, one additional task that has been used to test spatial learning and memory function is the eight-arm radial maze (Fig. 15-1D), which consists of a central platform with eight arms containing rewards radiating from it. The rat is required to obtain each of the rewards without reentering an arm where reward has already been obtained. Revisiting arms where reward was already obtained is considered an error of spatial working memory. The task can be made more difficult by imposing a delay between the retrieval of the first four and the last four rewards. Numerous laboratories have demonstrated that aged rats are impaired on this task, with and without the imposed delay (e.g., Barnes et al., 1980; J.E. Wallace et al., 1980; de Toledo-Morrell et al., 1984; Beatty et al., 1985; de Toledo-Morrell and Morrell, 1985; Gallagher et al., 1985; van Gool et al., 1985; Geinisman et al., 1986; CaprioH et al., 1991; Mizumori et al., 1996; McLay et al., 1999; Rossi et al., 2005). When this task is modified so that it can be solved using nonspatial strategies (i.e., a local-cue strategy), aged rats perform as well as young rats (Barnes et al., 1987).

B. Spatial Learning and Memory: Human Studies

Age-related declines in navigational skills are well documented in humans (for review see Rosenzweig and Barnes, 2003; Allen et al., 2004; Driscoll et al., 2005). An example from an early study that examined this issue used a large-scale real-world environmental setting to examine the navigational performance of aged (60–80 years) and middle-aged (26–45 years) residents. The task was to recall as many buildings as possible from the downtown area of Orange, California, and to locate them on a grid. To minimize the types of variables that can negatively impact aged subjects’ performance (i.e., speed, anxiety), the task was self-paced, and subjects were told that the purpose of the study was to help urban planners design better cities. Although aged subjects had actually lived in the area longer than the younger subjects, they had less complete knowledge of their home environment when asked to recall
environmental features and locate them accurately on a grid (Evans et al., 1984). A more recent study by Uttl and Graf (1993) assessed the spatial memory of several hundred young and old visitors (15–74 years of age) to a museum exhibit. In this study, participants were required to recall three pieces of information: (1) the route they traveled through the exhibit, (2) the landmarks they encountered on the route they traveled, and (3) the temporal order of landmarks they encountered. Aged subjects were able to recognize the landmarks they had encountered, but they had difficulty recalling the route they traveled through the exhibit as well as the temporal order of the landmarks they encountered (Uttl and Graf, 1993).

As noted in the previous section, the Morris water maze is one of the most widely used tests of spatial learning and memory for rodents. A task has been developed for humans that parallels the Morris water maze task used for rats. In the human version, subjects are explicitly instructed to learn the location of a landmark relative to extra-maze cues that are located outside of a large arena. Subjects are then required to place the landmark in the correct location after the environment has been manipulated in some way. As observed in aged rats, aged humans have more difficulty learning the correct location of the landmark than do young subjects (Newman and Kaszniak, 2000). These findings closely parallel those seen with aged rats in the Morris water maze or the circular platform task.

Virtual navigation tasks have become increasingly popular in assessing human spatial cognition. For example, Moffat et al. (2001) had subjects “navigate” a computer-generated maze that consisted of a series of interconnected hallways and alleys, some of which led to target goal positions within the virtual environment while others led to dead ends. Performance of the aged subjects was significantly impaired on this task; following the fifth learning trial, 86% of the young participants located the target goal without error, whereas only 24% of the aged subjects performed at this level. In addition to making significantly more spatial memory errors (i.e., traveled to more dead ends), aged participants traveled a greater distance and took longer to complete the task than did younger subjects.

A virtual analog of the Morris water maze, known as the computer-generated arena (C-G arena), has also been developed (Jacobs et al., 1997, 1998) and has been used to assess age-related changes in human spatial navigation (Thomas et al., 1999). For this task, participants are required to search and locate a target hidden within the virtual environment. Compared to younger adults, older adults had difficulty learning the location of the platform and travel a greater distance through the virtual environment in their search for the platform. In addition, on subsequent probe trials, aged subjects spent less time in the quadrant of the virtual environment that had previously contained the platform than did young subjects. This same pattern of results is demonstrated even when environmental support (i.e., a larger platform, more salient distal
cues) and more practice trials are allowed prior to testing (Thomas et al., 1999).
As noted earlier for the rodent studies, aged rats have particular difficulty with
tests that rely on knowledge of the environment. But if the task can be solved
using an alternative strategy, aged rats perform as well as do young rats (e.g.,
Barnes et al., 1980). Young adults in the Thomas et al. (1999) study reported
using a spatial strategy to solve the task, whereas aged adults were less likely to
report using a spatial strategy. Thus, these findings, together with those described
for rodents, support the idea that aged subjects preferentially employ non-spatial
strategies to solve spatial tasks when given an opportunity.

IV. INVOLVEMENT OF THE HIPPOCAMPUS IN SPATIAL
LEARNING AND MEMORY

A fundamental goal for research in the area of behavioral neuroscience is to
determine which brain structures are necessary for which behaviors. Some of
the very earliest attempts to find brain structures that participate in memory
relied heavily on lesion methods (for review see Olton, 1991). Defining a role
for the hippocampus in memory processes employed a number of lesioning
techniques, such as knife cut of the fimbria-fornix, aspiration of the hippo­
campus, electrolytic lesions, and chemical lesions. Although many of the tech­
niques that have been used to lesion the hippocampus are not selective and
may damage closely lying brain structures or fibers-of-passage, these approaches
have provided experimental evidence for a critical role of the hippocampus in
spatial memory. For example, Morris et al. (1982) removed the entire dorsal
and ventral hippocampus and a small area of overlying cortex in rats and then
assessed the effects of these lesions on spatial learning and memory using the
Morris water maze. Compared to control rats that had comparable cortical
lesions, the hippocampal lesioned rats took longer to locate the hidden platform
on the spatial version of the task. But when the platform was visible, lesioned
rats performed no differently from control rats. Moreover, on the probe trial,
when the platform is removed from the tank, control rats showed a preference
for the quadrant of the tank where the hidden platform was previously located,
whereas the lesioned rats did not show this preference. Animals with lesions
of the hippocampus and its closely related structures also show severe deficits
on other spatial tasks, such as the 14-unit T-maze (Bresnahan et al., 1988), the
Y-maze (Aggleton et al., 1986), the radial arm maze (Olton et al., 1978), and
the circular platform task (McNaughton et al., 1989). These deficits mimic
those observed in the memory-impaired aged rat on the same tasks; the deficits
associated with hippocampal lesions affect performance when the task is spatial
in nature but not when the task is nonspatial.

Neuronal recordings from the hippocampus have also provided evidence
that the hippocampus is involved in spatial learning and memory. When a rat
is allowed to explore an environment, pyramidal and granule “place cells” show patterned neuronal activity that is highly correlated with the rat's position in space (i.e., the place field; O'Keefe and Dostrovsky, 1971). When the activity of many place cells are recorded simultaneously, the position of the rat in a given environment can be accurately reconstructed from the cell firing activity alone (Wilson and McNaughton, 1993). There are several changes that occur to the “hippocampal maps” (i.e., distribution of place cell firing) of aged rats that may contribute to why or how spatial learning and memory deficits occur. For example, in young rats, the maintenance or stability of any particular map can remain intact for long periods of time (i.e., months) such that when a rat is returned to a previously explored environment, the hippocampal map for that particular environment is reliably retrieved (Thompson and Best, 1990). For the aged rat, retrieval of the correct map is somewhat less reliable; upon return to a previously encountered environment, the aged rat is more likely than a young rat to show a different population of activated place cells in a familiar environment (i.e., a different place map is retrieved; Barnes et al., 1997b). This finding correlates well with the bimodal performance of aged rats late in training on the spatial version of the Morris water task described earlier.

Several human studies using functional brain imaging have also supported the idea that the hippocampus is critically involved in spatial memory. For example, Maguire et al. (1998) used positron emission tomography (PET) to scan subjects’ brains while they navigated a virtual town. Brain activation was measured in a condition in which the subject navigated directly to a goal, compared to a control condition in which the subject was directed via arrows through the town. The right hippocampus was shown to be more active during navigation than during passive traversal of the town. In addition, the degree of hippocampal activation was positively correlated with more accurate navigation to the goal. A recent study conducted by Moffat et al. (2005) identified differences in brain activation for young and aged subjects during navigation of a virtual environment consisting of several rooms and interconnected hallways. Subjects were instructed to locate and remember the location of six objects within the environment, and performance of this task was compared to a control task in which the subjects followed a path through a similar environment. This study demonstrated that aged and adult subjects had different patterns of brain activation during navigation; in particular, activation of the hippocampus and parahippocampal gyrus was greater in young subjects and was positively correlated with performance on the navigation task (Moffat et al., 2005). Taken together, the results from these studies point to the hippocampus (see Figure 15-2) as a structure that may underlie age-related spatial memory. In fact, the hippocampal formation is among the brain structures that show the earliest signs of age-related changes in rodents, monkeys, and non-demented humans and is an important target structure in the etiology of
Alzheimer's disease (e.g., Hyman et al., 1984; Khachaturian, 1985; for a recent review see Hof and Morrison, 2004). The following discussion provides selected examples of work that attempt to correlate changes in the hippocampus in aging rats and humans with their memory performance. The experiments to be highlighted use neuroanatomical (including imaging studies), electrophysiological, and molecular methods for the correlation of brain function with behavior.

A. Neuroanatomical Findings

Early work on the question of whether aging is accompanied by significant neuron death reported that as much as 25–50% of the neurons in neocortical areas and some hippocampal subfields were lost in humans without Alzheimer's disease as well as in nonhuman primates and rodents (Brody, 1955; Ball, 1977; Brizzee et al., 1980; for review see Coleman and Flood, 1987). These studies, however, were not conducted using unbiased sampling methods, and thus the results were based on neuron density in the structure under investigation instead of on total neuron numbers (for review see Morrison and Hof, 1997). More recent studies that have employed unbiased stereological methods\(^3\) have not detected a significant age-related loss of principle cells in the rat (Rapp and Gallagher, 1996; Rasmussen et al., 1996), mouse (Calhoun et al., 1998), nonhuman primate (M.J. West et al., 1993; Peters et al., 1996), or human (M.J. West, 1993). These studies, therefore, have led to the conclusion that significant neuron loss is not an inevitable consequence of the normal aging process, at least with respect to the hippocampus and much of the neocortex.\(^4\)

In addition, although the rate of hippocampal neurogenesis declines with increasing age, new neurons do continue to be generated in the aged rat hippocampus (e.g., Kuhn et al., 1996; Kempermann et al., 2002; Heine et al., 2004). Granule cells are also born in the aged mouse dentate gyrus, and the rate of neurogenesis can be significantly increased by exercise (van Praag et al., 2005; Kronenberg et al., 2005). It remains to be determined, however, if the rate of neurogenesis affects memory function; one study has reported that exercise-induced increases in neurogenesis are associated with better performance on the Morris water maze (van Praag et al., 2005), whereas other studies have found no correlation (Bizon and Gallagher, 2003) or a negative correlation (Bizon et al., 2004) in the aged rodent.

\(^3\)Stereological methods are used to analyze biological tissue in 3D. This method uses unbiased sampling and estimation methods to determine parameters of volume, surface area, length, and number in the biological tissue being analyzed.

\(^4\)A recent study by D.E. Smith et al. (2004) has reported a 30% reduction in neuron number of area 8A of the dorsal lateral PFC.
If it is largely true that principle cell numbers are not altered in the aged hippocampus, then what is responsible for the age-related hippocampal-dependent cognitive changes that have been observed? Likely anatomical candidates include the dendrites and axons that connect cells to each other. Early studies that investigated dendritic branching suggested that age-related deterioration occurred in the hippocampus and entorhinal cortex (M.E. Scheibel et al., 1976; A.B. Scheibel, 1979), but these reports were later challenged, primarily on the basis that both demented and nondemented subjects were included in the analysis and nonstereological techniques were used. Subsequent studies demonstrated extensive dendritic branching in CA1, CA3, and the subiculum of both aged and young humans (Flood et al., 1987a; Flood, 1991; Hanks and Flood, 1991), and some studies have also reported increased den-
dendritic branching and length in the DG of aged compared to young adults (Flood et al., 1987b). Increased dendritic branching has also been reported in CA1 pyramidal cells of aged rats (Pyapali and Turner, 1996).

Additional studies have investigated possible changes in cell connectivity by assessing synapse numbers, but these attempts initially produced mixed results (Bondareff and Geinisman, 1976; Geinisman, 1979; Anderson et al., 1983; Scheff et al., 1985), either as an artifact of the method used to count synapses or because the cognitive status of the animals included was not known. More recent studies using unbiased stereological methods and behaviorally characterized rats have provided a clearer picture. For example, work by Geinisman and his colleagues (1992) demonstrated a decrease in the number of perforated and nonperforated axospinous synapses in the inner and middle molecular layers of the aged dentate gyrus. This finding was specific to aged memory-impaired rats; groups of young rats and aged rats that were not memory impaired were not significantly different from one another. Physiological measures obtained from the dentate gyrus support this finding, showing an age-related decrease in the presynaptic fiber potential elicited by stimulation of the perforant path (e.g., Barnes and McNaughton, 1980a; Foster et al., 1991), indicating the presence of fewer perforant path collateral axons originating from layer II entorhinal cortical cells. In addition, an age-related decrease in the field EPSP has been recorded from dentate granule cells (e.g., Barnes, 1979; Barnes and McNaughton, 1980a; Foster et al., 1991). Both of these findings support the idea that synapse loss is a feature of the aged dentate gyrus.

In area CA1, however, the number of axospinous synapses are not different between aged memory-impaired and unimpaired or young animals (Geinisman et al., 2004). This might seem surprising since the field EPSP in CA1 is reduced in the aged rats (Landfield et al., 1986; Barnes et al., 1992; Deupree et al., 1993). In contrast to the dentate gyrus, the presynaptic fiber potential in CA1 remains unchanged in aged rats (Barnes et al., 1997a; Rosenzweig et al., 1997; Barnes et al., 2000b), suggesting that the decrease in the field EPSP is a result of a loss of functional synaptic contacts rather than a loss of incoming axons. Additional analysis of the synaptic contacts in the CA1 region of aged rats has focused on changes in the size of the postsynaptic density (Nicholson et al., 2004), a region where neurotransmitter receptors, such as those for glutamate, are particularly dense (Peters et al., 1991). The size of the postsynaptic density at axospinous perforated synapses was reduced in memory-impaired aged rats (Nicholson et al., 2004). This finding, taken together with the physiological data, does suggest that a decrease in functional synaptic contacts is a feature associated with aging in this brain region. This raises the possibility that the population of synaptic contacts with smaller postsynaptic densities are the ones that are functionally silent (see Atwood and Wojtowicz, 1999).

Although neuroanatomical measures have been taken from human tissue, there are several methodological issues that impact the results obtained from
human tissue samples. For example, the cognitive status of the brain donor may not be known, and issues related to how the brain tissue was handled or preserved prior to analysis is not always consistent. Well-designed structural MRI studies can avoid some of these difficulties and therefore represent a powerful method that can be used to measure potential age-related neuroanatomical changes and to correlate such changes with cognitive function. The relationship between hippocampal size and memory function, however, is not as straightforward as one might first suppose. Studies that have used this technique have reported conflicting results; some studies demonstrate a significant positive correlation between hippocampal volume and memory function, some a significant negative correlation, while others have demonstrated no correlation (for recent reviews see Hedden and Gabrieli, 2005; Van Petten, 2004). There are numerous variables that may contribute to the variability across studies, including the age of the participants in the study, the types and sensitivity of the neuropsychological tests used to assess cognitive function, if or how normalization to head size and/or body size is carried out, and possibly the inclusion of subjects in the early stages of Alzheimer’s disease or some other underlying pathology. A recent comprehensive meta-analysis of 33 studies by Van Petten (2004) demonstrated that the relationship between hippocampal volume and episodic memory in healthy aged subjects was weak, due to significant variability across studies. Van Petten did find, however, that the trend was toward volume-memory correlations becoming more positive as the age of the sample under investigation increased (i.e., greater volume is associated with better memory performance). Since the current discrepancy in the literature may be partly due to the inclusion of subjects in the early stages of Alzheimer’s disease, assessing hippocampal volume in an organism that does not get Alzheimer’s disease is critical. For example, a recent study by Shamy et al. (2006) measured hippocampal volume in behaviorally characterized rhesus macaques. The results of this study indicate that memory-impaired aged monkeys differ by less than 6% from younger monkeys in hippocampal volume. These findings provide additional support for the idea that normal cognitive aging occurs independent of gross structural deterioration in the hippocampus.

B. Electrophysiological Findings

Despite changes at the morphological level, most of the basic electrical properties of the hippocampus remain constant over the life span. Studies investigating resting membrane potential, membrane time constant, threshold to action potential, input resistance, and the amplitude and duration of the $\text{Na}^+$ action potential have not revealed age-associated changes in these properties (for review see Barnes, 1994; Rosenzweig and Barnes, 2003). Some studies,
have shown an increase in the amplitude of the after-hyperpolarizing potential (AHP) for pyramidal cells of the CA1 subregion (e.g., Landfield and Pitter, 1984; Disterhoft et al., 1996; Thibault and Landfield, 1996), and this change has been correlated with cognitive functioning (Disterhoft et al., 1996; Tombaugh et al., 2005). The larger AHP may be due to changes in \( \text{Ca}^{2+} \) homeostasis, since the outward \( \text{K}^{+} \) current that underlies the AHP is \( \text{Ca}^{2+} \) dependent (Thibault and Landfield, 1996). In fact, aged CA1 pyramidal cells have a greater density of L-type \( \text{Ca}^{2+} \) channels, which may be a major contributing factor to the increased AHP (Thibault and Landfield, 1996). Although the larger AHP predicts slower firing frequencies, no firing rate change has been observed for CA1 pyramidal neurons in vivo. Pyramidal cells of CA3, however, have recently been shown to have elevated firing rates in vivo (I.A. Wilson et al., 2005), suggesting the possibility that the larger AHP in CA1 is an adaptive change that serves to normalize CA1 firing rates (i.e., doesn’t allow CA3 to hyperactive CA1).

One particular form of long-term synaptic plasticity, known as long-term potentiation (LTP), is thought to be a potential cellular mechanism for long-term memory (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973) and involves the strengthening of connections between neurons. A great deal of work has also been aimed at determining if changes in either the induction or the maintenance of LTP contributes significantly to the memory deficits associated with aging. Although variable results have been reported with respect to LTP induction across age, the differences can be attributed to the specific stimulation parameters used to induce LTP and the region of the hippocampus under investigation. When high-intensity stimulation protocols that are well above threshold for LTP induction are used, there are no age-related deficits in LTP induction at the CA3-CA1 Schaffer collateral synapse (e.g., Landfield and Lynch, 1977; Landfield et al., 1978) or the perforant path–granule cell synapse (e.g., Barnes, 1979). When stimulus parameters are set very close to the LTP induction threshold, however, aged rats consistently show LTP induction deficits at the Schaffer collateral CA1 synapse and the perforant path–granule cell synapse (e.g., Deupree et al., 1993; C.I. Moore et al., 1993; Barnes et al., 2000a).

After LTP has been induced, the durability or maintenance of LTP can also be measured and can be divided into an early phase, lasting one to three hours, and a late phase, lasting for more than 24 hours. New protein synthesis is not required to sustain the early phase of LTP, but new RNA and protein synthesis is required to maintain the late phase. Several studies have demonstrated that in the short term (i.e., over 1 hour), LTP decay rates are not different between aged and young rats (e.g., Landfield and Lynch, 1977; Landfield et al., 1978). Over a longer time course, however, clear age-related maintenance deficits do appear (e.g., Barnes, 1979; Barnes and McNaughton, 1980b; for review see Barnes, 2003). For example, Barnes and McNaughton (1980b) administered LTP-inducing stimuli at 24-hour intervals on 12 consecutive days and then
monitored the evoked field response for several weeks. Although there were no age-related differences in the final levels of LTP induction, LTP decayed nearly twice as fast in the aged rats over several weeks.

Several experiments have also demonstrated a significant correlation between LTP and memory deficits in aged animals (Barnes, 1979; Barnes and McNaughton, 1980b; Barnes and McNaughton, 1985; de Toledo-Morrell and Morrell, 1985; Bach et al., 1999). This was first demonstrated by Barnes (1979), who showed that aged rats took longer paths and a greater amount of time to solve the circular platform spatial memory task and also had greater difficulty learning a reversal or change of location for the escape box (Fig. 15-1B). These deficits in spatial memory performance were significantly correlated with the durability of synaptic enhancement in the dentate gyrus both within and between age groups. In subsequent studies, Barnes et al. (1980, 1985) also demonstrated a significant similarity between the rates of acquisition and forgetting of spatial memory and the induction and maintenance of LTP. Aged rats were slower to reach asymptotic performance levels and forgot the problem faster than did young rats.

Similarly, asymptotic LTP levels were reached more slowly and decayed more rapidly in aged rats. In both cases, the rate of decay for the memory of the escape box and LTP was nearly twice as fast for the aged rats. Bach et al. (1999) also found a significant relationship between LTP decay rates in area CA1 in vitro and performance on the Barnes maze in aged but not young mice. These findings are further supported by observations that old animals with spatial memory deficits on the eight-arm radial maze (Fig. 15-1D) also show faster decay rates for hippocampal LTP (de Toledo-Morrell and Morrell, 1985). Taken together, the currently available data indicate that learning and memory deficits observed in aged rats and mice parallel deficits in the induction and maintenance of LTP.

C. Molecular Findings: From Molecules to Memory

The neural signaling events necessary for long-lasting changes to occur in the brain are numerous and will either originate or ultimately converge at the nucleus where information from the entire cell is integrated and transmitted into the transcription of thousands of genes. In turn, this genetic transmission of information can have far-reaching effects on the expression of neuronal proteins, affecting the function not only of the individual neuron but potentially of entire neuronal networks. Long-term memory and LTP are two examples of long-lasting changes in neuronal function that are a result of changes in gene expression. The study of the molecular basis of memory function has resulted in the identification of several molecules now known to be needed for normal cognitive functioning. These molecules include the consti-
tutively expressed regulatory transcription factors, such as CREB, and several downstream immediate-early genes (IEGs), such as c-fos, zif268, and Arc. These molecules are the focus of the following discussion. First, it should be noted that the evaluation of IEG studies is complicated by a number of factors, including: (1) the different methods used to induce their expression (e.g., electrical stimulation that induces seizures or LTP, chemical stimulation with drugs, behavioral stimulation, such as exploration of a novel environment or learning and memory tasks, or no manipulation, so that the constitutive, or resting, activity of these genes can be evaluated); (2) the methods used to evaluate where and to what degree their expression takes place [e.g., in situ hybridization or immunohistochemistry to look at the proportions of cells that express a particular mRNA or protein, respectively, reverse transcriptase polymerase chain reaction (RT-PCR) to evaluate the number of gene copies for a given sample]; (3) the brain regions under investigation (e.g., different regions of the hippocampus, such as the dentate gyrus, cornu ammonis, subcortical or different cortical regions). In spite of the fact that the conclusions drawn are constrained and differ depending on the methods applied, there are several key molecules now known to be involved in memory processes. Moreover, age-associated alterations in the expression of some of these molecules are thought to contribute to the learning and memory deficits observed in the aged organism. For an overview of the cell-signaling cascades that are discussed in the text, see Figure 15-3.

1. cAMP-Response-Element-Binding Protein (CREB)

cAMP-response-element-binding protein (CREB) has been the focus of considerable attention in the field of learning and memory, although the exact mechanism through which CREB works is still debated (Mayr and Montminy, 2001; Cha-Molstad et al., 2004). CREB is a common target for many neuronal signaling pathways and can be activated by kinases, such as mitogen-activated kinase (MAPK), signaling pathways involving adenylyl cyclase, cAMP, and also Ca^{2+} (for overview see Nguyen and Woo, 2003; Carlezon et al., 2005). In the nucleus, CREB binds as a dimer to cAMP-response element (CRE) found in the promoter region of numerous genes, and it becomes “active” when it is phosphorylated at serine 133 (p-CREB). CREB may be present in the nucleus in one of two forms, as an activator of gene transcription (CREB-1) or as a repressor of gene transcription (CREB-2). In terms of memory function, overexpression of CREB-1 reduces the number of trials needed to train Drosophila on a memory task, whereas overexpression of CREB-2 blocks the formation of long-term memory (Yin et al., 1995). Similarly, results from studies using knockout mice with a targeted disruption of the alpha and delta isoforms of CREB suggest impairments in spatial memory (Bourtchuladze et al., 1994). Blocking CREB expression in rats with antisense oligonucleotides
Neurotrophin AMPA receptors, NMDA L-type Ca\(^{2+}\) G-protein coupled receptors

Figure 15-3 Simplified scheme of some of the cellular events involved in the regulation of CREB. Neurotransmitters and neurotrophins act at membrane receptors, such as TrkB in the case of neurotropins and AMPA, NMDA, and G-protein-coupled receptors in the case of neurotransmitters such as glutamate and dopamine, to trigger intracellular signaling cascades that culminate in phosphorylation (P) of CREB within the nucleus. Phosphorylation of CREB at serine 133 activates CREB-mediated gene transcription. The cell-signaling events depicted are shown as relatively separate processes, but there is often interaction among the different signaling cascades. The protein products of just a few of the numerous genes targeted by CREB are indicated. Abbreviations: AC, adenylyl cyclase; CaM, calmodulin; CaMK, Ca\(^{2+}\)/calmodulin-dependent kinase II and IV; CRE, cAMP-response element; MAPK, mitogen-activated protein kinase; PDE, phosphodiesterase; PLC, phospholipase C; RSK, MAPK-activated ribosomal S6 kinase; TrkB, neurotrophin tyrosine kinase receptor type 2.

Injected directly into the hippocampus also impairs long-term memory for the Morris water maze without impairing initial learning of the task (Guzowski and McGaugh, 1997). The role of CREB has also been investigated in plasticity with regard to its role in LTP. For example, mice with a partial knockout of CREB-1 show normal induction of LTP in area CA1 of the hippocampus; but in these animals, LTP decays to baseline more rapidly than it does for normal control animals (Bourtchuladze et al., 1994).

A number of studies have suggested that there is dysregulation of CREB activity in the aged brain. Monti et al. (2005), reported that basal levels of p-CREB were significantly increased in the hippocampus of aged rats. But when measured 24 hours after a fear-conditioning task, on which aged rats were impaired, the opposite was observed; aged rats had less p-CREB in the hippocampus than did young rats. At least two studies have also investigated...
potential age-related changes in the number of hippocampal neurons that express CREB. For example, Kudo et al. (2005) showed that a smaller proportion of cells in area CA1 express p-CREB in aged compared to young rats two hours after a contextual fear-conditioning task on which the aged rats were impaired. No age difference was found in the dentate gyrus or area CA3, nor was an age difference observed for levels of unphosphorylated CREB (Kudo et al., 2005). This finding is supported by Ramos et al. (2003), who did not find a significant age effect on the number of CA3 neurons that expressed p-CREB following a spatial working memory task, although these authors did report higher levels of p-CREB in the aged prefrontal cortex. Age-associated changes in CREB-1 but not CREB-2 activity have also been observed. Work by Brightwell et al. (2004) demonstrated a reduction in CREB-1 but not CREB-2 protein in the hippocampus of aged memory-impaired rats compared to nonimpaired aged rats or young rats following transfer training on the Morris water maze. This suggests that the dysregulation of CREB-1 (activator of gene transcription) but not CREB-2 (repressor of gene transcription) may contribute to the spatial memory deficits observed among some aged subjects, most likely as a result of alterations in downstream CREB-dependent gene transcription (e.g., BDNF or Arc).

2. Immediate-Early Genes

One of the next steps in the molecular events that lead to long-term memory formation involves the transcription of a class of genes known as immediate-early genes (IEGs). As the name suggests, IEGs are among the first group of genes to be expressed following synaptic activity, and they are defined as those genes expressed in the presence of protein synthesis inhibitors (Sheng and Greenberg, 1990). These genes can be broadly classified into (1) genes encoding transcription factors, such as c-fos and zif268 (also known as Krox-24, EGR-1, NGFI-A), that influence cellular activity by regulating the expression of target genes, and (2) genes coding for effector proteins, such as Arc (activity-regulated cytoskeletal gene; also known as Arg3.1), Narp (neuronal activity-regulated pentraxin), and BDNF (brain-derived neurotrophic factor), that directly affect cellular function (Hughes and Dragunow, 1995; Hughes et al., 1999). Correlative studies provided the first evidence that the expression of IEG RNAs and proteins can be increased in hippocampal neurons following training on behavioral tasks such as two-way avoidance (e.g., Nikolaev et al., 1992), brightness discrimination (e.g., Grimm and Tischmeyer, 1997), exposure to a novel environment (Papa et al., 1993; Hess et al., 1995b; C.S. Wallace et al., 1995; Pinaud et al., 2001), odor discrimination tasks (Hess et al., 1995a), and spatial learning tasks (e.g., Guzowski et al., 2000, 2001). In addition, several studies have demonstrated that hippocampal stimulation resulting in LTP also results in the expression of a number of IEGs in the hippocampus
(e.g., Cole et al., 1989; Worley et al., 1993; Lanahan et al., 1997; Guzowski et al., 2000; J.L. Lee et al, 2004).

Simply demonstrating that IEGs are induced, however, does not provide evidence that IEGs are necessary or sufficient for maintaining long-lasting changes in neuronal function. In order to demonstrate a more direct role for IEGs in memory function, a number of studies have selectively disrupted the expression of particular IEGs. For example, disrupting the expression of Arc and zif268 using antisense oligonucleotides or knockout technology, respectively, demonstrated that the durability of both long-term spatial memory and LTP are significantly compromised (Guzowski et al., 2000; Jones et al., 2001). These findings in adult animals mimic to some extent those seen in memory-impaired aged animals. Overall, studies like these, which employ strategies to selectively disrupt the expression of specific genes, together with the correlative studies discussed earlier, provide strong evidence that several of the IEGs are required for long-lasting functional changes in the brain.

Studies of IEG expression in the aged brain have focused mainly on potential changes in transcription factors such as zif268 and c-fos. One of the first studies systematically to investigate the transcription factor response of the aged hippocampus used LTP-inducing stimulation to induce gene expression (Worley et al., 1993). The authors report that although aged rats show accelerated rates of decay of the enhanced synaptic response following this treatment, the transcription factor responses do not differ between age groups. A later study that evaluated the expression of a panel of 19 transcription factors and effector IEGs following LTP induction using a reverse Northern strategy, however, found that c-fos was elevated in the hippocampus of aged rats compared to young rats (Lanahan et al., 1997). No age-related difference was found for the other 18 genes investigated in this study. The apparent contrast in these results as compared to the findings of Worley et al. (1993) can be explained by the different methods used to measure gene expression. Overall, these findings might be taken to imply that only very subtle differences in gene expression occur during aging or that the LTP induction process itself will not reveal age differences because it results in brain activation that is more synchronous and intense than would occur under normal conditions. If the latter interpretation is correct, then are there different approaches that might be taken to this question? Studies that have used behavioral treatments to induce IEG expression have found age differences for some genes, indicating that this approach may be preferable.

One example of this is provided by Touzani et al. (2003), who investigated behavioral activation of c-fos in adult and aged mice following performance

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5The study by Worley et al. (1993) measured the density of the mRNA from autoradiographs following in situ hybridization. The study by Lanahan et al. (1997) used a reverse Northern strategy, in which RNA is detected with a hybridization probe and then the amount of RNA present in the sample under investigation is analyzed using gel electrophoresis.
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on a radial-arm maze task. As expected, aged mice had difficulty learning the
maze task compared to the adult mice, and immunohistochemistry revealed
that aged mice had fewer fos-positive cells than did adult mice after a six-day
retention test in both the CA1 and CA3 regions of the hippocampus but not
in dentate granule cells. A second example of age-related changes in behavior-
ally induced IEG expression comes from the work on Arc. As discussed earlier,
Arc is an effector IEG whose role in long-term plasticity in the brain has been
well established (see Steward and Worley, 2002, for review). A study by Small
et al. (2004) used a fluorescent in situ hybridization protocol with high cellular
resolution (known as catFISH; see Guzowski et al., 2005, for a survey of this
technique) to map Arc expression in the hippocampus of aged and adult rats
after they explored a novel environment (Small et al., 2004). This study dem-
strated that in aged rats there is a decrease in the proportion of cells express­
ing Arc mRNA in the dentate gyrus but not in other hippocampal subfields.
Additional results from this group using real-time RT-PCR to quantify Arc
mRNA expression in the CA1 region of the hippocampus have revealed that
basal levels of Arc are lower in aged rats (i.e., in rats without behavioral or any
other inducing stimuli); but when Arc is induced by exploration of a novel
environment, memory-impaired aged rats show a more robust increase in Arc
expression than do adult rats. Taken together, these results indicate that despite
lower basal levels of Arc mRNA, the behavioral treatment used to induce Arc
ultimately brings Arc mRNA levels of the aged rats up to levels equivalent to
those of adult rats. This finding may indicate a compensatory mechanism in
the aged brain, although the avenue through which such a mechanism would
work remains undiscovered.

In addition to the studies that have investigated stimulation- or behavior-
induced levels of IEGs in the aged brain, there are studies that have sought
specifically to characterize basal levels of IEG expression.6 In the case of zif268,
Yau et al. (1996) found that its mRNA decreases with age in area CA1 but
not in other hippocampal regions, using in situ hybridization and densitometry
analysis. Using gene microarray technology, Blalock et al. (2003) reported that
zif268 is one of several IEGs down-regulated in the aged CA1. In addition,
Desjardins et al. (1997) observed a decrease in the number of cells that express
zif268 protein in the aged CA1, using immunohistochemistry and cell count
methods. Taken together, the results of these studies demonstrate that basal
levels of zif268 mRNA and protein are reduced in area CA1 of the aged brain,
likely as a result of fewer cells expressing zif268 mRNA and protein. In the
case of c-fos, Desjardins et al. (1997) reported no age difference in the number
of cells that express c-fos protein. D.R. Smith et al. (2001) found no age-related

6Although many IEGs are activity dependent and show very low levels of constitutive expres­
sion (e.g., Arc), some IEGs show relatively high constitutive levels of expression (e.g., zif268 and
C-fos).
change in basal levels of hippocampal $c\text{-}fos$ mRNA expression using Northern blot analysis. These authors also investigated potential age-associated changes in basal expression of $c\text{-}jun$ and activator protein-1 (AP-1). AP-1 is a protein complex composed of homodimers of Jun family proteins or heterodimers of Jun and Fos family proteins. If the levels of either $c\text{-}fos$ or $c\text{-}jun$ undergo age-associated changes in their expression levels, then the expression and DNA binding activity of AP-1 is likely to also be affected. D.R. Smith et al. (2001) reported that the basal levels of $c\text{-}fos$ and $c\text{-}jun$ mRNA were not different between aged and young rats, nor was AP-1 binding activity or composition different between age groups.

Finally, gene microarray studies have been applied to the question of whether there are broad patterns of gene expression disruption during aging (Jiang et al., 2001; C.K. Lee et al., 2000; Blalock et al., 2003; Verbitsky et al., 2004). One of these studies used behaviorally characterized aged and young rats in an attempt to correlate changes in basal levels of gene expression in area CA1 with cognitive decline. The basal expression of many genes were found to be up-regulated, including several associated with CA$^{2+}$ regulation and inflammation, while those found to be down-regulated in aged memory-impaired rats included genes associated with biosynthesis, energy metabolism, and activity-regulated synaptogenesis (Blalock et al., 2003). In addition, the basal levels of two effector IEGS, $Arc$ and $Narp$, were down-regulated in area CA1 of aged memory-impaired rats. A recent study has also reported on basal levels of $Arc$ protein, demonstrating that $Arc$ protein expression is higher in the hippocampus of aged compared to adult rats, using a Western blot technique (Monti et al., 2005). Because this is the first study to report on $Arc$ protein levels in the aged brain, additional work is needed to determine the functional implications. It is not known if elevated $Arc$ expression is a result of a larger proportion of cells expressing $Arc$ protein, the same number of cells expressing a larger amount of $Arc$ protein, or whether this change is subregion specific.

**V. NORMAL BRAIN AGING OUTSIDE THE HIPPOCAMPUS**

The hippocampus is not the only brain structure that shows early age-related vulnerability. Of the other brain regions known to show age-related changes, the prefrontal cortex, which is critically involved in working memory and executive functioning (e.g., Divac, 1971; Goldman-Rakic, 1987), is particularly susceptible to advancing age (e.g., Albert, 1997; Bartus et al., 1978; Rapp and Amaral, 1989; Schacter et al., 1996; R.L. West, 1996). Working memory can be readily assessed using a delayed-nonmatching-to-sample (DNMS) task. On one variant of this task, the T-maze alternation task, aged rats show working memory deficits as the delay between trials is increased (e.g., Ando and Ohashi, 1991; Ramos et al., 2003), and aged nonhuman primates also
show time-dependent deficits on other variants of the DNMS task (Moss et al., 1988, 1997; Rapp and Amaral, 1989). Working memory function is also affected in the aged human; work by Lyons-Warren et al. (2004) using a computerized spatial delayed-response (SDR) task, demonstrated a significant age effect when delay times were increased.

Executive functioning, which also relies on the PFC, is necessary for processes such as planning, cognitive flexibility, abstract thinking, rule acquisition, and inhibiting inappropriate actions and irrelevant sensory information. In humans, one way to measure executive function is with the Wisconsin card-sorting task. Aged humans have greater difficulty with this task relative to young adults, making more perseverative errors (Rhodes, 2004). Testing executive functioning in animal models is more difficult to accomplish, but analogous executive function tasks have been developed for nonhuman primates. Data from these experiments have confirmed an age-associated deficit, with aged monkeys making more perseverative errors than their younger counterparts (T.L. Moore et al., 2003).

In contrast to the hippocampus, neuron loss may contribute significantly to the cognitive deficits associated with aging and the PFC. Significant neuron loss (~30% reduction) has been found in selected areas of the aged PFC, particularly area 8A of the dorsolateral PFC (D.E. Smith et al., 2004). Longitudinal volumetric analysis of the human PFC has also revealed striking age-related declines in volume in the lateral PFC and orbito-frontal PFC (Raz et al., 2005). Functional imaging studies have also revealed interesting age-associated alterations in the activation of the PFC during memory retrieval tasks. For example, aged adult subjects sometimes show reduced PFC activity as compared to younger adults in areas that show the greatest activation in young subjects. This reduced activation is often correlated with poorer memory performance in the aged subjects. However, in some cases, older people may show equivalent or greater bilateral PFC activation when performing tasks that typically result in unilateral PFC activation for young adults. This pattern of activation is often correlated with better task performance, leading to the hypothesis that it could reflect a compensatory mechanism. A study by Gutches et al. (2005) suggests that this might be case; functional activation following successful encoding of pictures was lower in the parahippocampus of aged adults as compared to young adults, but activation of the PFC was equivalent for aged and adult subjects. Further, in aged adults, there was a negative correlation between parahippocampal and PFC activation, whereas for young adults the correlation was positive. In a similar vein, Grady et al. (2005) demonstrated a positive correlation between parahippocampal activity and recognition memory performance in young but not aged adults and positive correlations between PFC activation and memory performance in aged but not young adults. Studies like these have important implications for the administration or design of therapeutics aimed at treating age-associated cognitive decline. This was highlighted by Ramos et al. (2003), who demonstrated that treatment
Strategies aimed at restoring the function of the PFC may be different from those used to restore hippocampal function. Prior to this study, several studies indicated that increasing cAMP/protein kinase A (PKA) activity restored hippocampal function in memory-impaired aged rats and mice (e.g., de Toledo-Morrell et al., 1984; Randt et al., 1982; Barad et al., 1998). Ramos et al. (2003), however, demonstrated that enhancing PKA activity has the opposite effect on the function of the PFC in aged rats and monkeys, leading to poorer working memory performance, whereas PKA inhibition enhances working memory performance. These studies highlight the complex nature of the normal aging process.

VI. CONCLUSIONS

Although most of us will not suffer from a dementing condition as we age, all of us will experience some change in our memory function as we get older. The need for research aimed at identifying those changes in memory that occur during normal nonpathological aging is therefore of great importance. Rather than providing a comprehensive overview of all cognitive changes that occur in aged organisms, age-related changes in spatial memory function were highlighted. The choice of this particular behavior is not meant to imply that other age-associated cognitive changes do not occur or are not equally important, but, rather, changes in this particular cognitive domain are well documented in many species, including humans. This simplifies the task of applying knowledge gained from rodent or other nonhuman studies back to humans. Because the hippocampus is the brain structure that has been most closely associated with spatial cognition, age-associated alterations in the function of this structure were highlighted. It will be important for future work to focus on additional structures (e.g., prefrontal cortex) that also show significant age-dependent changes. Selective region-specific changes occur in the hippocampus, all of which probably contribute to some of the cognitive changes that occur with aging. For example, a change in functional cellular connectivity will alter the efficacy with which neurons can communicate with each other. Similarly, changes at the molecular level may change the ability of neurons to undergo plastic changes that are necessary for normal memory function, such as a change in dendritic spine shape or size or the distribution of glutamate receptors at the postsynaptic membrane. The advantages of gaining a better understanding of the kinds of cognitive changes that occur during normal nonpathological aging, together with an increasingly better understanding of the mechanisms that contribute to these changes, are twofold. First, strategies aimed at preventing or treating age-associated cognitive decline can be developed or improved; second, these investigations will provide information regarding how information processing, and the associated neural mechanisms, function in general.
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