Neurobiological Changes in the Hippocampus During Normative Aging

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The number of individuals older than 65 years is projected to exceed 71.5 million in the year 2030, which is twice the number alive during the year 2000. While this dramatic increase in the number of individuals at risk for Alzheimer and vascular disease will pose a significant challenge to the health care industry, many older individuals will not actually die of these age-related dementias. Instead, a significant proportion of those older than 65 years will have to cope with alterations in memory function that are associated with normative aging. A clear understanding of the neurobiological mechanisms underlying normal age-related changes will be essential in helping elderly populations maintain cognitive performance with increasing age. This review covers the major age-related alterations in the hippocampus, a critical structure for learning and memory.

It has been known for some time that many individuals will exhibit deficits in memory that are unrelated to neuropathologies. This subtle age-associated decline in normal memory function is not as drastic as the devastating effects of Alzheimer disease, but is nevertheless unsettling. Biological studies of the human nervous system are difficult for many reasons, including ethical issues of invasive studies in living persons and the nearly impossible task of controlling for all genetic and environmental variables among individuals. To investigate normal age-related impairments at the neuronal or synaptic level with the greatest range of experimental techniques, animal models must be used. However, animal studies have the limitation that animals cannot describe their experiences directly, making the investigation of certain cognitive functions, such as episodic memory, difficult. To make inferences about the aging process from the animal model back to the human, rigorous behavioral paradigms must be used to ensure that the same function is being examined across species. Fortunately, the domain of spatial memory provides a common ground between species and happens to be a domain where age-related deficits are described consistently for humans, nonhuman primates, and species such as dogs, rats, and mice. Because of its critical role in memory in general, and specifically in spatial memory, the hippocampus has been the focus of a productive line of research into the mechanisms of normative aging.

THE HIPPOCAMPUS AND SPATIAL MEMORY

The importance of the hippocampus in spatial memory was known decades before the discovery of place cells, hippocampal neurons that fire in a location-specific manner. Patients with damage to the hippocampal formation show impairments in spatial and episodic memory. This finding has been confirmed in targeted lesion studies in a variety of species from rodents to nonhuman primates. In rats, the Morris water maze is frequently used as a test of spatial memory. In this test, a rat learns the location of a hidden platform below the surface of a tank of opaque water. This platform is always in the same place relative to
the tank and the external cues of the testing room. Aged rats demonstrate impairments in learning this task despite good performance when the platform is visible, indicating that the impairment is attributable to a deficit in spatial memory, as opposed to deficits in visual perception or motivation to leave the water. Aged rats are also impaired on another test of spatial memory, the Barnes circular platform task. In this task, rats are placed on a circular platform with 18 holes at the periphery. One of the holes leads to a dark tunnel and is rewarding for the rat, as it allows the animal to escape the brightly lit platform. External cues hang on the walls and provide landmarks for the rat to use in learning the relative location of the reward hole. Aged rats have deficits in learning the position of the escape tunnel, taking significantly longer to reach asymptotic performance on the task. Following learning, aged animals are also significantly impaired in remembering the location of the escape tunnel. In a study of senescent mice, the same spatial memory deficits were observed as in rats; however, aged animals could perform equivalently to younger animals when the location of the escape tunnel was cued, similar to the results described above for the Morris water maze. There is now a large literature on spatial memory deficits in aged humans. Interestingly, an analog of the Morris water maze reveals memory deficits in humans that parallel findings in rats. In this experiment, subjects first were required to learn the location of a target in a 7-m enclosed space relative to the cues on the walls, and then had to place the target at its home position following various manipulations of the environmental cues (such as rotations of the external cues with respect to the entrance). Aged subjects were less accurate in locating the target than were the young controls, suggesting deficits in spatial memory that are consistent with the results of rodent experiments.

AGE-RELATED MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHANGES

Given that neuronal cell death underlies many neurological disorders, an obvious possibility would be that loss of neurons may contribute to age-related deficits in memory function. Early studies of autopsy material indeed suggested that humans had lost neurons during the aging process; however, these studies measured neuron density and were confounded by differential shrinkage of young vs old brain tissue during histological processing. More recent studies using modern design-based stereological techniques, which are unaffected by tissue shrinkage, have revealed an overall preservation of neuron number in the brains of aged rats, mice, monkeys, and humans; one study of hippocampal neuron numbers in the human brain described a loss of cells in the subiculum and hilus of the dentate gyrus, but the granule cells of the dentate gyrus and the principal cells of the hippocampus proper were unaffected.

If there is no great loss of principal neurons with aging, then the biological substrate responsible for age-associated memory impairment must lie elsewhere. Several studies have investigated the basic biophysical properties of aged neurons, another likely candidate for age-related declines in function. A variety of basic parameters such as the resting membrane potential, input resistance, membrane time constant, and certain characteristics of excitatory postsynaptic potentials (EPSPs) were investigated in both young and old rats, but the results of these studies predominantly show that basic electrophysiological properties of aged neurons compared with young neurons are preserved.

The preservation of neurons in both numbers and basic cellular properties leaves open the possibility that the cause of age-related alterations in memory function is rooted in the functional connections between these preserved elements. In fact, this is where most changes are observed. In the dentate gyrus, quantification of synapse number using stereological methods revealed reductions of about 24% of axospinous synapses in the middle and inner molecular layers of aged compared with young rats. This is consistent with experimental findings following stimulation of the perforant path input to the hippocampus, which demonstrate a significant reduction in the amplitude of the field EPSP in aged rats. This would be expected if functional synapses from this pathway onto granule cells were lost. Concurrent with these observations is a decrease in the amplitude of the presynaptic fiber potential, which suggests a reduction in the number of perforant path axon collaterals passing through the dentate gyrus. However, when the amplitude of the granule cell field EPSP is plotted against the presynaptic fiber amplitude, the aged granule cells show larger synaptic responses than do young granule cells. Thus, at equivalent levels of afferent fiber stimulation, aged granule cells exhibit stronger depolarizing responses, which appear to be explained by age-related increase in quantal size at this synapse. Taken together, these data indicate that there can be selective compensation in the aging hippocampus.

In the CA1 subfield of the hippocampus, different alterations of the functional connections are evident. In this subfield, there is no loss of synapses and no age-related change in the presynaptic fiber potential from the incoming Schaffer collaterals; yet, there is still a reduction in the field EPSP of CA1 principal cells. In contrast to the dentate gyrus, where synaptic input appears to be anatomically absent, the electrophysiological evidence suggests that the synaptic input to the CA1 principal neurons is functionally absent. An electron microscopic study of Schaffer collateral synapses in aged rats supports this view. Nicholson and colleagues measured the area of the postsynaptic density at Schaffer collateral synapses on CA1 neurons in stratum radiatum and reported a 30% reduction at axospinous perforated synapses in aged memory-impaired rats. Reductions in postsynaptic density area may contribute to the observed reduction in field EPSP in the CA1 subfield of aged rats. Because the unitary EPSPs are not different in size between young and old rats, these data suggest that the synapses with reduced postsynaptic densities are silent.

AGE-RELATED CHANGES IN HIPPOCAMPAL SYNAPTIC PLASTICITY

In 1949, the Canadian psychologist Donald Hebb, PhD, proposed a means by which synapses could be modified...
and thus perform the role of information storage. He suggested a process whereby the coincident firing of pre-synaptic and postsynaptic neurons strengthened the connection between the 2 cells. A biological process 2 decades later satisfying Hebb’s prediction was discovered when it was found that high-frequency stimulation of the rabbit dentate gyrus resulted in a long-lasting enhancement of synaptic strength, an N-methyl-D-aspartate (NMDA) receptor–dependent process now well known as long-term potentiation (LTP). Long-term potentiation consists of separate phases, including an induction phase and early and late maintenance phases.

Age-related changes in LTP induction in the hippocampus are not readily apparent when high-frequency and high-amplitude stimulation protocols are used that are well above the minimum threshold for LTP induction. However, when less robust protocols are used that are closer to threshold, aged rats show deficits in LTP induction at the Schaffer collateral input to CA1 synapses. Additionally, the synapses formed by perforant path input to the granule cells of the dentate gyrus in aged rats have been shown to have a higher depolarization threshold before LTP can be induced.

Studies of LTP maintenance show that in the early phase (1-3 hours after stimulation), there is no difference in the decay of LTP between young and old rats. When later time points are investigated, it becomes apparent that LTP decays more rapidly in the older animals. The maintenance of LTP was measured in animals that were also tested for spatial memory function in the Barnes circular platform task, and a correlation was found between the decay rates of LTP and performance on the task in both the aged and younger groups. Thus, the animals that showed the greatest propensity for forgetting the solution to the task were also the ones with the fastest LTP decay.

In addition to LTP, complementary processes of reducing synaptic strengths—long-term depression, which occurs at nonnaive synapses, and depotentiation, a reversal of LTP at nonnaive synapses—have been identified at hippocampal synapses. These processes also have been shown to change with age, with aged rats showing a heightened susceptibility to induction of long-term depression and a greater degree of depotentiation of previously strengthened synapses. These results, in concert with the changes in LTP described above, suggest that it is more difficult for aged synapses to form new memories and easier to forget them.

AGE-RELATED CHANGES IN HIPPOCAMPAL PLACE FIELD EXPRESSION

Perhaps the most striking correlate between neuronal physiology and behavior is represented by the activity of place cells in the hippocampus. Hippocampal place cells earned their name from their location-specific firing characteristics; when a rat is in a particular location in an environment, a subpopulation of hippocampal principal cells will selectively fire action potentials, while being nearly silent in other spatial positions in the environment. The term place field refers to the region of space that causes a place cell to selectively fire. Place fields are expressed the first time an animal moves through an environment, with the need for initial learning. In adult rats, these place fields are generally stable and are expressed on reintroduction of the rat to the same environment. However, on exploring the same environment a second time, the young animals fail to retrieve the first appropriate map. This reduction in the stability of hippocampal maps in young rats by an experimental blockade of LTP is particularly interesting given the deficits in LTP observed in aged rats and their inability to consistently retrieve the correct map between sessions.

The network activity of hippocampal place cells indicates that the hippocampus can generate distinct maps by recruiting independent populations to encode separate environments. Early studies of place field expression in aged animals indicated that senescence resulted in broader, less specific place fields that were also less reliable. In contrast, more recent studies produced contrary results: place fields were just as specific (and in some cases more specific) in aged than in adult rats. The reason for these incongruous results appears to be related to the ability of aged rats to reliably retrieve the correct map on reentry into a previously explored environment. In a study of place field expression by CA1 principal cells, Barnes and colleagues recorded from ensembles of hippocampal place cells while rats ran on the same elevated track maze for 2 sessions separated by approximately 25 to 60 minutes. The results showed that within individual sessions, aged rats expressed stable ensemble activity. That is, the same population of place cells expressed their respective place fields as the rat navigated the track for the duration of the recording session, as observed for the younger subjects. Place field maps were highly correlated between the first and second sessions in the young group, indicating that the animals were reactivating the same place cells for both exposures to the track. Additionally, many aged rats also were able to retrieve the appropriate map when they were reintroduced to the same environment for the second session; however, 30% of the time, an aged rat would activate a completely independent ensemble of place cells during the second session. This failure to retrieve the appropriate map between sessions explains the earlier description of unreliable and less specific place fields in aged rats because, in that study, animals were removed from the testing apparatus between trials to untangle recording wires.

Pharmacological blockade of NMDA receptors (thus preventing induction of LTP) in young rats during initial exposure to a novel environment does not affect the stability of place field expression within the session. However, on exploring the same environment a second time, the young animals fail to retrieve the first appropriate map.
Electrophysiological recordings provide excellent temporal information, but are limited in the number of neurons that can be sampled during a single experiment. The past few decades have seen impressive advances in imaging techniques that allow larger volumes of tissue to be investigated simultaneously, and these techniques are now applied to the study of normal aging. A recent study combined magnetic resonance imaging (MRI) with cellular compartment analysis of temporal activity by fluorescent in situ hybridization (catFISH) to investigate the relative effects of aging on multiple subfields of the hippocampal formation at the same time. This particular study highlights the benefit of innovative imaging technologies in studying the effect of aging on the hippocampus.

Figure. Hippocampal changes with age. The figure summarizes major changes that have been reported during the aging process for the pyramidal neuron population in the CA1 subfield of the hippocampus (A) and the granule cells of the dentate gyrus (B). In both regions, neuron numbers are preserved, but beyond that, the age-related pattern of change is not consistent between the 2 areas. A, In the aged CA1 subfield, there are (1) reductions in the area of postsynaptic density (PSD), resulting in electrophysiological silence in some synapses. Remaining functional synapses show (2) a preservation in the size of the unitary excitatory postsynaptic potentials (EPSP), but (3) decreases in long-term potentiation (LTP) and (4) increases in long-term depression (LTD), suggesting that it is more difficult for these synapses to encode and retain new information. Imaging studies reveal that in the CA1 subfield of aged rats, (4) the same number of neurons express Arc following behavior compared with young rats, a finding that is consistent with the preservation of resting metabolism in the CA1 region of aged monkeys, as revealed by magnetic resonance imaging (MRI). B, The dentate gyrus, on the other hand, (5) does show a loss of synaptic contacts during aging, but the remaining synapses (6) show increased unitary EPSP’s size, perhaps to compensate for the reduced number of synapses. These synapses also have (7) an increased threshold for LTP induction and a decreased durability of LTP, suggesting that is also harder for these neurons to encode and retain new information. (8) The number of granule cells that show behaviorally induced Arc expression decreases in the dentate gyrus of the aged rat, and resting metabolism revealed by magnetic resonance imaging is also significantly lower in this subfield for aged monkeys.

Small and colleagues first used MRI to measure cerebral blood volume, a correlate of metabolism used as a marker for neuronal activity, in rhesus monkeys ranging from young (7 years) to old (31 years). These subjects were also characterized behaviorally with the delayed nonmatching to sample task, a hippocampal-dependent test of memory function. The high resolution of the MRI scans allowed cerebral blood volume to be measured individually for the dentate gyrus, the CA1 subfield, the subiculum, and the entorhinal cortex. Significant changes were found for the dentate gyrus across age. The results revealed a significant inverse correlation between cerebral blood volume and age and a significant correlation between cerebral blood volume and performance on the delayed nonmatching to sample task.

The catFISH technique was then used to image the expression of Arc messenger RNA in young (9 months),
Normal aging brings with it deficits in cognitive performance despite a lack of pathologies such as Alzheimer disease. With a rising population older than 65 years, it becomes increasingly imperative to study the mechanisms by which abilities such as memory decline with normative aging. To date, an extensive effort has focused on characterizing the effects of age on the hippocampus, a critical region for learning and memory. These studies have revealed several deficits in both the structure and function of the hippocampus (Figure) as well as evidence of compensatory mechanisms (eg, the increased responsiveness of granule cells). Imaging studies have identified hippocampal subfields that are selectively affected by aging that will be useful in guiding future research. In addition, modern imaging techniques can examine larger volumes of the brain with increasing temporal and spatial resolution. This not only allows examination of all hippocampal subfields following exploration of an environment. The results of this analysis showed a significant age-related reduction in the percentage of Arc-positive cells following behavior that was restricted to the dentate gyrus, the same region that the MRI data indicated was most affected by aging in rhesus macaques.

CONCLUSIONS

middle-aged (15 months), and aged (24 months) rats. Arc is an immediate early gene that is induced following behaviorally relevant neural activity and can be used to visualize this activity in entire slices of tissue; in this case, it allowed for the functional imaging of entire subfields of the hippocampal formation with cellular resolution. Arc-positive neurons were quantified in the dentate gyrus, CA3, and CA1 subfields of the hippocampal formation following exploration of an environment. The results of this analysis showed a significant age-related reduction in the percentage of Arc-positive cells following behavior that was restricted to the dentate gyrus, the same region that the MRI data indicated was most affected by aging in rhesus macaques.

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