Stem cell aging

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Seeing is believing: old clones die young

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The ability of adult neural stem cells to produce new neurons (neurogenesis) declines markedly during aging, but exactly how this occurs is largely unknown. Using sophisticated in vivo imaging, a study in *Nature Aging* shows that aging affects several steps of neurogenesis — most notably, increasing the death of newborn clones.

Aging causes cognitive decline and increased susceptibility to neurodegenerative diseases, including Alzheimer's disease. The adult brain contains small reservoirs of neural stem cells (NSCs) that have regenerative potential and might help to counter aspects of brain aging and disease. NSCs are predominantly found in two regions (the subventricular zone (SVZ) and the dentate gyrus of the hippocampus[1,2]) and they have been found to be important for aspects of learning and memory in rodents[1,2]. NSCs are mostly quiescent (dormant), but they can receive signals to become proliferative and either self-renew (that is, give rise to other NSCs) or generate differentiated progeny, including new neurons — a process known as neurogenesis. During aging, neurogenesis markedly declines in rodents[1,2]. But the exact step in the path from quiescent NSCs to neurons that is most affected by increasing age is still not entirely clear. Understanding this question should help in the design of new targeted strategies to reverse aspects of aging in the brain. In this issue of *Nature Aging*, Wu et al.[3] use chronic intravital imaging to dynamically follow individual NSCs and their progeny in the mouse hippocampal niche.

In rodents, hippocampal neurogenesis sharply declines by early middle age (9 months)[4] and continues to decline as the animals age. Previous studies have used transcriptomic profiling and static lineage tracing to better understand the behavior of hippocampal NSCs[5,6], as well as changes in the hippocampal NSC lineage during aging[1,2,7,8]. These studies have revealed that this NSC reservoir is heterogeneous[5] and that NSCs in old brains enter a deeper state of quiescence or cannot activate as efficiently[7,8], contributing to reduced neurogenesis. But the precise dynamics of neurogenesis decline is not well understood, in part because of the lack of direct tracking ability. Wu et al.[3] addressed this gap by performing intravital imaging of genetically labeled NSCs in the hippocampal niche of young and middle-aged mice. To this end, they used a genetically engineered mouse that allows expression of a red fluorescent protein (tdTomato) in quiescent and activated NSCs (also known as radial glia-like (R) cells) and their progeny — neural progenitor cells (also known as nonradial glia-like (NR) cells or transiently amplified progenitors) and newborn neurons. They installed a cranial window in these mice and imaged the hippocampal niche daily using sophisticated two-photon microscopy, thereby enabling direct tracking of these labeled cells. The Jessberger laboratory previously used this imaging technique in young mice and observed differences in the dynamics of subpopulations of NSCs[9,10].

In the present study, Wu et al.[3] track the labeled quiescent NSCs and their progeny in the hippocampus for nearly 4 months in young (2-month-old) and middle-aged (12–14-month-old) mice. Wu et al.[3] show that aging is accompanied by a reduction in the ability of quiescent NSCs to exit quiescence, similar to other static lineage-tracing studies in the hippocampus[1,2,7,8] (Fig. 1). Importantly, the authors also find that each individual NSC leads to clones (that is, groups of cells that all originate from the same NSC) of smaller size during aging,

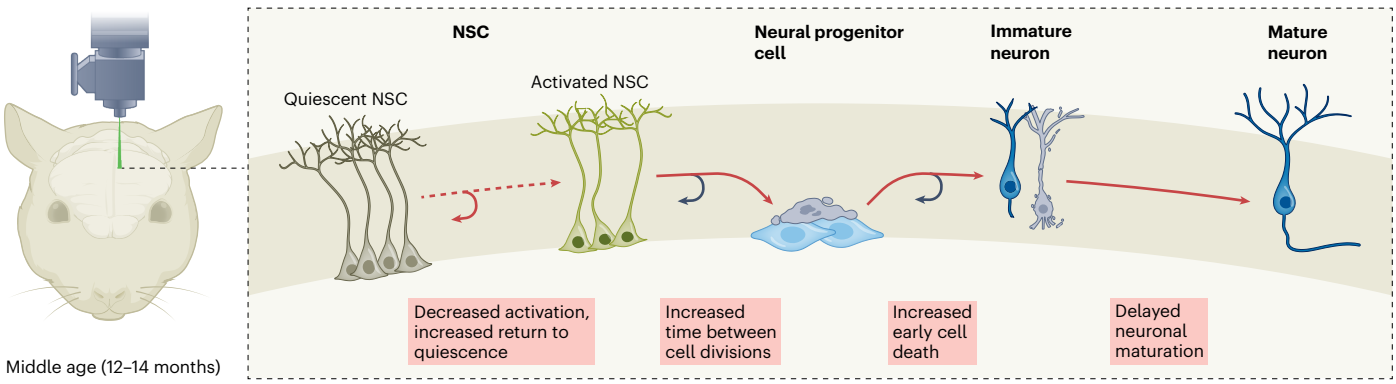

Fig. 1 | Aging affects neurogenesis dynamics. Quiescent NSCs (tan) are the resident NSC population in the adult brain. Intravital imaging of the hippocampal NSC niche reveals several changes that occur during aging (highlighted by red arrows and red boxes). Quiescent NSCs activate and produce less-proliferative activated NSCs (green) and exhibit an increased tendency to return to long-term quiescence. NSCs undergo several rounds of division to produce progeny such as neural progenitor cells (light blue), which give rise to neurons (dark blue). Aging causes the time between these divisions to increase, but overall division potential and differentiation capacity remains unaffected. Importantly, aging also causes increased early cell death in newborn cells within NSC clones (gray cells) and delayed neuronal maturation. All these factors contribute to an age-related decrease in clonal output for an individual quiescent NSC.

which corroborates a finding from a recent lineage-tracing study in the aging hippocampus[5]. Wu et al.[3] also show that aging causes the time between divisions to increase for both NSCs and progenitors and that, at least for NSCs, this is driven by an increased return to quiescence (Fig. 1). This is also consistent with previous studies in the hippocampal NSC niche[7,8]. Wu et al.[3] observe in vivo that after activation, a portion of young NSCs undergo a burst of proliferation and become depleted whereas middle-aged NSCs tend to return to long-term quiescence after activation and become proliferative later.

The authors find that even though NSC clone size decreases with age, the cell division potential and the type of division – self-renewing versus differentiating divisions – are actually unchanged with age. So why do clones of NSCs become smaller with age? Wu et al.[3] discover that there is a notable increase in NSC progeny death in middle-aged mice. Specifically, cells in middle-aged mice die shortly after being born (within 7 days) (Fig. 1). Thus, the reduction of clone size (and decrease in neurogenesis) is heavily influenced by the early death of newborn cells in NSC clones. The mechanisms by which these newborn clones die is not yet known and could be influenced both by intrinsic and extrinsic factors (such as a lack of support factors from other cells, which may decline during aging, or the accumulation of neurotoxic factors with age). It will be important to explore how these newborn cells in NSC clones are dying, whether it be through apoptosis, autophagic cell death, necrosis or some other type of cell death. It is also unclear whether existing cells in the niche may become senescent during aging, resulting in the production of proinflammatory cytokines (or other factors) that could contribute to the death of newborn clones.

Finally, Wu et al.[3] explore how aging affects the final step in the process of neurogenesis – the production of fully mature neurons. In the hippocampus, immature neurons migrate to the granule cell layer where they mature and differentiate into dentate granule cells. Leveraging chronic intravital imaging, the authors observe the dynamics of cell migration and neuronal maturation in the hippocampus (Fig. 1). They find that aging does not affect migration speed or distance traveled by newborn neurons. However, the length and number of dendritic branches is significantly decreased in middle-aged mice over the time period observed (about 1 month after the birth of a new neuron in the adult mouse). Thus, aging causes delayed neuronal maturation, which had previously been observed[11]. As hippocampal neurons produced in older animals eventually gain mature characteristics[11,12], aging may cause only a brief delay in neuronal maturation. It will be interesting to determine whether such a short delay can have a functional effect on learning and memory behaviors.

Are the defects observed exacerbated with increasing age and disease? At 12–14 months of age, mice are middle-aged (the equivalent of 45 years old in humans) and already exhibit marked defects. It will be interesting to conduct these studies at a range of ages to test whether the changes observed by Wu et al.[3] are linear with age or whether there are different inflection points in the aging process. Injury or neurodegenerative diseases could also exacerbate age-dependent differences. Finally, there could be individual-to-individual differences, with individuals exhibiting better or worse aging trajectories in terms of their neurogenesis steps, and this could predict future susceptibility to disease.

Although many aspects regulating NSC function are similar between the two main neurogenic niches (the hippocampus and the SVZ), distinct differences have also been observed. Unlike the present study, a static lineage-tracing study of NSCs in the SVZ niche found that aging did not affect clone size[13]. Although this could be due to differences in the labeling technique used to identify NSCs or in the length of time that individual clones were tracked, this may also reflect different underlying biology between these niches. In addition, hippocampal NSCs largely undergo asymmetric divisions[5,6] whereas SVZ NSCs undergo more symmetric self-renewing divisions[14]. It will be interesting to use chronic intravital imaging to directly compare NSC dynamics in the SVZ and the hippocampus during aging. We might also consider stem cell niches in other tissues. This type of dynamic tracking has been used to study tissue homeostasis and/or repair in other stem cell niches, such as in hair follicles, intestine, bone marrow, muscle and mammary gland. It will be interesting to compare how aging affects different types of stem cells, particularly the early cell death of newborn clones observed during aging in the hippocampus.

Recent technological advancements in intravital imaging have enabled continuous chronic intravital imaging in freely moving young adult mice, which revealed that NSC activation is regulated by day–night cycles in the SVZ niche[15]. In the present study, Wu et al.[3] perform intravital imaging in anesthetized mice for periods up to 45 minutes a day. In future work, it will be informative to use continuous intravital imaging in freely moving mice during day and night to investigate aspects of NSC activation, proliferation, differentiation and survival during aging, and to uncover potential circadian changes. Although the extent of adult neurogenesis has been debated in humans, NSCs are present in the human brain and their ability to give rise to new neurons also declines as a function of age[1,2] – it would be exciting to study this in real-time. Although this type of imaging is of course not possible in humans with current technology, positron emission tomography tracers are being developed to track stem cells to noninvasively monitor potential NSC activity in humans.

At the molecular level, previous studies have revealed that aging causes many changes in the NSC lineage, including changes in the epigenome and transcriptome, decreased protein homeostasis, increased inflammation and altered metabolism[1,2]. It will be important to determine the causal link between these age-dependent molecular changes and the cellular steps affected during aging that are identified in the present study. Another goal for the future will be to use intravital imaging to explore how interventions that have been shown to reverse the decline in neurogenesis (exercise, young blood and dietary interventions)[1] act on the various cellular processes affected by age.

Overall, the power of chronic intravital imaging in mice has revealed fundamental new NSC biology. This approach enables real-time imaging of the dynamics of NSCs and their ability to give rise to new neurons in young and old individuals. Wu et al.[3] find that aging causes increased early cell death in newborn clones in the hippocampal niche, raising the exciting question of whether this cell-death phenotype is caused by intrinsic or extrinsic mechanisms. They also strengthened previous findings in NSC aging biology by showing that aging results in an enhanced return to long-term quiescence and delayed neuronal maturation. This study offers insights into how aging affects NSC function, which could serve as a foundation for targeted therapies to improve neurogenesis and counter brain aging and diseases.

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References

1. Navarro Negredo, P., Yeo, R. W. & Brunet, A. *Cell Stem Cell* **27**, 202–223 (2020).
2. Kuhn, H. G., Toda, T. & Gage, F. H. *J. Neurosci.* **38**, 10401–10410 (2018).
3. Wu, Y. et al. *Nat. Aging*, https://doi.org/10.1038/s43587-023-00370-9 (2023).
4. Ben Abdallah, N. M., Slomianka, L., Vyssotski, A. L. & Lipp, H. P. *Neurobiol. Aging* **31**, 151–161 (2010).
5. Bonaguidi, M. A. et al. *Cell* **145**, 1142–1155 (2011).
6. Encinas, J. M. et al. *Cell Stem Cell* **8**, 566–579 (2011).
7. Harris, L. et al. *Cell Stem Cell* **28**, 863–876 (2021).
8. Ibrayeva, A. et al. *Cell Stem Cell* **28**, 955–966 (2021).
9. Bottes, S. et al. *Nat. Neurosci.* **24**, 225–233 (2021).
10. Pilz, G. A. et al. *Science* **359**, 658–662 (2018).
11. Trinchero, M. F. et al. *Cell Rep.* **21**, 1129–1139 (2017).
12. Morgenstern, N. A., Lombardi, G. & Schinder, A. F. *J. Physiol.* **586**, 3751–3757 (2008).
13. Bast, L. et al. *Cell Rep.* **25**, 3231–3240 (2018).
14. Obernier, K. et al. *Cell Stem Cell* **22**, 221–234 (2018).
15. Gengatharan, A. et al. *Cell* **184**, 709–722 (2021).

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Competing interests

The authors declare no competing interests.