A reduction in the ability to generate new neurons in the brain has been suggested to contribute to cognitive decline with advanced age. In an outbred model strain of Long-Evans rats, cognitive performance as a function of age is variable in assessments of hippocampal-dependent spatial memory. Recent research indicates that greater hippocampal neurogenesis accompanies diminished cognitive abilities in older Long-Evans rats. These findings imply that the role of neurogenesis might change between youth and old age, and that further work is needed to understand the potential benefits and liabilities that new neurons may afford an aging brain.

Studying Cognitive Decline in Aging Rodents

Substantial evidence indicates that learning and memory involving the hippocampus (part of the medial temporal lobe system of the brain, shown in Fig. 1) are vulnerable to age-associated dysfunction both in pathological conditions and in the normal aging process (see Tuszynski Perspective at http://sageke.sciencemag.org/cgi/content/full/2004/19/pe20). Such memory deficits are of great concern to an aging human population, in which expectations of longevity have doubled in the developed world during the past century. The identification of neurobiological alterations that contribute to age-related memory loss is a topic of considerable interest (see Thal Perspective at http://sageke.sciencemag.org/cgi/content/full/2004/23/pe26), but investigating such questions in humans poses formidable challenges.

Aged laboratory rodents are an important system for studying the causes of age-related memory loss. Behavioral assessments that require the integrity of the medial temporal lobe system, including the hippocampus, reveal impairments in aged rats as compared to young adults (1–3). In outbred rat populations, a common feature is individual variability of performance: Deficits are seen in some aged rats, but others perform on a par with young cohorts, demonstrating preserved cognitive function at very advanced ages (3). Naturally occurring models of aging in rats thus mimic the variability of cognitive decline seen in humans, and they demonstrate that cognitive dysfunction during aging is not inevitable or strictly linked to chronological age. Such animals provide a useful way to evaluate the functional significance of neural alterations in the aged brain and to detect markers of brain aging that may not be apparent when comparisons are based on chronological age alone.

Individual differences in cognitive decline are apparent in aged (25 months old) male Long-Evans rats as measured by means of a widely used behavioral test—the Morris water maze—in which rats learn the location of an escape platform submerged in a pool of water by remembering visual cues around the maze. Long-Evans rats are ideally suited for these experiments because they have pigmented eyes, affording excellent visual acuity even at advanced ages, and because they are outbred, so that genetic variability is regularly introduced into the population. Old rats in this study population have no difficulty in swimming to a visible platform, indicating visual, sensorimotor, and motivational abilities that are comparable to those of young rats. However, old Long-Evans rats are impaired relative to younger rats in locating the submerged platform (3) (Fig. 2), which requires the effective use of spatial information and is dependent on the functional integrity of the medial temporal lobe system of the brain. The performance of individual aged rats varies substantially, however, with some aged rats performing as well as young rats and others demonstrating impairment on the task (Fig. 2, inset panel). Such differences are reliable: If tested in a new spatial environment weeks later, rats that were impaired in the initial assessment again perform poorly, whereas the rats that demonstrated preserved function remain proficient at the task (3).

This rat model has proved useful for examining the neurobiological changes that underlie age-related cognitive decline. For many years, neuronal loss was assumed to be an inevitable consequence of advancing age and the primary basis for impaired cognition in aging (4). However, recent studies in rats reveal that frank neural loss is not pervasive in normal aging. Quantitative unbiased stereology, a neuroanatomical technique that can be used to determine the number of neurons in structures associated with the medial temporal lobe system, has been used to demonstrate that neuronal numbers in aged cognitively impaired Long-Evans rats do not differ reliably from those in young rats or in age-matched cohorts with preserved cognition (5). This finding has been documented for the principal neurons of the hippocampal formation; the entorhinal cortex; and the parahippocampal region, including the perirhinal and postrhinal cortices (5, 6) (Fig. 1). These structures work together as a functional system for processing the information used for spatial learning and other forms of declarative memory. The lack of frank neural loss in normal aging has also been confirmed in other rodent models and in primates, including humans (7–10). Additional studies, which support the absence of widespread neurodegeneration in normal aging, have investigated the immunohistochemical localization of glial fibrillary acidic protein (GFAP), a marker for astrocytes (a type of supporting non-neuronal brain cell, known collectively as glia or glial cells). Glial cells, which characteristically increase in size and number in response to injury and neuronal degeneration, are unchanged.
Fig. 1. Medial temporal lobe memory system and hippocampal circuitry in the human and rat brain. The top left panel shows a schematic sagittal view of the human brain, with the medial temporal lobe region shaded light blue and the hippocampus highlighted in dark blue. The circuit diagram in the top right panel shows the information flow in the medial temporal lobe memory system. Structures generally considered part of this memory system are shown in light blue. The neocortex, which conveys information about the environment, projects to the hippocampus through the entorhinal cortex via the parahippocampal gyrus. The lower panel shows the location of the hippocampus in a schematic view of the rat brain and highlights the basic circuitry within the hippocampus in a coronal section. Projections from the entorhinal cortex (green) make connections with granule cells (dark red) of the dentate gyrus [including the GCL (bright red) and hilus]. New hippocampal neurons (one example is shown in yellow) are generally born in the subgranular zone of the dentate gyrus, which comprises the border between the GCL (red) and the hilus. Granule cells project to the hippocampus proper (regions CA3 and then CA1) before exiting the hippocampus and sending information back to neocortical structures (see circuit diagram).
in number in Long-Evans rats, irrespective of age or cognitive status (11). Similarly, other markers for gliosis (extensive production of glial cells, which can accompany neuronal death) such as OX-44 (which labels another type of glial cell) are not elevated in aged cognitively impaired rats in this behavioral model (11, 12).

Continuing Neurogenesis in Adulthood
Stability in the number of neurons during aging is significant because other evidence indicates that new hippocampal neurons are generated throughout the entire life span (see Wise Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/22/pe13). In the hippocampus, neuronal progenitors are located in the subgranular zone, which comprises the border between the granule cell layer (GCL) and the hilus of the dentate gyrus [a region of the hippocampus that receives signals from other parts of the brain (Fig. 1)]. A substantial body of work supports a role for adult-generated neurons born in this region in memory processes in young adult animals (13–18). In young rats, many of the newly generated hippocampal cells differentiate into mature hippocampal neurons in the adult and are incorporated into the existing circuitry of the brain (19–21). These new neurons also have electrophysiological properties, conduct action potentials, and make functional synapses consistent with the adult granule cell (neuronal) phenotype (22). A number of studies have shown that the proliferation and survival of newly generated hippocampal neurons can be regulated by a variety of physiological and/or environmental conditions, including exposure to sensory-rich environments and learning procedures (13–15, 23–26). Indeed, neurogenesis may even be critical for certain forms of hippocampal-dependent learning in young rats (17).

It has been suggested that recently generated neurons in adulthood may play a role in establishing new memories by contributing to plasticity (the ability to form new or more effective routes of communication between neurons) within the hippocampus. One intriguing suggestion is that recently generated granule cells may favor synaptic plasticity as compared to older granule cells [reviewed in (27)]. If that is the case, then persistent neurogenesis in the mature adult might contribute to increased synaptic change, and diminished neurogenesis in the aged brain might reduce such plasticity. A loss in the capacity to generate new neurons might therefore play a role in the age-related deficits in cognitive performance involving the hippocampus. This hypothesis could still be consistent with the observed stability of total neuronal number, because ongoing apoptotic cell death also occurs throughout life in the hippocampal GCL (28). If the apoptotic loss of neurons is diminished in tandem with a decrease in the addition of new cells, no net change in the number of neurons would be detected.

Neurogenesis and Cognitive Impairment in Rats
We have studied the birth, survival, and differentiation of hippocampal cells in behaviorally characterized Long-Evans rats using the label bromodeoxyuridine (BrdU), which is incorporated into newly synthesized DNA, to identify dividing cells. Despite the fact that various neurobiological alterations in the hippocampus have been found to be predictive of cognitive impairment in this study population (29–34), we failed to detect any relationship between new cells born in the dentate gyrus and the spatial learning performance of rats at any age (35). Other investigators have also failed to find a relationship between individual differences in water maze performance and the birth of cells in the GCL in aged Fisher 344 female rats [(7), but see also (36); it should be noted that a relationship has been reported between greater rates of neuronal proliferation and better water maze performance in aged male Sprague-Dawley rats, but the source of the conflicting findings from Drapeau et al. (36) and those reported by Bizon and Gallagher (35) and Merrill et al. (7) remains unclear at present, as discussed in (37)]. We have now completed a second study designed to assess the survival and differentiation of newly generated hippocampal cells in the same rat model (37), and the results are summarized in Fig. 3. Contrary to expectations, greater numbers of BrdU+ cells in the GCL of aged rats were associated with worse cognitive status. Double-labeling studies confirmed that the majority of the BrdU+ cells were of the neuronal phenotype.

This somewhat surprising result is difficult to reconcile with the view that neurogenesis confers a benefit throughout life and that promoting neurogenesis could potentially counteract a decline in cognitive abilities that would otherwise occur during aging. At the very least, these data suggest that endogenous mechanisms promoting new neuronal survival at advanced ages are insufficient to restore cognitive abilities. Of course, an apparent lack of benefit could be explained if the new neurons produced in aged brains are dysfunctional, so that aged rats are unable to use recently generated neurons in hippocampal functions. Because the cellular environment in the aged brain is substantially different from that in the young adult, some aspect of the full functional integration of new neurons into hippocampal circuitry could be compromised. If that were...

Fig. 2. Young and aged rat performance in the hidden platform version of the Morris water maze used to evaluate hippocampal-dependent cognition. These representative data show that, as a group, aged (25 months old) male Long-Evans rats are impaired relative to young (6 months old) rats. However, the inset graph shows the substantial variability in performance that is typical among aged rats in this study population. Approximately half of the aged (A) rats perform below the range of young (Y) rats (a higher learning index score reflecting poorer performance), whereas the rest perform as well as young rats.

http://sageke.sciencemag.org/cgi/content/full/2005/7/re2
an alternative explanation of our results and other new findings. Neurogenesis could help in treating cognitive impairment. Have in age-related memory loss and whether promoting will be critical for understanding what role this process might generated and existing granule cell neurons in the aged brain connectivity, and ultimately the functional status of recently (Fig. 1). Future work directed at determining the longevity, (blue). The BrdU-positive cell is double-labeled with the neuronal but not the astroglial marker. (C) A graph showing the relationship between the number of BrdU+ cells and spatial learning index among aged Long-Evans rats. There is a significant positive correlation, indicating that higher numbers of BrdU+ cells are associated with worse behavioral performance. [Figure adapted from (37)]

the case, then diminished cognitive function might occur because of a greater loss of functional circuits. Recall that apoptosis occurs throughout life in persistently neurogenic regions of the brain including the hippocampus (28), with evident coordination of the processes of cell birth and death in the adult (28). Our data showing unchanged numbers of neurons in the dentate gyrus during aging (5) would suggest that any addition of new neurons to the GCL is offset by the loss of more mature granule cells. Thus, in aged rats with elevated neurogenesis, higher neuronal turnover might contribute to a worse cognitive outcome if new neurons are functionally compromised while at the same time more mature cells are lost.

A related consideration involves the long-term survival and connectivity of the new cells born in the aged brain. Thus far, we have only examined 3- to 4-week-old cells and defined these cells as neurons by double-labeling them with the neuronal marker NeuN. This marker labels an antigen expressed by virtually all mature granule cells. However, even if these neurons are expressing NeuN, it is possible that they are not forming anatomical connections typical of mature granule cells. Two elegant studies, using neuronal tracing in combination with markers to label recently generated neurons, have demonstrated that neurons born in the young adult hippocampus send projections to CA3 pyramidal neurons, as would be expected if these neurons are functionally contributing to hippocampal processes (19, 21) (Fig. 1). Future work directed at determining the longevity, connectivity, and ultimately the functional status of recently generated and existing granule cell neurons in the aged brain will be critical for understanding what role this process might have in age-related memory loss and whether promoting neurogenesis could help in treating cognitive impairment.

While we await further studies, it may also be timely to consider an alternative explanation of our results and other new findings. Most notably, an elevation in markers of neurogenesis was recently reported in humans with the age-related memory disorder Alzheimer's disease (http://sageke.sciencemag.org/cgi/content/full/2001/1/oa5); and see Shors Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/49/pe35]. Thus, endogenous mechanisms in both normal and pathological aging are capable of stimulating hippocampal neurogenesis. It is reasonable to ask whether such mechanisms could have an unexpected deleterious effect in the context of an aged brain. Is it possible that neurogenesis, or key factors that regulate neurogenesis, represent a case of antagonistic pleiotropy, referring to biological adaptations that have evolved for the advantage(s) they confer in youth but may have deleterious effects later in life? It is interesting to note that exposure to sensory-rich environments, which increases the birth of new hippocampal granule cells in both young and aged rodents, also results in increased expression of amyloid precursor protein (APP) in young rats (39) and induces greater amyloid plaque formation in transgenic mouse models of Alzheimer's disease (40). Although the soluble APP fragments generally promote cell survival, neurite outgrowth, and synaptogenesis in young rats, excess insoluble aggregated amyloid is a feature of the signature pathology of Alzheimer's disease and animal models thereof (41).

In the context of normal aging, it is important to consider other neurobiological changes associated with hippocampal dysfunction that may have a secondary effect on the survival of cells born in the GCL. For example, there is strong evidence that oxidative stress (http://sageke.sciencemag.org/cgi/content/full/2001/1/oa5) is increased in cognitively impaired relative to cognitively unimpaired aged Long-Evans rats (12). In order to combat such cellular stress, certain survival factors might be up-regulated in the dentate gyrus, an effect that could influence the survival of recently generated hippocampal cells in turn. Growth factors such as brain-derived neurotrophic factor (BDNF) have been implicated in the survival of hippocampal granule cells during development and in the adult brain (42, 43). Moreover, BDNF mRNA expression is significantly elevated in Long-Evans rats as a function of age, and hippocampal BDNF expression is inversely correlated with spatial learning abilities among aged rats (44).

**Conclusion**

An important goal for future work will be to determine the characteristics of the cellular environment in the aged brain that are responsible for the birth, differentiation, and integration of new neurons born in the hippocampal formation. One issue will be to clarify the parameters of neuronal turnover in the dentate gyrus in aged Long-Evans rats: Does the neuronal cell cycle differ as a function of age and/or cognitive status? Does increased

![Fig. 3. (A) Bright-field photomicrograph showing immunohistochemical localization of BrdU-positive cells in the GCL (gcl) and hilus of a young rat killed 3 weeks after BrdU administration. (B) High-powered photomicrograph taken from a representative rat, showing immunofluorescent localization of BrdU (red), the neuronal marker NeuN (green), and the astroglial marker glial fibrillary acidic protein (blue). The BrdU-positive cell is double-labeled with the neuronal but not the astroglial marker. (C) A graph showing the relationship between the number of BrdU+ cells and spatial learning index among aged Long-Evans rats. There is a significant positive correlation, indicating that higher numbers of BrdU+ cells are associated with worse behavioral performance. [Figure adapted from (37)]](image-url)
cell death accompany the elevated neurogenesis that we observe in the dentate gyrus of aged cognitively impaired rats? Preliminary work using cDNA microarrays indicates that expression of cell cycle regulatory genes and genes involved in neuronal differentiation can be distinguished in aged rats with impaired or preserved cognitive function. Future experiments using these new molecular techniques in combination with both novel and established behavioral paradigms should prove very informative for determining the functional status of hippocampal circuitry in the aged brain, and will help yield an understanding of the possible role of new neurons in overcoming cognitive decline in old age.

References