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New neurons in an aged brain

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Abstract

Adult hippocampal neurogenesis is one of the most robust forms of synaptic plasticity in the nervous system and occurs throughout life. However, the rate of neurogenesis declines dramatically with age. Older animals have significantly less neural progenitor cell proliferation, neuronal differentiation, and newborn neuron survival compared to younger animals. Intrinsic properties of neural progenitor cells, such as gene transcription and telomerase activity, change with age, which may contribute to the observed decline in neurogenesis. In addition, age-related changes in the local cells of the neurogenic niche may no longer provide neural progenitor cells with the cell-cell contact and soluble cues necessary for hippocampal neurogenesis. Astrocytes, microglia, and endothelial cells undergo changes in morphology and signaling properties with age, altering the foundation of the neurogenic niche. While most studies indicate a correlation between decreased hippocampal neurogenesis and impaired performance in hippocampus-dependent cognitive tasks in aged mice, a few have demonstrated that young and aged mice are equivalent in their cognitive ability. Here, we summarize the different behavioral paradigms to test hippocampus-dependent cognition and the need to develop neurogenesis-dependent tasks.

Keywords

aging; neurogenesis; neurogenic niche; cognition

1. Introduction

At the core of the developing nervous system, neural stem cells (NSCs) proliferate and differentiate into all cell types of the nervous system. They are unique in their unlimited self-renewal and multi-lineage potential. NSCs generate neural progenitor cells (NPCs), which are limited in proliferation and differentiate into neurons or glia (1). Newborn neurons migrate to their target location, extend their dendrites and axons, and integrate into the nascent circuit as neurons communicate with one another via synapses to function efficiently and effectively. From the development of the nervous system and for the entire life span of the individual, neurons are continuously undergoing synaptic plasticity and remodeling as they extend and withdraw their processes, forming new and severing old targets.

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Neurogenesis, the generation of new neurons, is a key contributor to synaptic plasticity in the adult brain. While NPCs exist in many regions throughout the adult brain (2), they produce new neurons in only two specific regions under physiological conditions: the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus. NPCs in the SVZ supply new neurons to the olfactory bulb; neurons born in the SGZ integrate into the existing hippocampal circuit. The extent to which newborn neurons contribute to the functions of the olfactory bulb and hippocampus remains controversial (3, 4), but newborn neurons confer synaptic plasticity to two regions involved in learning and memory. Although neurogenesis in the SGZ and SVZ is involved in different functions, there are many underlying similarities in the mechanisms that regulate adult neurogenesis in these two regions.

In this review, we will focus mainly on rodent studies investigating the effects of aging on neurogenesis and the function of the hippocampus. While there are differences in the rate of hippocampal neurogenesis and newborn cell maturation between adult rat and mouse (5), it remains unknown if aging differentially affects hippocampal neurogenesis and cognitive function in rats and mice, as there has yet to be a detailed comparative study. Here we discuss rats and mice in depth, as the age-related decline in neurogenesis is prominent and has been extensively studied in both organisms (6).

2. Age-related changes in neurogenesis

While hippocampal neurogenesis persists throughout adulthood, the rate at which new neurons are generated dramatically declines as the animal becomes older. Aged animals have significantly less neural progenitor proliferation, neuronal differentiation, and newborn neuron survival compared to younger animals (7-9). The age-related decline in NPC proliferation and neuronal differentiation in mice begins at 1-2 months of age and progressively decreases each month thereafter (10, 11) until it is barely present in aged (18 months) mice (7). The newborn cells in aged mice differentiate less into neurons and more into astrocytes compared to younger adult mice (7, 12, 13). Furthermore, the number of surviving cells decreases with aging (12, 13). The age-related decline in neurogenesis has been extensively documented (6); however, the cause of this phenomenon is poorly understood.

One possible explanation is that the population of NSCs is significantly diminished or absent in aged animals. Sex-determining region Y-box 2 (Sox2) is a transcription factor found in embryonic stem cells and was recently identified as a NSC marker in the adult hippocampus (14). Interestingly, the number of NSCs (Sox2+) did not decrease in the SGZ in aged rats, but their proliferative activity was greatly reduced compared to young adult rats. This finding suggested that the age-related decline in neurogenesis was largely a result of the NSCs entering a quiescent state (15). Results from the SVZ of mice were slightly different as the number of Sox2+ cells in the SVZ was reduced by 50% in aged compared to adult mice, demonstrating that the population of NSCs declined with increasing age in mice (16). Additionally, a smaller percentage of the Sox2+ cells was actively dividing in the aged SVZ (22-26 months) (16) and the overall proliferating population (Ki67+ cells) was considerably diminished in the SVZ of middle-aged rats (12 months) (17). The cellular loss occurred in both mitotic and non-mitotic populations in the SVZ of middle-aged rats (17). The number of primary neurospheres isolated from the SVZ of adult and middle-aged rats was similar (17), but fewer primary neurospheres were isolated from the SVZ of aged rats (16). However, after selecting for Nestin-GFP+ cells from the SVZ, the number of primary neurospheres from adult and aged rats generated was comparable, suggesting that the NSC population was similar between the adult and aged rats (16). NSCs isolated from the SVZ of aged rats proliferated more slowly compared to those from the SVZ of adult rats (16). These

studies confirmed the presence of NSCs in the germinal regions of the aged brain and suggested that the decline in neurogenesis might stem from the inactivity of the NSCs.

In addition to Sox2, the basic helix-loop-helix gene Hes5 can be considered a marker for NSCs as most Hes5+ cells co-label with Sox2 and other stem cell markers such as brain lipid binding protein (BLBP), the intermediate filament protein Nestin, and glial fibrillary acidic protein (GFAP) (18). The Hes5+ NSC population can be further subdivided into three groups: quiescent radial cells, quiescent horizontal cells, and proliferative horizontal cells. The total Hes5+ cell population in the SGZ of aged mice was reduced by approximately 30% from the young adult mice. However, there was approximately an 80% decrease in the proliferative horizontal Hes5+ cell population in aged mice, suggesting that the loss of this specific subpopulation accounted for reduced neurogenesis in aged mice. The lack of proliferation was not a result of increased cell cycle in aged NSCs as the length of S-phase was not altered with aging (19). These studies demonstrated the presence of both radial and horizontal NSCs, albeit at a slightly reduced number, but the proliferative activity of NSCs was dramatically absent in the aged mice.

The quiescent regulation of neurogenesis can occur at both intrinsic and extrinsic levels, and one or both of these modulatory systems may be disrupted with aging. In a transgenic mouse with green fluorescent protein (GFP) under the Nestin promoter, NSCs were labeled with GFP. Isolation of Nestin-GFP+ cells from the embryonic lateral ganglionic eminence and adult and aged SVZ in mice revealed that gene expression profiles of NSCs changed with age (16). NSCs from adult and aged SVZ exhibited reduced expression of stem cell markers Nestin and Musashi but increased levels of Sox2 compared to embryonic NSCs. Expression of two transcription factors, Er81 and Dlx2, which are critical during neural development, in aged NSCs was reduced compared to that in the adult SVZ, supporting the hypothesis that aged NSCs are intrinsically different from adult NSCs.

NSC proliferation and neurogenesis are also dependent on telomere length and telomerase activity. Telomerase functions to maintain telomere length and prevent chromosome shortening during repeated cell division of stem cells. Telomerase activity is detectable in areas of neurogenesis and is reduced during neuronal differentiation (20, 21). Consistent with the function of telomerase, telomere length in cells is longer in the SGZ than in the granular zone of the DG (22). As found in other stem cell populations, both telomerase activity and telomere length in neurogenic regions were significantly reduced in aged compared to adult mice (22, 23). Furthermore, shorter telomeres of NSCs correlated with decreased neuronal differentiation and neurite length (23), and newborn neurons with telomere damage were particularly prone to apoptosis (21). Aged NSCs cannot continue to proliferate and differentiate into neurons with shorter telomeres due to the lack of telomerase activity (23). The impact of telomerase activity on NSCs was observed *in vitro*, where neurospheres isolated from the SVZ of telomerase-deficient adult mice were significantly smaller compared to those from wildtype mice (24-26). In adult mice deficient in telomerase activity, neurogenesis defects in the SVZ were characterized by reduced proliferation (Ki67+ cells), fewer NSCs (Sox2+ cells), and fewer immature neurons (Doublecortin – Dcx + cells) (26). The density of Sox2+ cells, Ki67+ cells, and Dcx+ cells could be partially restored in telomerase-deficient mice by four weeks of reactivating endogenous telomerase activity, implicating telomerase activity as a potential target to stimulate neurogenesis in aged mice (26). In the SVZ, telomerase activity remained relatively stable between two and four months of age and significantly reduced by 12 months of age (23). However, it remains unknown whether the temporal decline of telomerase levels occurs concurrently with that of neurogenesis or if one precedes the other. Characterizing the temporal pattern of neurogenesis and telomerase decline will provide further evidence to determine if

telomerase activity is a consequence of senescent NSCs or the cause of decreased proliferation in aged mice.

3. Age-related changes in the neurogenic niche

In addition to intrinsic factors, extrinsic factors in the local microenvironment strongly regulate NSCs and neurogenesis (Figure 1). Although NSCs in the aged SVZ are characterized by substantially less proliferation, differentiation into neurons and survival, they can continue to generate functional neurons *in vitro* (16). A smaller population of the neurons derived from aged NSCs fire action potentials, but they exhibit similar electrophysiological profiles, except increased K^+ current density, compared to neurons derived from adult NSCs. This finding indicates that aged NSCs have the ability to differentiate into neurons but the aged neurogenic niches are no longer conducive for neurogenesis.

NPCs found in the germinal regions of the brain generate neurons in the presence of the neurogenic microenvironment. The neurogenic niche supports and promotes neurogenesis through both secreted factors and cell-cell contact. NPCs from the SVZ become oligodendrocytes and astrocytes when transplanted into ectopic regions of the adult brain (27). In contrast, progenitors isolated from spinal cord can differentiate into neurons when transplanted into the DG (28). Astrocytes represent one of the major contributors to the neurogenic niche, as those isolated from the adult hippocampus but not spinal cord promote neuronal differentiation of NPCs in co-culture through both soluble factors and cell-cell contact (29). In addition to NPCs, astrocytes are closely associated with endothelial cells, as astrocytic endfeet wrap tightly around the blood vessels. The vasculature modulates the activity of NPCs, as was evident by the presence of NPC clusters in close proximity to blood vessels (30, 31). Endothelial cells secreted soluble factors that increased NPC proliferation, neurogenesis (32), maturation, and migration, in part by secreting brain-derived neurotrophic factor (BDNF) (33). These studies demonstrated the critical role of both astrocytes and endothelial cells in regulating neurogenesis by maintaining and providing a permissive microenvironment.

Microglia, the resident macrophages of the brain, and astrocytes are considered to be the major immune cells in the brain, as an intact blood brain barrier (BBB) keeps most immune cells from entering. Microglia in the resting state continuously survey the local environment, with their dynamic processes interacting with other cell types (34). Immune signaling activates and mobilizes microglia to sites of injury and cell death, where microglia phagocytose cellular debris (35). Depending on the mode of activation, activated microglia can release pro-inflammatory or anti-inflammatory cytokines, modulating the immune response in support of or suppression of neurogenesis (36, 37). Classically activated microglia secrete the cardinal pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), which can inhibit NSCs from differentiating into neurons in favor of astrocytes (38).

Impaired neurogenesis in the aged brain may result from one or a combination of the many environmental factors that are upregulated or downregulated with increasing age. For example, basal levels of glucocorticoids increased with age in rats and humans (39, 40). Adrenalectomy, which removes corticosteroids, in aged rats dramatically increased cell proliferation, implicating glucocorticoids as negative influences on neurogenesis in aged rats (41, 42). Both age-related hippocampal neurogenesis and cognitive decline can also be prevented when the adrenalectomy is performed in middle-aged (10 months) rats and analyzed at 23 months of age or older, suggesting the absence of corticosteroids during the later stages of aging is critical for preserving neurogenesis and cognitive function (43).

Interestingly, the weight of the adrenal glands correlated negatively with newborn cell proliferation and survival and spatial memory in aged rats, illustrating the strong impact of adrenal glands on hippocampal neurogenesis and function (43).

Additionally, inflammation and oxidative stress are two strong inhibitors of neurogenesis, and both are increased in the hippocampus of aged mice compared to younger mice (44). The density of activated microglia increased with age as well as the levels of pro-inflammatory cytokines in the brain (45). Microglia isolated from aged mice released more TNF- α and IL-6 upon activation compared to those from young mice, suggesting that microglia from aged mice mounted a greater inflammatory response (46). In addition to inflammation, microglia play a key role in modulating levels of oxidative stress by producing the antioxidant glutathione (47, 48). Microglia from aged mice had significantly lower glutathione levels than microglia from young mice (46), and oxidation products were found in microglia in aged mice (49), suggesting that microglia were less effective in attenuating oxidative stress in aged mice.

The major cell types of the neurogenic niche exhibit age-related changes that may be responsible for altering the local microenvironment and the low neurogenesis seen in aged animals. The levels of hippocampal growth factors, known for promoting neurogenesis, including fibroblast growth factor-2 (FGF-2), insulin growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF), were significantly reduced in aged rats compared to young rats (50). The overall density of hippocampal astrocytes, immunopositive for GFAP, remained the same throughout adulthood; however, astrocytes in aged animals had greater immunoreactivity with GFAP and longer processes. Interestingly, the density of astrocytes that expressed FGF-2 declined with age in the DG as well as other regions of the hippocampus, suggesting that the local environment for NPCs progressively became less supportive of neurogenesis with age (50).

Vascular changes with age are also likely to contribute to the decline in neurogenesis. The density of arterioles decreases with age, which may be explained by the impaired angiogenesis characteristic of aged rats (51, 52). The vasculature served as a source of growth factors, and the concentration of IGF-1 decreased in the plasma with age (51), which would result in reduced levels of hippocampal IGF-1. In addition to supplying the hippocampus with neurogenic factors such as IGF-1 and VEGF, the vasculature and the BBB are critical in preventing the entry of most proteins and cells from the bloodstream into the brain. However, with age, there is a breakdown of the BBB and an increase in permeability, allowing the passage of abnormal proteins and cells into the brain that can activate microglia and increase inflammation (53). In senescence-accelerated mice (SAMP8), an aging mouse model, structural changes in the vasculature, including swelling of astrocyte endfeet and perivascular cells with lipofuscin-like granules, were seen in aged but not young SAMP8 mice (54). While it is evident that the neurogenic niche changes with age, it is difficult to identify the primary cell type responsible for the change, given the fact that age impacts each cell type.

4. Integration and maturation of newborn neurons in the aged brain

Newborn neurons in the adult DG undergo a maturation process similar to that generated during development and eventually integrate into the existing circuit of the hippocampus (55-59). This process begins with the initial proliferation of NPCs in the SGZ, which are found clustered among blood vessels, indicative of an intimate relationship between the vasculature and neurogenesis (30). In the first two weeks, new cells migrate from the SGZ and into the granule cell layer where they begin to extend their axons towards the CA3 pyramidal cells of the hippocampus (58, 60, 61). At two to four weeks of age, the

morphological appearance of these immature neurons begins to resemble that of their neighboring mature neurons, as they now display elaborate dendritic arborizations and have developed dendritic spines (58). A striking difference between the newly differentiated and mature neurons remains in their physiological properties, specifically in connection with long-term potentiation (LTP) and GABAergic inputs. LTP refers to the enhanced postsynaptic potential following a brief response to a single pulse following high frequency stimulation and is thought to be an integral component of learning and memory. Newborn immature neurons exhibit a lower threshold for LTP, which may be important for both integration into the local network and contribution to hippocampal functions such as learning and memory (62, 63). GABA is typically known as an inhibitory neurotransmitter for neurons; however, immature neurons initially receive excitatory GABAergic synaptic inputs from local interneurons, which eventually become inhibitory synaptic inputs as the neurons mature. During this time, the neurons begin to receive excitatory glutamatergic inputs from the entorhinal cortex (55, 56). At four to six weeks of age, the newborn neurons are characterized by a critical period of synaptic plasticity, similar to that found during development, with enhanced excitability, larger LTP amplitudes, and reduced induction thresholds (62). Eventually, the new neurons adopt the same morphological and physiological characteristics as the pre-existing neurons and contribute to hippocampal functions.

While newborn neurons in the young adult hippocampus have been extensively studied in regards to their physiological and morphological properties, little is known about newborn neurons in the aged hippocampus. There is, however, some evidence to suggest that there is no difference in the rate of maturation and spine formation of newborn neurons between the aged and young adult brain (11, 13, 64). By stereotactically injecting a GFP retrovirus into the DG to look at spine morphology in the newborn neurons of both young and aged mice, no differences were observed in either spine density or the number of mushroom spines (11, 13). One study, however, suggested that, although the end goal of becoming a mature neuron was attained, the maturation process of newborn neurons in the aged brain was delayed (65). Newborn neurons of both middle-aged and aged rats show decreases in the number of dendritic nodes as well as delayed expression of the mature neuronal marker NeuN. It should be noted that most studies in this area have used mice as the model organism, but the study in which delayed maturation was observed used rats. Little is known about the electrophysiological properties of newborn neurons in the aged brain. One study found no difference in the input resistance of newborn neurons in young adult (two to three months) and aged (16 and 21 months) mice (64); however, it remains unknown if the characteristic critical periods and electrophysiological profile of newborn neurons apply to those generated in the aged brain.

5. Topographic changes of neurogenesis during aging

Ever since the influential case of Henry Molaison, previously known as H.M., who had most of his medial temporal lobe removed to treat epilepsy, the hippocampus has been known to play a crucial role in certain types of learning and memory (66, 67). In addition to the vast amounts of data supporting the link between the hippocampus and memory, the hippocampus has been implicated in other functions such as stress and anxiety (68). There is increasing evidence to suggest that different topographic regions of the hippocampus are involved in each of these functions (69-72). For example, across the dorsoventral axis of the hippocampus, spatial learning and memory functions are more closely tied to the dorsal hippocampus whereas emotion and anxiety are associated with the ventral hippocampus (68, 70, 71). Although it is clear that adult neurogenesis declines with age, little is known about whether this decline follows a topographic pattern in the hippocampus. One study used a stereology-based method to observe topographic differences across aging (73). Multiple

endogenous markers were used in both young (2-month-old) and middle-aged (10-month-old) mice to determine the changes in the densities of neural progenitors and their progeny. A combination of six endogenous markers was used to identify primary progenitors (Sox2⁺/GFAP⁺/S100β⁻ and BLBP⁺), intermediate progenitors (Sox2⁺/GFAP⁺/S100β⁻), glial lineages (Sox2⁺/GFAP⁺/S100β⁺), neuronal lineages (Dcx⁺), and proliferating cells (PCNA⁺). The results of this study indicate that the ventral part of the hippocampus is more susceptible to the effects of aging. Although both primary progenitors and neuronal lineages decreased from young to middle aged animals in all topographic regions, significantly more of this reduction occurred in the ventral section of the hippocampus when compared to the dorsal section. Interestingly, despite the age-related decline in neurogenesis, no topographic differences were observed in gliogenesis during aging.

6. Function of the hippocampus and, more specifically, the DG

It is generally accepted that the hippocampus plays a crucial role in spatial reference learning and memory. Using hippocampal lesions and complete removal of the hippocampus, hippocampus-dependent behavioral tasks have been designed to test this function (74, 75). The Morris Water Maze (MWM) is the most widely used and is considered a standard for hippocampus-dependent behavior. In this spatial navigation task, the animal is placed in a circular pool and trained to swim to a hidden platform just below the surface of the water using spatial cues placed around the room. Latency to reach the platform is used as a measure of spatial learning. In general, once the animal is successfully trained to find the hidden platform, the platform is removed from the pool and probe trials are used to assess the animal's ability to remember the spatial location of the platform. During the probe trial, the animal is allowed to swim freely for a set period of time. If the animal has learned the task using a spatial strategy (relying on the hippocampus), the animal spends the majority of its time swimming in the quadrant that contained the previously hidden platform. Time spent in the target quadrant during the probe trial is indicative of spatial memory. Animals without an intact hippocampus are unable to learn the location of the platform (74). If the hippocampus is inactivated after training, the animals exhibit impaired spatial memory.

Based on the hippocampus-dependent nature of both the spatial learning and memory aspects of the MWM, studies investigating the functional role of hippocampal neurogenesis utilized this behavioral paradigm to determine if the absence of neurogenesis negatively impacted spatial learning and memory. Aged mice, with significantly lower hippocampal neurogenesis, took considerably longer to swim to the platform during training trials, indicating that their spatial learning was impaired compared to young mice (13). While the cognitive impairment of aged rats compared to young rats was apparent using the MWM, the performance of aged rats could vary substantially. Based on their MWM performance, aged rats could be separated into two different groups by designating half the animals above the median as aged-impaired and the other half as aged-unimpaired (76). Aged-impaired rats were slower to learn the platform location and searched less in the target quadrant compared to aged-unimpaired. Behavioral performance in the MWM positively correlated with cell proliferation and the number of surviving newborn neurons, both of which were greater in the aged-unimpaired than in the aged-impaired rats. However, when aged rats were categorized as aged-unimpaired using a stricter criterion, there appeared to be no correlation between cell proliferation and MWM performance (77). In this study, aged rats were designated as aged-unimpaired only if they searched the target quadrant during the probe trial to a similar extent as young rats. The high variation in MWM performance of aged rats did not correlate with low variation in cell proliferation levels of aged rats, suggesting that cell proliferation cannot be used as a predictive measure of cognitive abilities (77-79). Collectively, these studies demonstrate that, when comparing aged to young animals, aged

animals were impaired in both hippocampal neurogenesis and behavioral performance, suggesting there is a positive correlation between neurogenesis and cognition. However, conflicting results were reported when studies attempted to correlate neurogenesis and behavioral performance within only the aged animals; the discrepancies might be due to a number of experimental differences, including rodent strains and MWM paradigms (Table 1 – list of studies included in this review). Conceptually, if neurogenesis were directly related to hippocampal function, then an aged animal with more neurogenesis would perform better in the MWM than an aged animal with less neurogenesis. Unfortunately, both the behavioral performance and overall process of aging, e.g. changes in immune system, are highly variable even within the same age group, which is in contrast to the relatively consistent neurogenesis levels among animals of the same age. While the concept of correlating individual performance with neurogenesis levels is logical and reasonable, it may be unfair to expect the neurogenesis to completely account for the variability in behavior of aged animals when other aging factors may be having a larger impact.

Another potential explanation for cognitive differences is embedded in the design of the MWM paradigm. Unfortunately, there is no standard protocol for the MWM paradigm, and experiments vary in the number of training days, trials, and number of probe trials, and when probe trials are administered. The probe data of aged mice can be interpreted as either a deficit in spatial memory or a reflection of insufficient training, as aged mice may simply take more trials to learn the platform position. However, if young and aged mice were trained to the same criterion (equivalent swim distance or latency to platform), subsequent performance on a probe trial was also similar in young and aged mice, demonstrating that aged mice were not impaired in spatial memory (80).

In addition to MWM, contextual fear conditioning (CFC) is commonly used as a secondary complement to MWM. While both tasks are considered hippocampus-dependent, CFC uses different motor and sensory components and allows for another method to independently test hippocampal function. The CFC test is based on Pavlovian conditioning, where a conditioned stimulus (CS) becomes associated with an unconditioned stimulus (US). The level of association learning can be measured by the conditioned response (CR) in response to presentation of the CS only. In the CFC task, animals must make an association between an aversive experience (foot shock - US) and a spatial context (CS). Briefly, the animal is placed in a box (context 1) and administered a foot shock. Following the experience, the animal is either placed again in context 1 or into a new context 2 to determine whether the animal makes an association between the foot shock and the specific spatial context. Memory, in this case, is measured by the animal's natural response of freezing (CR), which only occurs in context 1 if the animal has learned the task appropriately. Probe trials can be administered to test for retention of the contextual memory.

Similar to the variability in the MWM, middle-aged mice (8 months) could be separated into two groups based upon performance in CFC (81). Middle-aged mice freezing less than 61% of the time in the context were designated as weak-learners, compared to the 91% freezing observed in young (2 months) and middle-aged learner mice. Thirty percent of middle-aged mice were designated as weak-learners due to their minimal contextual freezing. This variability in performance within the middle-aged animals may partially account for the conflicting results of whether aged animals are impaired in CFC. In a trace fear conditioning paradigm, aged rats (24-33 months) exhibited a short-term memory deficit (one to two days post conditioning) compared to young rats (3-6 months) when placed back into the conditioning context (82, 83). In contrast, another study found that aged rats (12 months) did not exhibit a short-term memory deficit (two days post conditioning), which does not correlate with the decline in NPC proliferation in aged rats (84). The conflicting results may largely be due to the difference in the age of rats used in the two studies, as one-year-old

rats, which could be considered middle-aged, had more cell proliferation than two-year-old rats (85). Although middle-aged rats had decreased cell proliferation compared to young rats, the reduced neurogenesis might not manifest as impaired cognition in middle-aged rats. It remains unknown why two middle-aged animals with a similar level of neurogenesis can differ so dramatically in cognitive performance. Perhaps a more stringent behavioral test would be sensitive to detect these slight differences in neurogenesis.

In addition to the variability among the animals of the same age group, many features can be manipulated in the CFC paradigm, including the presentation of tones, number of trials, and timing of retention trials, to influence behavioral performance. In a delayed fear conditioning paradigm, aged rats (23 months) exhibited long-term (52 days post conditioning) retention deficits but not short-term retention deficits (10 days post conditioning) (86). Interestingly, young rats (4 months) froze more during the long-term than short-term time point, whereas there was no change in the amount of freezing in aged rats over time (86). Given the length of time between conditioning and testing, long-term retention deficits seen in aged rats might be more suggestive of age-related changes in memory consolidation than hippocampal neurogenesis as retention becomes less dependent on hippocampus over time (86). The increase in freezing by young rats over time is attributed to generalization, which refers to the enhanced conditioning response to multiple stimuli. When discussing fear conditioning in aged animals, it is important to consider how aging affects generalization, an interesting feature of CFC memory that occurs in young but not aged rats (87). It is unknown how hippocampal neurogenesis affects generalization in young and aged animals.

Although CFC and MWM are standard hippocampus-dependent behavioral tasks and have proven useful in many ways, there is still a need for more sensitive tasks. While it is clear that neurogenesis persists in the hippocampus and these newborn neurons functionally integrate themselves into the existing hippocampal circuitry, the contribution that these newborn neurons make to the overall function of the hippocampus is still unclear. Although a few studies did not find a correlative relationship between neurogenesis levels and hippocampus-dependent behavior, the majority of studies have reported a positive correlation (6). Many of the discrepancies seen with neurogenesis levels and hippocampus-dependent behavior could be attributed to slight variations in each of the studies (strain, species, side-effects of manipulations, age, etc...), but perhaps we simply need more sensitive tasks. Neurogenesis is restricted to the DG and therefore it is highly likely that the contribution of newborn neurons is more crucial to DG function specifically. Aside from spatial learning and memory, the hippocampus, and primarily the DG, has been implicated in spatial pattern separation (88, 89). Spatial pattern separation refers to the ability to discriminate between two closely related but distinct pieces of spatial information. Recently, studies have suggested that, as two pieces of spatial information become more similar, neurogenesis becomes necessary to distinguish between the two. If the two pieces of spatial information are relatively different, even animals without neurogenesis can distinguish between the two (90, 91). To test spatial pattern separation, a recent study developed a novel touchscreen task that appears to be very sensitive to changes in neurogenesis (90).

7. Touchscreen tasks

One of the main sources of variability in behavioral tests is the investigator who handles the animals and administers the tests. For example, in the MWM, investigators differ in how they introduce the mouse into the water, how they guide the mouse to the platform during training, and how the mice are handled after each trial. These tiny manipulations have a tremendous impact on the behavioral performance of the animals. One of the benefits to using mouse touchscreen operant chambers is that each individual trial is run by the mouse

and therefore eliminates any variability introduced by the investigator. Each mouse touchscreen chamber is outfitted with an infrared touchscreen, a reward dispenser (either pellet or liquid) with light illumination to let the animal know a reward has been given, and head entry detectors so the animals can activate the next trial.

Recently, a hippocampus-dependent paradigm was designed for the touchscreen operant chamber (92). In the paired associates learning (PAL) object in place test, mice were trained to match three different objects to three unique spatial locations on the touchscreens. During testing phases, two objects are presented, one in the correct spatial location and one in the incorrect spatial location. Mice received a reward only when they nose-poked the object in the correct spatial location. Inactivating the hippocampus with lidocaine impaired the ability of the mice to identify the object in the correct spatial location, illustrating that the associative learning was dependent on an intact hippocampus. Interestingly, if neurogenesis was ablated by irradiation or lentivirus-mediated knockdown expressing a dominant negative Wnt (dnWNT) protein, mice performed this task equally well compared to mice with normal levels of neurogenesis, indicating that neurogenesis was not necessary for this particular hippocampus-dependent learning task (90).

A spatial pattern separation task using mouse touchscreens has been shown to be especially sensitive to changes in neurogenesis levels (90). The two-choice spatial discrimination task was designed to test the spatial discrimination ability of the mouse. Briefly, mice were presented with two illuminated boxes during each trial. Mice were trained to nose-poke one of the boxes, which was fixed in its spatial location until a certain criterion was met. Then reversal trials were performed, and mice were now rewarded only when choosing the other box. By manipulating the degree of separation between the two boxes, this task became sensitive to neurogenesis levels. If the boxes were spaced farther apart (high separation), mice with or without neurogenesis performed equally well on this task. However, at low degrees of separation, where the boxes were spaced in close proximity to each other, mice without neurogenesis were severely impaired in discriminating between the two spatial locations. This study not only highlighted the advantages to using the touchscreens but also provided evidence for a task that is highly sensitive to neurogenesis levels. Aged animals also exhibited poor spatial pattern separation on the touchscreen task, but their performance improved with voluntary wheel running, which increased neurogenesis (91).

8. Effects of running and enrichment on neurogenesis in aged mice

Neurogenesis is a dynamic process and is regulated by many factors. Manipulations of the environment, such as enrichment and exercise, have been shown repeatedly to increase neurogenesis (93, 94). Environmental enrichment can be defined as an environment that enhances cognitive, sensory, social, and motor stimulation. In many cases, it includes an enlarged housing cage consisting of toys, tunnels, larger social groups (allowing for more social interaction), and, in some cases, running wheels. Previously, most enriched environments included running wheels for motor stimulation; however, it was not until one study identified voluntary exercise as one of the most salient components of environmental enrichment that the effects of each were investigated separately (95). Both voluntary exercise and environmental enrichment enhance adult hippocampal neurogenesis, albeit through different mechanisms. Voluntary exercise increases cell proliferation in the DG, leading to an overall total increase in the number of newborn neurons, whereas environmental enrichment promotes the survival of newborn neurons. Both environmental manipulations are consistent with increases in trophic factors, such as BDNF and nerve growth factor (NGF), as well as increases in spine density (see (96) for review), which could partially explain the observed increase in neurogenesis and enhanced learning and memory. The reasons why each manipulation acts differently to increase neurogenesis remain unclear,

but one clear difference between voluntary exercise and environmental enrichment is the rigorous physical activity animals undertake when allowed access to a running wheel (97). This increase in motor activity is associated with changes in the vasculature of not only the brain but also the periphery, resulting in increased blood flow and BBB permeability. This change in both brain and peripheral vasculature could help increase the circulation of pro-neurogenic factors such as VEGF to other areas of the brain (98).

It is well established that aging is associated with a decline in cognitive function as well as neurogenesis levels, but these declines may be prevented or even reversed even at advanced stages of aging. Voluntary exercise and environmental enrichment have both been shown to dramatically reduce the effects of aging on neurogenesis and cognition in mice (13, 91, 99). Aged animals placed in an enriched environment for roughly half their lives showed a five-fold increase in the number of newborn granule cells, which might be primarily due to an increase specifically in the neuronal differentiation of newborn cells (99). Labeling with the thymidine analog bromodeoxyuridine (BrdU) showed no significant difference in the total number of newborn cells in control and enriched mice, suggesting that cell proliferation remained unaffected by enrichment. However, analysis of the phenotype of these newborn cells revealed significantly more of the newborn cells differentiated into neurons. The increase in neurogenesis was reflected in cognitive function, as the enriched mice were significantly better in spatial learning in the MWM than controls that were housed in standard caging, supporting the positive correlation of neurogenesis and cognition.

While most studies house animals in enriched environments, a weekly three-hour exposure for 18 months was sufficient to increase both BrdU and Dcx densities as well as improve novel object recognition memory in aged rats (100). In the novel object recognition paradigm, animals were presented with two objects during the study phase. During the retention phase, animals were presented with one familiar object, which was explored during the study phase, and one novel object. Time spent exploring the novel object was indicative of recognition memory. While the extent of hippocampus involvement in this task remains controversial (101, 102), rats with hippocampal lesions spent less time with the novel object during the retention phase, suggesting the contribution from the hippocampus was critical for this task (103). Rats exposed to enriched environment weekly spent significantly more time with the novel object during the retention phase, suggesting that enriched environment improved recognition memory in aged rats (100).

Voluntary exercise has also been shown to reverse many of the age-related deficits observed in animals (13). Aged mice (18 months) allowed access to a running wheel for seven weeks exhibited a five-fold increase in the total number of newborn neurons. This increase in neurogenesis positively correlated with an improvement in the hippocampus-dependent MWM task. Interestingly, this study demonstrated that even at late stages of aging, when deficits were most dramatic, running was still able to partially reverse the age-related deficits of cognition and neurogenesis. These studies suggested that running and enriched environment altered the local environment in aged brains, allowing newborn cells to retain the potential to become functional neurons comparable to young brains. These environmental manipulations are sufficient to both protect and reverse many of the age-related deficits, even at advanced stages of aging.

9. Effects of aging on neurogenesis in non-human primates

Adult neurogenesis is present in many mammals including humans (104); however, the effects of aging on higher order mammals, such as non-human primates, has been observed in only a handful of studies (105-108). Consistent with findings in rodent models, these studies indicated a reduction of neurogenesis and cell proliferation in aged animals in both

New and Old World primates (105-108). Middle-aged marmosets (3.5-7 years) had significantly less newborn cell survival and fewer of the newborn cells differentiated into neurons compared to young marmosets (1.5-3 years) (108). There was a 90% decrease in newborn neurons from young (< 3 years) to aged marmosets (8-15 years), illustrating the age-related decline in neurogenesis in New World primates (109). Consistent with these findings, Old World aged cynomolgus macaques (23 years old) had both significantly less cell proliferation (BrdU+ cells) and immature neurons (TOAD-64+ cells) compared to young adult cynomolgus macaques (5 years old) (107).

The cognitive decline associated with aging has been shown repeatedly to correlate with neurogenesis levels in rodents; however, few studies have looked at the effects of aging on both hippocampal neurogenesis and cognitive function in non-human primates. Age-related cognitive deficits can be detected in a variety of behavioral tasks in non-human primates. A visual pattern discrimination (VPD) task, which has been shown to involve an intact hippocampus (110, 111), can be used to assess the animal's cognitive capabilities (106). Young and aged cynomolgus macaques were tested in two versions of the VPD task. In VPD1, macaques were shown a pair of visual patterned cards. Two cards were presented simultaneously and macaques were trained to identify one pre-defined "reward" card in each trial. A total of 13 cards was used randomly and 12 were pre-defined as "non-reward" cards. In VPD2, macaques were again shown a pair of cards, and during this task 12 cards were pre-defined as "reward" cards and one as a "non-reward" card. The percentage of correct trials served as a measure of cognitive ability. While both young and aged macaques were able to learn both tasks, aged macaques learned the tasks significantly more slowly compared to young macaques, demonstrating the age-related cognitive deficit in non-human primates. Correlating with learning performance on the VPD tasks, aged macaques had significantly less cell proliferation (endogenous proliferative marker Ki67+ cells) when compared to young macaques.

Although it is clear that aging is associated with diminished cell proliferation and newborn neurons, little is known about whether these are caused by NSCs becoming quiescent or an overall reduction in NSC population with increasing age in primates. As discussed earlier, there are no age-related changes in the density of NSCs in aged rats, as assessed with SOX2 as an NSC marker (15). However, this density may not be conserved in primates, based on the observation that NPC population decreases with age in humans, as measured by magnetic resonance spectroscopy (112). Although there are obvious differences in procedure and analysis of NPCs in rats versus humans, this discrepancy may be due to a differential aging process of NPCs in rodents and primates. To investigate this hypothesis, Aizawa et al. examined NPC populations detected by triple immunostaining for FABP7 (fatty acid binding protein 7), Sox2, and GFAP in both young and aged macaques and mice (105). Confirming both previous studies, a reduction in the number of NPCs was observed in the aged macaques but the NPC population remained stable with age in mice. Remarkably, this reduction in NPC population appeared to be specific to primates, whereas the decline in proliferation of NPCs was conserved between mice and primates.

In addition to differences in total NPC numbers, morphological changes of these cells with age were observed in aged macaques and mice (105). In both young and aged animals, the processes of NPCs in mice were much longer than those in the macaques. With age, there was no shortening of the processes of NPCs in mice. However, aged macaques showed a significant decrease in process length when compared to young macaques. By quantifying the angle at which the NPCs extended their processes, in relation to a vertical line perpendicular to the SGZ, young macaques and both young and aged mice sent processes at an angle nearly perpendicular to the SGZ. In contrast, NPCs in aged macaques exhibited a more horizontal orientation of the processes, which was significantly different than young

macaques and both young and aged mice. While there was a similar decline in the number of dividing cells and immature neurons in both mice and macaques with age, the observed primate-specific changes might reflect a difference in the aging of long life span animals where more advanced age-related changes occur.

10. Effects of aging on neurogenesis in humans

The first evidence of neurogenesis in humans was observed in postmortem tissue from cancer patients, who had previously received one BrdU injection to assess proliferative activity of cancer cells (104). Staining the postmortem tissue for BrdU revealed the presence of dividing cells in the SGZ as well as the SVZ of the human brain. Determined by their cross-reactivity with GFAP and neuronal markers such as NeuN, calbindin, and NSE, BrdU⁺ cells were found to co-localize with neuronal as well as astrocytic markers. A fraction of the BrdU⁺ cells failed to differentiate into neurons or astrocytes, indicative of an undifferentiated NPC population.

A more recent study identified NPCs in the adult human brain *in vivo* using magnetic resonance spectroscopy (112). Two techniques, proton magnetic resonance spectroscopy (H-MRS) and proton nuclear magnetic resonance spectroscopy, can be used to identify metabolites specific to NPCs in the human brain *in vivo*. By first determining the unique NPC spectra profile in mice *in vitro*, Manganas et al. identified a reliable biomarker of NPCs, 1.28-ppm, that can also be confirmed in rats *in vivo*. In adult humans, the biomarker is detected in the hippocampus but not in the cortex, illustrating the specificity of this biomarker in humans as well as the utility of this method to detect levels of NPCs. Interestingly, there was a significant decrease in the signal of the 1.28-ppm biomarker from preadolescents to adults, consistent with data that there is an age-related decrease of neurogenesis in mammals.

Numerous studies have observed age-related cognitive deficits in humans (113); however, it remains unclear whether there is a direct link to neurogenesis in humans. There is evidence demonstrating the close association of known enhancers of neurogenesis in non-human mammals, such as exercise and mental enrichment, with improvements in cognition in aged humans, but this is merely correlational (114, 115). Although it remains uncertain whether these positive regulators of neurogenesis in non-human mammals have the same effect in humans, on the newborn neuron population, the beneficial effects of exercise and mental stimulation on cognition remain unmistakable (114, 115).

11. Conclusion

New neurons are continuously generated in the adult brain throughout life. These newborn neurons go through an extended maturation process and eventually functionally integrate themselves into the existing DG circuitry. Although the exact role these newborn neurons play remains unclear and many of the links to hippocampus-dependent learning and memory are merely correlational, new behavioral tasks that are more sensitive to functions specific to the DG may be necessary to elucidate a causal link between the two. It is clear, however, that aging is associated with a dramatic decrease in both neurogenesis and hippocampus-dependent learning and memory. Interestingly, although many ongoing changes are present in the local microenvironment of the aging brain, newborn neurons appear to retain the potential to become fully mature and functional granule cells. The potential growth and maintenance of the newborn neurons can be enhanced, even at late stages of aging, by exposing animals to both physical and mental stimulation. Perhaps, there's some truth to the phrase that 'a healthy body equals a healthy mind'.

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Research Highlights

- Adult hippocampal neurogenesis and cognitive function decline with age.
- Regulation of neurogenesis altered at both intrinsic and extrinsic levels with age.
- Newborn neurons can develop into fully functional neurons in an aged brain.

Figure 1.

The effects of aging on the neurogenic niche in the SGZ. Fewer newborn neurons are accompanied by the increase in reactive astrocytes and activated microglia as well as changes in the BBB in the aged SGZ.

Behavioral Test	Age	Sex	Strain/Species	Paradigm	Effects on cognition and neurogenesis	Reference
Morris water maze	3 vs 19 mo	Male	C57BL/6 mouse	Training: 4 trials/day for 5 days Probe: 4 hours after last training trial	Reduced neuronal differentiation correlates with impaired spatial learning in aged mice	13
	3-5 vs 19-21 mo	Male	Balb/C mouse	Training: 5-6 trials for 1-2 days Probe: 30 days after last training day	Aged mice exhibit similar spatial memory compared to young mice if trained to a similar degree	80
	2 vs 21 mo	Female	Fischer 344 rat	Training: 2 trials/day for 10 days Probe: last training day	Number of newborn cells does not correlate with spatial memory in aged rats	77
	3 vs 20 mo	Male	Sprague-Dawley rat	Training: 4 trials/day	Number of new neurons correlates with behavioral performance in aged rats	76
	7, 13, 25 mo	Male	Long Evans rat	Training: 3 trials/day for 8 days Probe: every 6th trial	Number of newborn cells does not correlate with spatial learning in aged rats	78
Contextual fear conditioning	7 vs 25 mo	Male	Long Evans rat	Training: 3 trials/day for 8 days Probe: every 6th trial	Number of newborn cells and neuronal differentiation does not correlate with spatial learning in aged rats	79
	4, 10, 23 mo	Male	Fischer 344 rat	Training: 3 trials of delay conditioning Intertrial interval: 90-120 min Retention trial: 10 and 52 days	Aged mice are impaired in long-term but not short-term memory	86
	3, 9, 24 mo	Male	Fischer 344 rat	Training: 2 trials of delay conditioning Intertrial interval: ? Retention trial: 24 hour	Aged rats impaired in short-term memory	82
	3-6, 8-12, 16-20, 24-33 mo	Male	Sprague-Dawley rat	Training: 10 trials of trace conditioning Intertrial interval: 8.5 min Retention trial: 48 hour	Aged mice are impaired in short-term memory	83
	2, 5, 12 mo	Male	Sprague-Dawley rat	Training: 10 trials of trace conditioning Intertrial interval: 4 min Retention trial: 48 hour	Reduced number of newborn neurons does not correlate with unimpaired short-term memory in aged rats	84
Touchscreen task	3 vs 22 mo	Male	C57BL/6 mouse	Training: two-choice spatial discrimination task	Aged mice are impaired in the two-choice spatial discrimination task	91