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Review

Brain aging and rejuvenation at single-cell resolution

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SUMMARY

Brain aging leads to a decline in cognitive function and a concomitant increase in the susceptibility to neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. A key question is how changes within individual cells of the brain give rise to age-related dysfunction. Developments in single-cell "omics" technologies, such as single-cell transcriptomics, have facilitated high-dimensional profiling of individual cells. These technologies have led to new and comprehensive characterizations of brain aging at single-cell resolution. Here, we review insights gleaned from single-cell omics studies of brain aging, starting with a cell-type-centric overview of age-associated changes and followed by a discussion of cell-cell interactions during aging. We highlight how single-cell omics studies provide an unbiased view of different rejuvenation interventions and comment on the promise of combinatorial rejuvenation approaches for the brain. Finally, we propose new directions, including models of brain aging and neural stem cells as a focal point for rejuvenation.

INTRODUCTION

Aging is associated with a decline in brain function and a striking increase in the prevalence of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. Indeed, the main risk factor for these neurodegenerative diseases is old age.[1] Even in the absence of disease, aging is associated with cognitive decline.[2] In humans, aging is often characterized by decline across multiple cognitive domains such as fluid intelligence, processing speed, attention, memory, and learning.[2] Hence, a systematic understanding of the changes that occur in the aging brain is critical to designing new strategies for countering age-related cognitive decline and neurodegenerative diseases.

In the past, most studies on brain aging have focused on select aspects, including performance on cognitive and behavioral tasks,[2–5] loss of synaptic plasticity and neural circuits,[6] changes in gene expression from bulk profiling of brain tissues,[7–9] DNA damage and repair,[10] compromised brain metabolism,[11] and comparisons between normal aging and neurodegenerative disease.[12] This has provided invaluable information on how the global state of the brain changes during aging. However, the ensemble of cellular changes in the brain during aging, and how they differ in diverse brain cells, is still not fully understood. The brain is arguably the most complex of all organs, consisting of many different cell types and subtypes, with specialized functions, and with intricate interactions between cell types. For example, neurons encompass many specialized subtypes with different functions across brain regions.[13,14] Non-neuronal cell types—oligodendrocytes, astrocytes, neural stem cells (NSCs), cells of the brain vasculature and meninges, and immune cells of the brain—have emerged as key players in brain aging.[15–19] Thus, important questions arise: are all brain cells aging at the same pace and in the same manner? Are there shared hallmarks of aging across brain cell types or regions? How are interactions between these diverse cell types changing with age? Can specific aspects of cellular brain aging be rejuvenated by specific interventions?

The advent of single-cell omics technologies has resulted in an unprecedented wealth of data on gene expression, chromatin state, and other types of biomarkers in individual cell types. Unlike earlier single-cell-based techniques (immunohistochemistry, cell sorting, lineage tracing, etc.), single-cell omics technologies are high-dimensional and largely unbiased, capturing information across hundreds to thousands of molecular entities. High-dimensional data are powerful for machine learning modeling, notably to build "aging clocks" and for generating novel hypotheses about brain aging. Accordingly, single-cell omics technologies have been instrumental in establishing new systematic understandings of age-related changes at the cell-type level across multiple tissues and organ systems, including the brain.[20–23] Given the rapid development of single-cell

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technologies to study the diverse cell types and regions of the brain, there is a need to synthesize and understand the multitude of aging- and rejuvenation-induced changes in the brain.

Of particular interest are the interactions that occur between different cell types of the brain and how these interactions are impacted by aging. The high-dimensional nature of single-cell omics provides an opportunity to identify putative cell-cell interactions, which can be experimentally validated. The recent development of spatial technologies to profile tissues *in situ* at single-cell resolution provides an additional level of spatial insight that can be leveraged to identify cell-cell interactions in the context of brain aging. Spatially resolved datasets are also critical to compare aging of similar cell types but across different regions of the brain, which is particularly interesting given the highly specialized function of distinct brain regions.

An important goal for the study of brain aging is to identify avenues for rejuvenating the brain, which can slow or reverse different aspects of brain aging and cognitive decline. Several promising interventions, including physical exercise, dietary restriction, and the introduction of young circulating blood factors, have been shown to at least partially rejuvenate certain functions of the aged brain.[24–26] With single-cell omics, the response of different cell types to diverse rejuvenation interventions and their relative contributions to functional brain rejuvenation can be investigated in a systematic manner. Importantly, such analysis should lead to a better understanding of the shared and unique pathways by which different rejuvenation interventions achieve their effects—paving the way toward identification of synergistic combinations of multiple rejuvenation interventions.

In this review, we will focus on vertebrate brain aging and rejuvenation, mostly discussing work in mice and humans. While invertebrate nervous system aging has provided key insights into brain aging and its effect on organismal lifespan,[27–30] the vertebrate brain contains cell types not present in invertebrates, such as endothelial cells and specialized immune cells. We will also focus on "physiological" aging rather than specific age-related pathologies, though we will highlight interesting connections between brain aging and susceptibility to injury and neurodegenerative disease.

Here, we present an overview of recent insights into brain aging and rejuvenation that have been provided by single-cell omics technologies, and we highlight promising future directions that could lead to new discoveries and interventions. We review the key aging-related changes occurring in multiple different cell types of the adult brain and describe how cell-cell interactions change during the course of aging. We discuss the emergence of single-cell omics in systematically profiling rejuvenation interventions in the brain and in comparing their effects across cell types and regions. We also consider the potential role of single-cell omics in profiling cell-type-specific and pathway-specific rejuvenation responses to develop combinatorial rejuvenation interventions. We outline shared cell-type-specific signatures between aging and disease. Finally, we discuss promising *in vitro* models for studying human brain aging and highlight insights from non-mammalian vertebrate species.

SINGLE-CELL OMICS TECHNOLOGIES USED TO STUDY BRAIN AGING

Single-cell omics technologies have emerged as a powerful tool to study brain aging because they enable a high-throughput and high-dimensional understanding of age-dependent molecular and cellular changes.[20,31] In this section, we describe the single-cell-based technologies mainly used to understand brain aging and rejuvenation as well as their advantages and limitations. Most studies for the aged brain have leveraged single-cell transcriptomics, including single-cell RNA sequencing (scRNA-seq) and single-nuclei RNA-seq (snRNA-seq) (Figure 1). While scRNA-seq detects both cytosolic and nuclear transcripts and recovers more transcripts, snRNA-seq exhibits less bias in cell-type coverage, particularly for cells that are difficult to isolate, such as neurons, and it can be applied to frozen tissues[32] (Figure 1). Several single-cell transcriptomics methods have been developed, including droplet-based methods and combinatorial indexing-based technologies.[33] Spatial transcriptomics technologies have also recently emerged, with some of them exhibiting single-cell resolution (e.g., Multiplexed Error-Robust Fluorescence In Situ Hybridization, or MERFISH). A key advantage of spatial single-cell transcriptomics approaches compared with dissociated ones is that they avoid bias from cell dissociation and preserve the spatial context of individual cells and their neighbors. A limitation of spatial transcriptomics is that the methods largely used for brain aging (e.g., MERFISH) are based on predesigned gene panels, which introduces a bias in the genes examined (Figure 1). Finally, single-cell technologies have also started to be used for profiling chromatin accessibility, chromatin modifications (e.g., DNA methylation), genome architecture, whole-genome sequence, and even protein abundance for the aging brain (Figure 1). Compared with more traditional approaches, single-cell omics studies of brain aging have allowed unbiased discoveries and comparison between cell types, but they also have limitations such as data sparsity and high costs. Consideration of the relative strengths and limitations of each method is important to evaluate the findings obtained from single-cell omics studies of brain aging and rejuvenation. In addition, the various techniques used could underlie some of the differences between studies, and we indicate this below wherever appropriate.

AGING OF SPECIFIC BRAIN CELL TYPES

The brain is composed of many cell types, including neurons, glia, NSCs, and neural progenitors, and cells of the brain borders, and it also experiences infiltration by immune cells, especially during aging (Figure 2). Here, we systematically evaluate the processes most impacted during aging in each of these cell types. We mostly review recent single-cell omics studies describing changes in gene expression and chromatin accessibility in different cell types, paying special attention to the spatial context when available. We also discuss emerging data about somatic mutation accumulation in specific brain cell types. A summary of key findings is provided in Table 1, and comparisons between cell types are in Table 2.

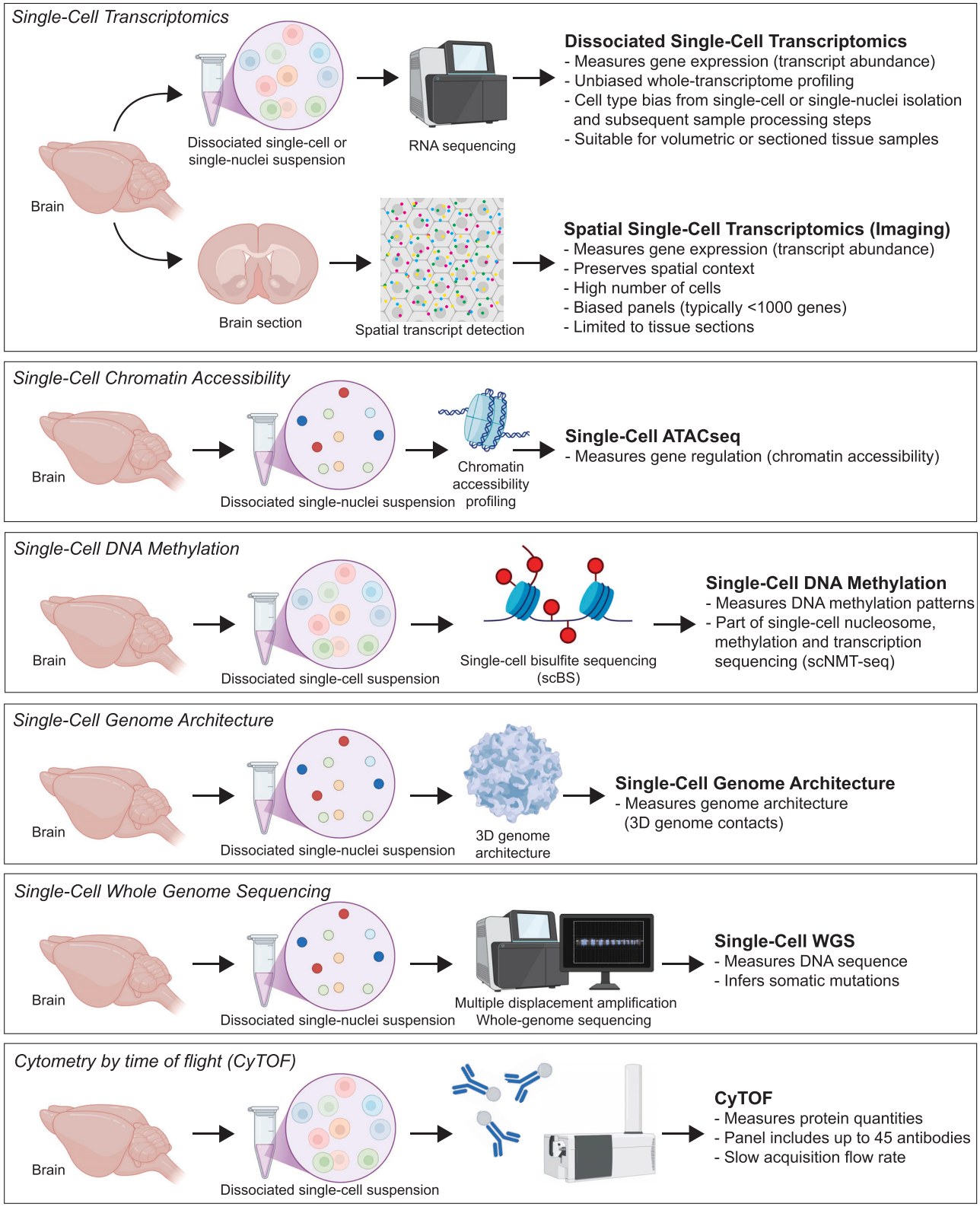

Figure 1. Overview of main single-cell omics technologies for the brain

Depiction of the experimental pipelines for single-cell and single-nuclei transcriptomics (dissociated and spatial), chromatin accessibility, methylation, genome architecture, genome sequencing, and cytometry by time of flight (CyTOF). Key attributes, advantages, and disadvantages are listed next to each technology.

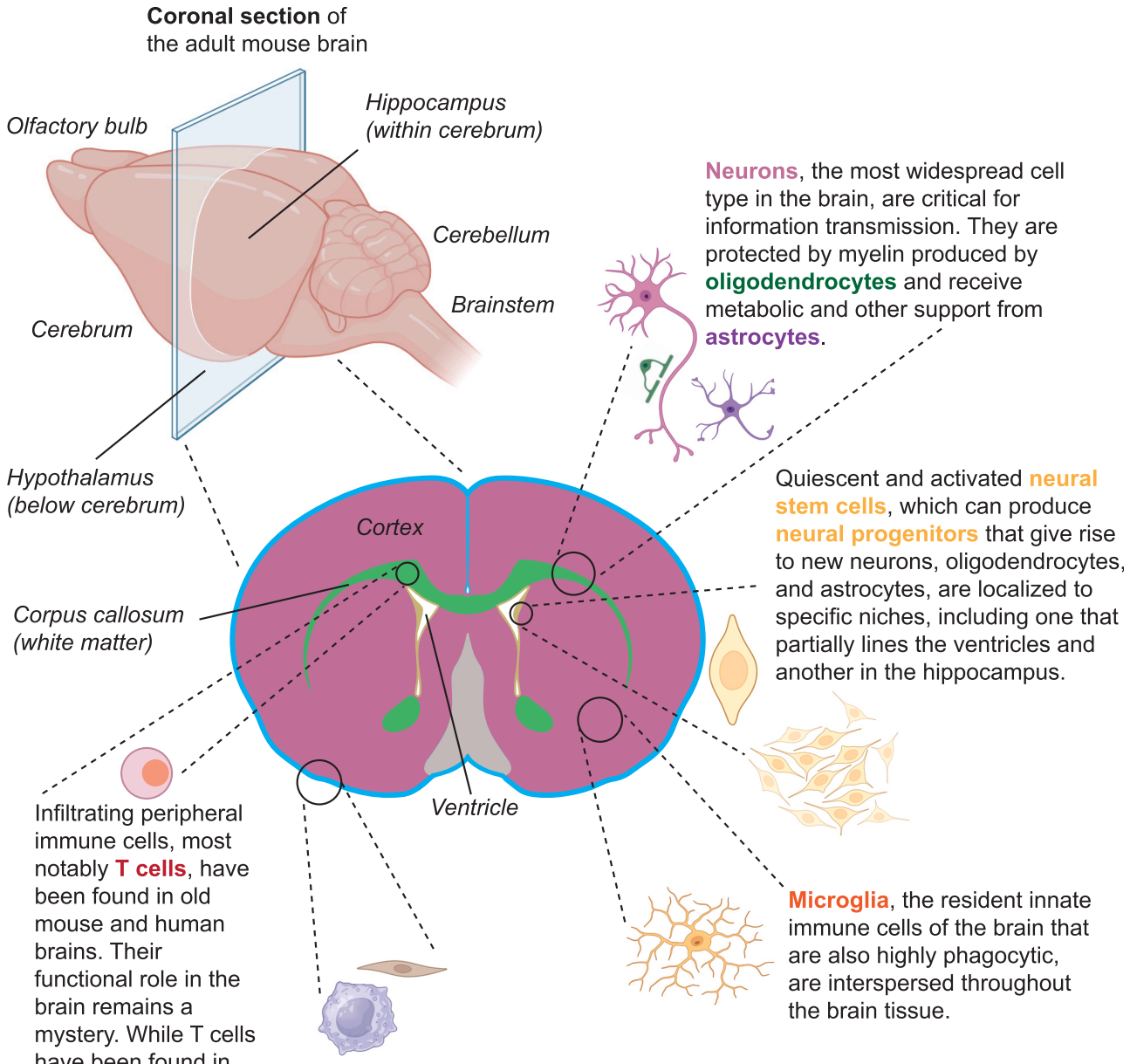

Figure 2. Cell types of the vertebrate brain
Depiction of a coronal section from a mouse brain with cutouts showing common cell types of the brain that are highlighted in this review.

Neuronal cell types

Neurons are critical for receiving and transmitting information in the brain. Apart from a few exceptions, they are generated during development and remain present throughout the life of an individual. They also exhibit striking diversity both within and across different regions of the brain and are often organized in subregions.[13,14] For example, distinct neuronal subtypes show laminar organization in the cortex.[55] Additionally, highly heterogeneous neuronal subtypes are present in the hypothalamus[56,57] and the cerebellum.[13] Here, we survey the transcriptional,

Table 1. Insights from select key single-cell omics studies of aging in the vertebrate brain

Insight	Source data	Species	References
Neurons show changes in pathways relating to protein synthesis, metabolism (including mitochondrial function and ion homeostasis), and neurotransmission with age	single-cell RNA-seq; single-nuclei RNA-seq; spatial transcriptomics	mouse	Ximerakis et al.[34]; Hajdarovic et al.[35]; Allen et al.[18]
Neurons show heterogeneity in age-related transcriptional changes across different subtypes and brain regions	single-cell RNA-seq; single-nuclei RNA-seq; spatial transcriptomics	mouse	Ximerakis et al.[34]; Hajdarovic et al.[35]; Hahn et al.[36]
Multiple cell types of the female hypothalamus show increased expression of *Xist* with age	single-nuclei RNA-seq	mouse	Hajdarovic et al.[35]
Transcriptional profiles of multiple cell types can be used to predict age	single-cell RNA-seq; single-nuclei RNA-seq; spatial transcriptomics	human, mouse	Emani et al.[37]; Buckley et al.[38]; Sun et al.[19]
Excitatory neurons show loss of heterochromatin with age	single-cell ATAC-seq	mouse	Zhang et al.[39]
Neurons accumulate somatic insertions/deletions faster than oligodendrocytes, but oligodendrocytes accumulate somatic single-nucleotide variants faster	single-cell whole-genome sequencing	human	Ganz et al.[40]
Oligodendrocyte precursor cells decline in abundance with age, while oligodendrocytes increase in abundance	single-cell RNA-seq; single-nuclei RNA-seq; spatial transcriptomics	human, mouse	Emani et al.[37]; Ximerakis et al.[34]; Allen et al.[18]; Sun et al.[19]
Microglia, as well as other glial cell types such as astrocytes and oligodendrocytes, show increased expression of inflammation-related genes with age	single-cell RNA-seq; spatial transcriptomics	human, mouse	Sankowski et al.[41]; Hammond et al.[42]; Allen et al.[18]
White matter tracts such as the corpus callosum are hotspots for aging-enriched glia	spatial transcriptomics	mouse	Hahn et al.[36]; Allen et al.[18]
Aged oligodendrocytes, astrocytes, and microglia upregulate genes associated with these cell types' respective "reactive" states	single-cell RNA-seq; spatial transcriptomics; single-cell ATAC-seq	mouse	Allen et al.[18]; Sziraki et al.[43]
Neuronal progenitors decrease in abundance and neurogenic potential with age	single-cell RNA-seq; spatial transcriptomics	mouse, killifish	Kalamakis et al.[44]; Ximerakis et al.[34]; Xie et al.[45]; Lu et al.[46]; Wu et al.[47]; Ayana et al.[48]; Sun et al.[19]
Endothelial cells show transcriptomic evidence of metabolic shifts, oxidative stress responses, and changes in immune-related genes with age	single-cell RNA-seq; spatial transcriptomics	mouse	Ximerakis et al.[34]; Chen et al.[49]; Allen et al.[18]
Border-associated macrophages show increased inflammatory signaling with age, as do other cells found at the brain's borders such as pericytes and ependymocytes	single-cell RNA-seq; single-nuclei RNA-seq; spatial transcriptomics; single-cell mass cytometry	mouse	Mrdjen et al.[50]; Ximerakis et al.[34]; Dani et al.[51]; Allen et al.[18]
Various immune cell types including T cells and natural killer cells infiltrate the brain with age	single-cell RNA-seq; spatial transcriptomics	human, mouse	Groh et al.[52]; Dulken et al.[53]; Jin et al.[54]; Allen et al.[18]; Sun et al.[19]

Summary of select key single-cell omics studies of brain aging and their associated findings.

chromatin, and genomic changes that occur in neurons during aging as identified unbiasedly via single-cell omics studies.

Transcriptomic changes in neurons and heterogeneity across subtypes and regions

Single-cell transcriptomics has become a widely used tool to characterize the heterogeneity of neuronal cell types and their changes during aging. scRNA-seq of whole brains from young and old male mice revealed that all mature neurons exhibit shared aging-related pathways such as dysregulated expression of some metabolic, ion homeostasis, and neurotransmission pathways.[34] However, neuronal subtypes also display heterogeneity in age-associated regulation of some genes.[34] For example, dopaminergic neurons show downregulated expression of ribosomal protein-encoding genes with age, while GABAergic and glutamatergic neurons show upregulated expression of the same genes with age.[34] Similarly, a spatially resolved single-cell transcriptomic atlas of brain coronal sections containing frontal cortex and striatum from juvenile, young, and old female mice revealed that genes involved in neurodegenerative diseases, oxidative response, and mitochondrial function are upregulated with age in all neurons but that this is particularly pronounced for inhibitory neurons.[18] In the hypothalamus—a region with many neuronal subtypes involved in many physiological functions in the body (e.g., sleep, hunger/satiety, stress, fertility)[56,57]—scRNA-seq has been performed in young and old female mice.[35] Clustering of hypothalamic neuron

Table 2. Summary of main insights for brain aging in different cell types

Responses / Cell types	Increased inflammation	Change in abundance	Increased reactivity	Metabolic shifts	Aging clocks	Somatic mutation accumulation
Neurons	?	?	N/A	✓	✓	✓
Oligodendrocytes	✓	↑	✓	?	✓	✓
Oligodendrocyte precursor cells	?	↓	N/A	?	✓	?
Microglia	✓	?	✓	?	✓	?
Astrocytes	✓	?	✓	?	✓	?
Neural stem cells/neural progenitors	✓	↓	N/A	?	✓	?
Endothelial cells	✓	?	N/A	✓	✓	?
Border-associated macrophages	✓	?	N/A	?	?	?
Ependymocytes	✓	?	N/A	?	✓	?
Pericytes	✓	?	N/A	?	✓	?
T cells	?	↑	N/A	?	✓	?
Natural killer cells	?	↑	N/A	?	?	?

Green checkmarks and black arrows: conclusions highlighted by one or more studies cited in this review. Question marks: conclusive results have not been reported, or results from different studies conflict. "N/A," "not applicable."

subtypes shows both subtype-specific and shared gene pathways that are impacted by aging.[35] Subtype-specific changes in gene expression generally involve neuropeptide processing genes, while shared pathways generally implicate neurodegenerative disease pathways, ribosomes, and oxidative phosphorylation.[35] Interestingly, during aging of female mice, *Xist*, the key regulator of X chromosome inactivation, increases in expression in hypothalamic neurons (and in several other cell types) and can be used to predict neuronal age.[35] Consideration of sex-specific differences in aging will be crucial for the study of neuronal aging. Comparisons across single-cell transcriptomics studies indicate that neuronal subtypes display shared aging signatures, particularly for genes associated with neurodegeneration[18,35,58] or mitochondrial function and metabolism,[18,34] as well as distinct subtype-specific aging-related changes.[34,35]

Individual neuronal cell types display transcriptomic shifts during aging that can be region-specific.[36] snRNA-seq profiling of the anterior hippocampus and caudate putamen regions in young and old mice found caudate putamen-specific aging gene signatures for medium spiny neurons, which are not observed in any other brain regions, and hippocampal dentate gyrus (DG)-specific aging signatures for granule cells, which are not observed in the granule neurons of the cerebellum.[36] These transcriptomic differences may reflect functional specialization of neuronal subtypes in different regions of the brain.

Are neurons exhibiting more or fewer transcriptional changes than other cell types in the brain? Due to the high abundance of neuronal cell types, there is greater power for detecting low-magnitude but statistically significant changes in gene expression in neurons. Accordingly, single-cell transcriptomics on entorhinal cortex samples from young and old cynomolgus monkeys (*Macaca fascicularis*) show the largest number of differentially expressed genes in neurons compared with other cell types, which is likely due to the very high abundance of neurons relative to other cells in this dataset.[58] Likewise, predictive modeling using a large-scale snRNA-seq atlas of aging human brains revealed that the transcriptomes of intratelencephalic cortical neuron subtypes (along with oligodendrocytes and

oligodendrocyte progenitor cells [OPCs]) are highly predictive of age.[37] However, studies using snRNA-seq[18,36] and spatially resolved single-cell transcriptomics[18,19] have shown a comparatively lower magnitude of transcriptomic changes in neurons than in non-neuronal cell types (e.g., glia) during aging, after controlling for differences in the abundance of cell types. The lower magnitude of transcriptomic changes during aging of neurons could be the consequence of their longer lifespan relative to some other cell types.

Overall, neuronal cell types experience both shared and divergent transcriptomic changes across different brain regions and subtypes, but these changes are of generally lower magnitude than in other cell types of the brain.

Epigenomic changes in neurons: chromatin accessibility and genome architecture

Several studies have characterized age-related changes in epigenomics, including chromatin accessibility, at single-cell resolution. Single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq)[59,60] has been used to profile nuclei from brains of young, middle-aged, and old mice.[39] This profiling revealed a general age-dependent loss of heterochromatin in excitatory neurons but not in other cell types.[39] However, there is increased chromatin accessibility in specific heterochromatin domains of excitatory neurons in old mouse brains.[39] Accordingly, the expression of genes within these domains is increased with age, and these genes are enriched for repetitive elements or pseudogenes and for the humoral immune response and cell-adhesion pathways.[39] A separate study used paired snRNA-seq and scATAC-seq profiling of the dorsal hippocampus in young and old male mice under normal conditions and with environmental enrichment.[61] The paired analysis revealed that aging has a stronger effect on chromatin accessibility and gene expression than environmental enrichment, especially in the excitatory neuron populations of the dorsal hippocampus.[61]

Interestingly, integrated profiling of single-cell transcriptome, chromatin accessibility, and three-dimensional genome architecture in the cerebellum of humans and mice across life showed pronounced age-related changes of three-dimensional genome structure in cerebellar granule cells across both species.[62] These three-dimensional changes are characterized by ultra-long-range intrachromosomal contacts that are rarely found in neurons from young adults.[62] Yet, the transcriptional and chromatin accessibility changes in cerebellar granule cells during aging are quite low.[62] Thus, neurons may exhibit greater age-related changes in other aspects of gene regulation, such as genome organization. It will be important to understand the role of this remodeling outside of gene expression.

Overall, single-cell epigenomic analyses of neurons have revealed age-related changes that are subtype-specific and different from hallmark transcriptional changes discussed above. Further studies will be necessary to identify epigenomic changes in additional neuronal cell types and in different brain regions and to determine the role of these epigenomic changes in neuronal aging—either on gene expression or otherwise.

Accumulation of somatic mutations in neurons

Emerging evidence highlights the accumulation of somatic mutations in neurons with age, which may underlie some of the transcriptional and chromatin changes observed. Single-cell whole-genome sequencing identified somatic single-nucleotide variants (sSNVs) in neurons from the prefrontal cortex and hippocampus from healthy human individuals at different ages.[63] The rate of increase in sSNVs across age is linear in both of these regions, with a higher rate of increase in the hippocampus.[63] Similarly, another single-cell whole-genome sequencing study of human brain aging revealed that both sSNVs and indels (insertions and deletions) accumulate with age across cell types.[40] Interestingly, neurons accumulate indels faster—but sSNVs slower—than oligodendrocytes in the same individual.[40] Chromatin accessibility and snRNA-seq profiling further revealed that neuronal mutations are enriched in open chromatin regions that are transcriptionally active, while oligodendrocyte mutations are concentrated in inactive genomic regions.[40] Single-cell genomics of the mouse hippocampus indicated age-associated accumulation of neuronal mutations in sites not bound by the NPAS4-NuA4 complex, which is involved in synaptic activity-dependent DNA repair.[64] Given the long-lived nature of neurons, the role of somatic mutations in neuronal aging and their accumulation across life may be critical for dysregulated neuronal gene expression and function during aging.

Glial cell types

Glia encompass various non-neuronal cell types with different origins (neural and immune) that are present in both mice and humans: astrocytes, oligodendrocytes, and microglia.[65] In contrast to neurons, astrocytes and oligodendrocytes are born later in development, and some of these cells can be produced in adulthood.[66] Astrocytes provide metabolic support to neurons and make contacts with synapses.[67] Mature oligodendrocytes are generated from OPCs, and they ensheath neurons in myelin for protection and rapid signal transmission.[68] Unlike astrocytes and oligodendrocytes, which have a neural origin, microglia represent brain-resident innate immune cells, with an important role in phagocytosis and clearing of damaged cells, aggregated proteins, or debris[69] as well as remodeling of extracellular matrix[70] and synapses in specific brain regions.[71] Here, we describe transcriptional changes in glia in mice and humans, highlighting common and divergent changes with age in different glia types.

Comparison of several scRNA-seq and spatial transcriptomic studies of the aging mouse and human brain shows that one common hallmark especially prominent in glia is a striking increase of immune and inflammatory gene expression with age.[18,36,41,42,47,72] The number of microglia increases with age in the mouse midbrain, corpus callosum, and external capsule, as shown by a spatial transcriptomic study with single-cell resolution.[73] Spatial transcriptomic studies have also revealed an increase in oligodendrocyte abundance with age in the mouse brain.[18,19] Mouse oligodendrocytes show decreased expression of *Mog*, which encodes a key myelin component, with age[34]—a change that may be linked to age-related defects in myelination.[74]

Aging-associated changes in microglia (and other glia) appear to be driven by changes in specific subpopulations.[18,42,50] A subpopulation of oligodendrocytes expressing immune genes is enriched in the aged mouse brain, and spatial transcriptomics has identified white matter (myelin-rich regions) such as the

corpus callosum as a hotspot for aging-enriched glia.[18,36] Subpopulations of senescent or proliferative microglia have also been detected in the aged mouse brain.[46,75] In a scRNA-seq study of adult human microglia, an age-associated gene co-expression module related to lipid metabolism is enriched in an interferon-responsive microglia subpopulation.[76] This is consistent with the inflammatory lipid droplet-accumulating microglia state described in the aged mouse brain.[77] In mice, microglia and astrocytes both upregulate genes associated with these cell types' "activated" (or "reactive") states with age, though astrocytes are more spatially heterogeneous in their aging-related profiles than microglia.[18] It will be important to determine whether these phenotypes are conserved in humans, though snRNA-seq, which is commonly used to profile human brain samples, may lack the sensitivity to detect microglial activation.[78]

Outside of white matter tracts, glia may exhibit more region-independent aging signatures than neurons. Indeed, a comparison of snRNA-seq data from the aging mouse hypothalamus and hippocampus showed stronger correlation between these two regions in age-related gene expression changes in glia than in neurons.[35] Interestingly, one key commonality between neurons and many glial cells (oligodendrocytes, astrocytes, and microglia) in the female hypothalamus is an increase in *Xist* expression with age,[35] which is also observed in female hippocampal neurons.[79] It will be important to investigate whether neurons and glia also show different regional patterns of age-related transcriptional changes in the human brain.

scATAC-seq (and scRNA-seq) of the mouse brain has shown increased reactivity in oligodendrocytes and microglia with age,[43] similar to the aforementioned findings from spatial transcriptomics. Somatic mutations have also been examined in human glia. Interestingly, some age-associated mutational signatures in oligodendrocytes behave in a "clock-like" manner (i.e., accumulating at a constant rate over time[80]), with at least one of these signatures possibly corresponding to mutations occurring during OPC proliferation.[40] Further exploration will be needed to identify changes at the chromatin and genomic levels that may underlie the increased expression of immune genes in subpopulations of glial cell types and identify conserved features between mice and humans.

Overall, inflammation is a conserved hallmark of brain aging, and single-cell omics has been instrumental in identifying glia, especially microglia, as important and conserved players in this process. Increased expression of immune genes in aged glia does indeed correspond to increases in the encoded proteins (e.g., Dulken et al.[53]). However, it will be important to determine if changes in transcripts in glia with age correspond to changes in proteins in mouse and human brains by performing validation or proteomics studies (e.g., Soto et al.[81] and Kjell et al.[82]). In addition, the mechanisms by which immune-related signals in glia impact brain health are not fully understood. Given that glia have varied functional interactions with neurons, each other, and other brain cell types, comprehensive functional studies will be indispensable to reveal pathways that could be therapeutic targets for reversing detrimental effects of inflammation and other aging processes in the old brain.

Neural stem and progenitor cells

NSCs and neural progenitor cells (NPCs) have been a central focus of brain aging studies due to their potential to generate new neurons (neurogenesis), astrocytes, and oligodendrocytes in the adult brain.[83–86] The regenerative niches of the adult mammalian brain are the subventricular zone (SVZ) of the lateral ventricles and the DG of the hippocampus. NSCs in the SVZ generate new neurons in the olfactory bulb,[87] which is important for olfactory discrimination and memory,[88–90] whereas NSCs in the DG generate new granule neurons in the hippocampus,[91–93] which contributes to learning and memory formation in mice.[83,84,94–96] With age, the rate of neurogenesis in both the SVZ[85,86,88,89,97] and DG[92,96,98,99] niches declines in mice. While the extent of neurogenesis is controversial in adult humans,[100] some studies have shown a decline in neurogenesis during human aging.[101,102] Here, we discuss recent single-cell studies of NSC aging in mice and humans and how they can uncover mechanisms that drive age-dependent neurogenesis defects.

Single-cell transcriptomic studies have characterized the quiescent and activated (proliferative) pools of NSCs during aging in mouse neurogenic niches—SVZ[44,45,53] and DG.[103,104] These studies have shown that the total number of NSCs (including quiescent and activated NSCs) in the SVZ[44,53] and DG[104] declines with age. In addition, the fraction of quiescent NSCs increases in both niches with age,[44,104] and old quiescent NSCs are less able to activate.[44,45] Interestingly, in the DG, quiescent NSCs downregulate the expression of genes associated with cell cycle and neurogenesis in middle-age,[104] which could underlie defects in proliferation and differentiation along the neurogenic lineage. scRNA-seq has also revealed that NSCs upregulate interferon signaling and inflammation pathways with age,[44,53,103] and this could contribute to the increase in quiescence with age in the SVZ.[44] Numerous other NSC-specific molecular changes have been identified in aging, including changes in proteostasis,[105–108] and epigenetic landscape,[109–111] among others. Complementary imaging-based approaches have been essential to understand *in vivo* NSC dynamics with age, including reduced NSC migration in the SVZ[112] and increased cell death in hippocampal NSC clones.[113] Targeting molecular pathways identified by scRNA-seq or other methods has the potential to rejuvenate quiescent NSCs and boost neurogenesis in the brains of old mice.

Single-cell transcriptomic studies have highlighted inflammation as a central signature of old age in the SVZ and DG neurogenic niches.[43,44,53,103] These single-cell omics studies have also revealed that T cells infiltrate the SVZ neurogenic niche with age,[53] and this correlates with upregulation of the interferon response pathway in many cells in the SVZ neurogenic niche.[19,53] Infiltrated T cells and inflammation may be responsible for the age-dependent decline in NSC activation.[53] Consistently, inhibition of inflammatory cytokine CXCL10 with a neutralizing antibody increases the proportion of neuroblasts in the old niche.[44] Thus, targeting T cells or inflammatory cytokines could represent a therapeutic strategy to increase neurogenesis with age.

Interestingly, a multi-omics approach combining scRNA-seq, chromatin accessibility, and DNA methylation showed that quiescent SVZ NSCs and astrocytes (from the cortex and

striatum) are transcriptionally similar but differ in their DNA methylome.[114] Thus, DNA methylation could be a stemness signature,[114] and it will be interesting to determine how this signature changes with age.

Single-cell omics studies, including spatial transcriptomics, have also characterized the proliferation and differentiation of NSCs in the SVZ and DG, highlighting their decline with age.[19,46,47] A single-cell method using combinatorial indexing profiled the transcriptome and chromatin landscape of proliferating progenitor cells *in vivo* from the brains of young and old mice.[46] This study showed a decline in NPC number and a decrease in self-renewal potential of those progenitors, which may underlie the neurogenesis decline with age.[46] In mice, neuroblasts—more committed progenitors originating from NSCs—decline in number earlier in the DG than in the SVZ and the olfactory bulb,[43] pointing to important regional differences.

NSCs within the SVZ niche are heterogeneous[86,115,116] and their regional location within the niche determines differentiation potential.[117,118] Regional dissection of the SVZ coupled to scRNA-seq has also shown differences between septal and lateral location of the niche with respect to the lateral ventricle.[118] These regional differences could be due to distinct intrinsic cellular fates or different responses to external stimuli. Heterogeneity within the SVZ may allow for the production of distinct populations of neurons in specific physiological contexts such as pregnancy.[119] Spatial transcriptomic technologies would help further probe the regional differences of NSCs in response to environmental stimuli.

Interestingly, single-cell transcriptomics has also helped to characterize the NSC pool in the aging human brain. Adult human hippocampal neurogenesis has been controversial,[100–102] and single-cell omics strategies could give an unbiased characterization of NSCs and their progeny during human aging. Two studies identified immature neurons in the adult and aged brain (40–82 years old in Zhou et al., and 67–92 years old in Wang et al.),[120,121] but one study did not (~53 years old).[122] A recent study of 62 individuals ranging from 0 to 92 years old used snRNA-seq coupled to machine learning to identify immature neurons in the adult human hippocampus.[120] This approach avoids relying on mouse-specific NSC markers, which have little overlap with human-specific markers.[120,121,123] Interestingly, one of the studies that identified immature neurons in the human hippocampus showed that they decrease with age.[120] Adult human neurogenesis in the SVZ has been less controversial, and scRNA-seq of the human SVZ of adults aged 38–72 identified distinct subpopulations of NSCs that have enrichment for neuronal or oligodendrocyte progenitor pathways.[124,125] Overall, more work will be needed to further characterize neurogenesis in humans during aging.

In addition to NSCs and NPCs, other cells with regenerative potential include OPCs, which give rise to mature oligodendrocytes in different brain regions. Similar to what has been found for NSCs, scRNA-seq, snRNA-seq, and spatial transcriptomics have all shown a decrease in abundance of OPCs in both mice and humans during aging.[18,34,37]

Overall, technological advances have been crucial to developing a better understanding of aging stem and progenitor cells of the brain by identifying age-related changes in numbers and unbiasedly characterizing molecular signatures. Combining the observation of age-related changes in regenerative cells with functional readouts will be essential to understand the causal effects of these molecular changes on NSC function during aging.

Cell types of the brain's borders

The brain's interactions with the periphery are critical in aging-related signaling, as highlighted by the rejuvenating effects on the brain of young blood[126] and cerebrospinal fluid (CSF).[127] In both mice and humans, border tissues of the brain include blood vessels, lined by endothelial cells as well as vascular smooth muscle cells and pericytes (mural cells), which all form the blood-brain barrier.[26,128] Border tissues also include the choroid plexus, which produces CSF and forms the blood-CSF barrier.[129] Finally, border tissues of the brain also encompass the meninges (including the leptomeninges), a multilayered structure composed of immune cells, fibroblasts, and other cell types that surrounds the brain.[130]

Cells of the brain's borders are very diverse but generally experience increased immune and inflammatory signaling with age, similar to many other cell types in the brain. scRNA-seq of mouse brains also revealed increased senescence and hypoxia signatures as well as evidence of metabolic shifts in endothelial cells with age.[34,131] scRNA-seq and spatial transcriptomics on the mouse brain have highlighted increased interferon signaling with age in ependymocytes, which form the actual blood-CSF barrier, as well as in pericytes.[18,34]

A study using flow cytometry to enrich for brain endothelial cells prior to scRNA-seq profiling showed that in the mouse hippocampus, the subclasses of endothelial cells—arteries, veins, and capillaries—exhibit distinct patterns of transcriptional changes with age, with the most pronounced changes occurring in capillaries and relating to immune genes as well as transforming growth factor β (TGF-β) signaling and oxidative stress responses.[49] The increase in inflammation could be a consequence of the infiltration of immune cells such as T cells in the old brain (see the following section).

An immune cell type found in proximity to all of the brain's borders is the border-associated macrophage (BAM).[132] BAMs are in contact with the CSF and regulate its dynamics.[132] Single-cell mass and fluorescence cytometry of the mouse brain immune compartment showed that BAMs display altered surface marker expression with age.[50] One notable change observed with age was an expansion of BAM subpopulations expressing proteins of the antigen-presentation machinery, indicative of an inflammatory phenotype.[50] Consistently, in the mouse choroid plexus, predicted inflammatory signaling mediated by choroid plexus macrophages (a subset of BAMs) increases with age.[51]

Spatial transcriptomics of mouse brains revealed that a subpopulation of endothelial cells, characterized by high expression of xanthine dehydrogenase (*Xdh*; an enzyme involved in purine oxidation[133]), is enriched in the brains of old mice, though *Xdh*-high endothelial cells and other subpopulations do not display region-specific localization.[18] This could indicate that age-related metabolic changes in endothelial cells are driven more by exposure to circulating blood factors than by the surrounding brain environment.

Insights from single-cell chromatin-level profiling of brain border tissues have been sparse, though a bulk ATAC-seq study that included fluorescence-activated cell sorting (FACS)-sorted endothelial cells of the mouse SVZ neurogenic niche identified several cell-adhesion pathways enriched among the genes with more open chromatin in old endothelial cells of this brain region.[111] No single-cell whole-genome sequencing studies to date have examined somatic mutations in the brain's border tissues, and this would be a fruitful area for future study in an aging context.

Also critical for additional investigation will be determining which changes in the brain borders' cells are linked to different permeability to peripheral signals with age—including blood or CSF proteins (e.g., Yang et al.[134]) as well as infiltrating peripheral immune cells, as discussed in the following section.

Infiltrating and peripheral immune cell types

Recent evidence, including single-cell omics atlases, has revealed that during aging, the brain is infiltrated with non-resident immune cells and affected by peripheral immune cells, including T cells. Despite their relative sparsity in comparison with other cell types, these immune cells exhibit stark changes during aging and can have dramatic effects on resident cell types of the brain (see "interactions between cell types during aging").

T cells infiltrate the brain with age

T cells are key players in the adaptive immune response and have been implicated in aging across multiple tissues.[135] scRNA-seq has revealed the infiltration of the brain by T cells during aging, with increased numbers of infiltrating cytotoxic (CD8+) T cells in the old mouse subventricular zone,[53] in multiple regions of the mouse brain,[18,19] and in old human and mouse white matter regions.[52] Infiltrating CD8+ T cells in the SVZ exhibited transcriptional signatures consistent with activation, tissue retention, and effector memory state[53] and are clonally expanded, suggesting they have recognized an antigen.[53] Infiltrating CD8+ T cells in old white matter regions of both mouse and human brains contribute to axon degeneration and cognitive deficits.[52] scRNA-seq uncovered multiple T cell subtypes, including central memory, effector, and interferon-stimulated T cell subsets.[52,136] Single-cell whole-transcriptome and T cell receptor (TCR)-targeted sequencing of CSF from patients of different ages revealed that TCR sequences from cognitively impaired donors are most similar to TCR sequences from the oldest ages in cognitively normal individuals,[137] suggesting that aging and cognitive impairment may be associated with similar shifts in infiltrating T cells. Hence, single-cell transcriptomics has been instrumental in revealing the infiltration of cytotoxic and clonally expanded T cells in the brain with age.

Although less common than CD8+ T cells, spatially resolved single-cell transcriptomics show that CD4+ T cells also infiltrate the brain with age.[19] Given that CD4+ T cells generally play a more supportive and modulating role in immune response compared with CD8+ T cells, the infiltrating CD4+ T cells could have beneficial roles in the aging brain.

Another interesting subset of T cells is regulatory T (T_{reg}) cells, which may also have a beneficial role in the aging brain. Consistently, scRNA-seq studies in young and old mice with brain-specific injection of IL-2, a cytokine that increases the number of T_{reg} cells,[138–140] have suggested that T_{reg} cells could suppress neuroinflammation, partially restore age-related transcriptional signatures of brain glia, and reduce decline in spatial learning during aging.

Other infiltrating immune cells in the brain

Single-cell omics studies have revealed aging-associated changes in other infiltrating immune cells in the brain.[34,141]

Infiltrating monocytes, white blood cells that perform phagocytosis and antigen presentation, have been discovered in the brain using single-cell omics tools.[34,137,142] scRNA-seq of CSF from 45 cognitively normal human donors across different ages and 14 human donors with cognitive impairment revealed an increase in the expression of lipid transport genes in monocytes during aging and decreased expression of these genes in cognitively impaired donors.[137] In mice, scRNA-seq of young and aged brains revealed that monocytes (as well as resident microglia) had elevated senescence-related transcriptional signatures during aging.[142] Further single-cell characterizations will be necessary to determine compartment-specific heterogeneity (e.g., CSF compared with brain) and species-specific heterogeneity (e.g., mouse compared with human) of monocyte aging in the brain.

Natural killer (NK) cells, which are white blood cells with cytotoxic properties, have also been identified to infiltrate the brain across single-cell transcriptomic studies.[54,143,144] Importantly, NK cells are particularly enriched in the DG of aged mice and potentially supported by IL-27 secretion by neuroblasts in this brain region.[54] These NK cells exhibited increased activation, cytotoxicity, and adhesion based on transcriptional signatures.[54] Fate mapping further revealed that local expansion of NK cells contributes to their age-associated increase in the DG, and immunostaining confirmed accumulation of NK cells in the aged human DG.[54]

In addition to the aforementioned immune cells (neutrophils, monocytes, NK cells), other immune cells such as macrophages,[50,53] dendritic cells,[50] and B cells[137] have also been observed in single-cell omics studies. However, most infiltrating immune cells have not been studied at depth in the aging brain, likely due to the low numbers of these cells relative to other brain cell types. Future efforts to build a comprehensive atlas of the infiltrating immune cell repertoire in the aging brain will likely yield new insights into how neuroinflammation and other aging-related changes are regulated by infiltrating immune cells.

INTERACTIONS BETWEEN CELL TYPES DURING AGING

The brain is a highly complex organ, consisting of many different interacting cell types. It is therefore essential to understand how cell-cell interactions are impacted by aging. Single-cell technologies, notably spatial approaches, enable large-scale characterization of cell-cell interactions across entire tissues. Here, we discuss insights on cell-cell interactions in brain aging, focusing on direct cell-cell contacts and paracrine signaling (e.g., secretion of cytokines).

Decrease in synaptic neuron-astrocyte program with aging

Astrocytes are abundant in the brain, play a supportive role for neuronal function, and are essential in the formation of long-term memories.[145] An extensive snRNA-seq atlas of the

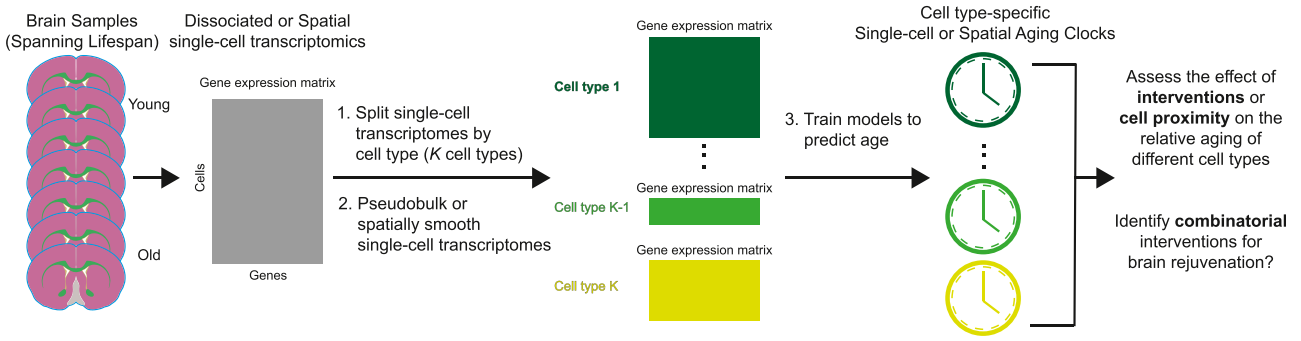

Figure 3. Cell-type-specific aging clocks

Illustration of the main steps in building cell-type-specific transcriptomic aging clocks: collect single-cell transcriptomic data using scRNA-seq or spatial transcriptomics on brain samples for multiple ages, split single-cell transcriptomic data into individual cell types, pre-process the cell-type-specific transcriptomic data using pseudobulking or spatial smoothing to reduce sparsity of gene expression, and train machine learning models to predict age from each cell-type-specific transcriptomic data to develop cell-type-specific transcriptomic aging clocks. Cell-type-specific transcriptomic aging clocks can be used to determine the effect of different interventions on the transcriptional aging of different cell types and the proximity effect of certain cell types on the transcriptional aging of nearby cells.

prefrontal cortex across 191 human donors spanning 22–92 years of age revealed a new synaptic neuron-astrocyte program (SNAP), where cortical neurons with higher expression of synaptic component genes are associated with astrocytes with higher expression of synaptic function and cholesterol synthesis genes.[146] Interestingly, SNAP decreases in the brain during aging and in patients with schizophrenia,[146] suggesting that aging and cognitive states may both be influenced by dysregulated co-expression of this neuron-astrocyte program. The SNAP cell-cell interaction may involve the synthesis of cholesterol in astrocytes to support neuronal synapses and dendritic spines, which require high amounts of cholesterol.[147] Further experiments will be needed to identify the causal factors underlying SNAP and assess the functional impact of this synaptic neuron-astrocyte program on brain aging.

Potential influence of NSCs on other cells

Spatial aging clocks are cell-type-specific machine learning models that have been developed to predict the age of individual cells from spatially resolved single-cell transcriptomics data across adult mouse lifespan (Figure 3). Spatial aging clocks have been used to identify cell types with strong influences on the relative aging of nearby cells in the brain.[19] Spatial aging clocks predicted that NSCs and neuroblasts have the most pro-rejuvenating effect on nearby cells.[19] Differential expression analysis using imputation[148] has implicated extracellular vesicles/exosomes in NSCs as potential mediators of the pro-rejuvenating effect of NSCs on neighboring cells and fatty acid oxidation as one of the pathways that could be responsive in these neighboring cells.[19] The co-expression of the exosome marker CD9 and the fatty acid oxidation marker CPT1A has been confirmed by immunostaining.[19] Further characterization of the effect of NSCs on other cell types through experimental perturbation studies will be crucial to establish a causal link between NSCs and other cell types during aging.

Impact of T cells on microglia and oligodendrocytes

Infiltrating T cells can have dramatic effects on the state of microglia and oligodendrocytes in the aging brain. scRNA-seq of young and old mouse brains revealed that old oligodendrocytes and microglia exhibit a strong response to interferon in the white matter—brain regions that contain high proportions of oligodendrocytes.[136] Interestingly, these interferon-responsive cells are localized close to cytotoxic CD8+ T cells based on immunostaining.[136] Activated microglia and inflamed oligodendrocytes are also spatially enriched near infiltrating T cells across multiple brain regions in spatially resolved single-cell transcriptomics.[19] Interferons are a family of secreted cytokines that bind to specific receptors and encompass three major isoforms (α, β, and γ) that are secreted by different cell types.[149] As cytotoxic T cells secrete interferon-γ,[150,151] T cells may trigger microglia and oligodendrocyte inflammation via secretion of interferon-γ (although interferon-α and interferon-β, secreted by other cells, may contribute to this response). Consistently, scRNA-seq showed decreased proportion of interferon-responsive oligodendrocytes and microglia in immunodeficient mice compared with control mice, suggesting that infiltrating T cells cause interferon-responsive oligodendrocytes and microglia.[136] Interferon-γ injection leads to myelination damage in white matter, suggesting an overall detrimental effect.[136] T cells may act on microglia and oligodendrocytes through interferon-γ signaling, shifting their transcriptional states in aging. Inflamed microglia and oligodendrocytes could, in turn, contribute to T cell infiltration in a positive feed-forward loop.

How detrimental is this interferon response on neighboring cells? Spatial aging clocks revealed that the presence of T cells is associated with a strong pro-aging effect on nearby cells, including microglia and oligodendrocytes, as measured by their transcriptional signatures.[19] These findings suggest a detrimental effect of T cells on neighboring cells, likely due to inflammation. The pro-aging effect of T cells on microglia and oligodendrocytes persisted even after controlling for activation status of microglia and inflammation status of oligodendrocytes.[19] Imputation analysis indicated that the pro-aging effect of T cells on nearby cells is mediated through interferon-γ signaling, which was further validated via immunostaining.[19] Whether experimental modulation of interferon-γ production or secretion in T cells is sufficient to alter the aging of nearby cells remains to be determined.

Infiltrating immune cells affect NSCs and neuroblasts

Despite their overall low abundance in the brain, infiltrating immune cells such as T cells and NK cells can have dramatic effects on NSCs and neuroblasts, which are key cells involved in neurogenesis. scRNA-seq of the mouse SVZ neurogenic niche from young and old male mice shows age-associated T cell infiltration of the SVZ and concomitant upregulation of transcriptional signatures for interferon response across other cell types in the niche, including astrocytes, quiescent and activated NSCs and NPCs, and endothelial cells, in addition to microglia as described above.[53] Upregulated interferon response markers (STAT1 and BST2) in cells near T cells have also been confirmed in the SVZ and other brain regions (cortex, striatum, and corpus callosum) by immunostaining.[19,53] Importantly, NSCs with strong interferon-γ response also exhibit reduced proliferation *in vivo*,[53] suggesting that T cells may negatively affect neurogenesis in the SVZ via secretion of interferon-γ. Consistently, inflammatory interferons have been shown to reduce NSC proliferation *in vitro*.[44]

Other immune cells could also impact NSCs. scRNA-seq has revealed that NK cells accumulate in the DG of the hippocampus in aged brains.[54] Neuroblasts in this region exhibit a senescence-associated secretory phenotype (SASP) and are eliminated by NK cells.[54] This occurs through NK cell activation and cytotoxicity induced by upregulation of major histocompatibility complex (MHC) class-I-related ligands in senescent neuroblasts.[54] Experimental depletion of NK cells using antibodies can restore neurogenesis,[54] suggesting that at least in the hippocampal neurogenic niche, NK cells may drive the age-related decline in neurogenesis.

Putative cell-cell interactions predicted from single-cell omics

Several computational methods have been developed to predict putative cell-cell interactions from scRNA-seq datasets by modeling enrichment of ligand-receptor pairs.[152,153] Due to their ease of use for assessing cell-cell interactions, these computational methods have become popular tools in the analysis of single-cell omics, including in the context of aging.

Application of the CellChat model[154] on scRNA-seq data from the young and aged SVZ niche indicates an overall reduction in intercellular interactions during aging but enhanced microglial interactions with multiple cell types. Aging was associated with reduced TWEAK (a tumor necrosis factor family ligand) signaling from multiple other SVZ cell types to endothelial cells, characterized by decreased expression of the TWEAK receptor gene.[155] Experimental validation of the role of TWEAK signaling in SVZ aging is still needed.

Another method, CellPhoneDB,[156] has been used with scRNA-seq of the young and old choroid plexus.[51] This study predicts enhanced IL-1β signaling from macrophages to endothelial cells (and mesenchymal cells) during choroid plexus aging, which has been validated by immunostaining.[51] CellPhoneDB also predicts that in the aged primate entorhinal cortex, receptor-ligand pairs are enriched for pro-inflammatory, cell-adhesion, and neuroactive pathways.[58]

Application of NicheNet[157] to scRNA-seq data of young and old spinal cords from mouse models of multiple sclerosis identifies *Spp1* as a top age-associated ligand with putative interactions with integrins (*Itgb1*, *Itgav*, and *Itgb5*) in microglia.[158] Immunostaining confirms overlap between these integrins and microglia in young and old brain lesions,[158] suggesting a potential role for this interaction in extracellular matrix remodeling.

Finally, deployment of iTALK[159] to snRNA-seq profiles from hippocampus of young and aged macaques indicates strong interactions between NSCs and astrocytes.[121] In particular, growth factor signaling (VEGF-VEGFR and fibroblast growth factor [FGF]) is enriched in the young macaque hippocampus,[121] and this type of signaling is known to mediate the positive effect of astrocytes on neurogenesis.[160,161] Consistently, spatial transcriptomic profiling of the adult mouse brain across life suggests that *Vegfa* expression is associated with the beneficial effect of NSCs on nearby cells.[19] Secretion of VEGF by hippocampal NSCs is important for maintaining the neurogenic niche and proximity of NSCs to the vasculature.[162,163] Additionally, interactions involving cytokines, integrins, and the BMP pathway are predicted to be enriched in the old macaque hippocampus.[121] As some of these molecules have previously been linked to induction of quiescence in stem cells,[164–167] cell-cell interactions between NSCs and astrocytes may contribute to decreased NSC proliferation during aging.

It is important to note that these computational methods provide only inferred cell-cell interactions. Experimental validation is necessary to confirm putative interactions and test functional significance. Despite this limitation, these methods provide an accessible first step into probing the interplay of different cell types during brain aging and disease.

Spatial dependencies between aging of brain cell types

Spatially resolved single-cell transcriptomics have also been applied to characterize cellular architectures across different brain regions.[18,148,168–171] Spatial transcriptomics technologies can provide new insights into cell-cell interactions beyond what can be achieved using dissociated single-cell transcriptomics due to preserved spatial architecture.[18,19] For example, astrocytes exhibit higher activation scores in proximity of vascular cells (particularly those localized to the meninges).[18] Meanwhile, microglia exhibit higher activation scores in proximity to oligodendrocytes (but not to vascular cells).[18] Thus, different cell-cell interactions may drive activation of microglia compared to activation of astrocytes. Interestingly, activation scores of astrocytes and microglia are also spatially correlated with oligodendrocyte inflammation levels.[18] Finally, activated microglia and inflamed oligodendrocytes are spatially localized closer to infiltrating T cells in the aging mouse brain.[19] These observations suggest a prominent role for spatial relationships between cells in driving the activation of microglia and inflammatory status of glia, but the whole cast of mechanisms underlying these spatial associations remains to be determined.

INTERVENTIONS FOR REJUVENATING CELL TYPES OF THE BRAIN

An important aspect of aging biology is to identify "rejuvenating" interventions that boost cell function and restore tissue homeostasis during aging. Research in the field has delineated several

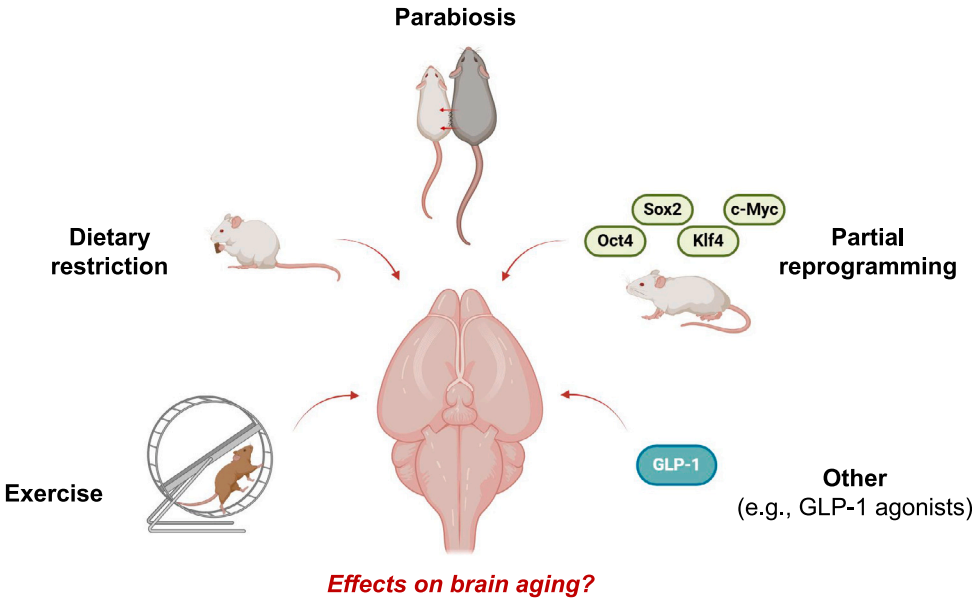

Figure 4. Rejuvenation interventions for the brain
Depiction of classical rejuvenation interventions such as exercise, dietary restriction, and parabiosis as well as promising newer interventions such as partial reprogramming and GLP-1 agonists.

promising rejuvenation interventions for rescuing age-related deficits in the brain[24,26,97] (Figure 4). However, the cellular and molecular changes underlying these rejuvenation effects are not fully understood for most interventions. Single-cell omics technologies provide a useful lens to investigate the cellular and molecular changes that occur during aging and rejuvenation at scale (Table 3).

Dietary interventions

Dietary restriction is a gold standard intervention that has been shown to improve physiologic and cognitive functions, slow or even reverse age-related decline across multiple tissues and organs, and increase median and maximum lifespan across different species.[180,181] Several studies have examined the impact of long-term and acute dietary restriction on the rodent brain at single-cell resolution.[36,172]

To determine the cellular and molecular changes underlying dietary restriction, snRNA-seq was performed on brain tissue from aged rats (27 months old) that were subjected to either *ad libitum* feeding or long-term 70% calorie restriction for 9 months[172] and from young rats (5 months) fed *ad libitum*. The age-dependent decrease in inhibitory neurons and endothelial cells is restored to more youthful levels in response to calorie restriction.[172] Remarkably, the expression of several genes associated with DNA-damage response is rescued by calorie restriction.[172] These findings raise the possibility that calorie restriction may have beneficial effects on the aged brain by improving responses to DNA damage.

Another study performed snRNA-seq of the hippocampus of aged female mice fed *ad libitum* or with 40% acute dietary restriction for 4 weeks.[36] Analysis of this dataset revealed that a dietary restriction signature (generated from bulk RNA-seq) is specifically upregulated in glia, including oligodendrocytes, OPCs, astrocytes, and microglia, but not increased in any neuronal cell types.[36] These observations suggest that the transcriptomic response to dietary restriction is primarily driven by glial cells, though the low transcriptional changes in neurons during aging may also contribute to the limited impact of dietary restriction on neuronal cell types.

While dietary restriction does provide rejuvenating effects on the transcriptional signatures of cells in both studies, the exact cell types impacted vary depending on the study.[36,172] These different single-cell transcriptional responses could be due to differences in dietary restriction interventions (in terms of duration, timing of feeding, and nutritional composition). Single-cell transcriptional responses to dietary restriction may also differ because of the distinct brain regions probed or differences between species (rat vs. mouse). The expanded use of single-cell omics to disentangle the differences between dietary restriction regimens on various cell types of the brain should lead to a better understanding of the effect of nutrient-based interventions on brain aging.

Exercise

Like dietary restriction, exercise is a widely studied and well-validated intervention for rejuvenating the function of multiple tissues and slowing or reversing age-related decline.[182–184] Unlike dietary restriction, however, exercise does not appear to extend maximal lifespan.[185] Here, we examine insights obtained from single-cell transcriptomic studies into the effect of exercise on the brain.

Exercise has rejuvenating effects on several regions of the brain. An atlas of aging and exercise has been generated on multiple organs, including multiple brain regions and spinal cord.[173] In this study, young and old mice are either sedentary or subjected to long-term voluntary exercise for 12 months. Analysis

Table 3. Insights from select key single-cell omics studies of rejuvenation interventions in the vertebrate brain

Intervention	Protocol	Key cell-type impacts	References
Diet	9 months of 70% calorie restriction in old rats	• Restoration of endothelial and inhibitory neuron populations • Restored DNA damage response gene expression	Ma et al.[172]
	4 months of 40% acute dietary restriction in old mice	• Oligodendrocytes, OPCs, astrocytes and microglia are transcriptionally more rejuvenated than neuronal cell types	Hahn et al.[36]
Exercise	5 weeks of voluntary wheel running in young and old mice	• Increased proportion of NSC and neuroblasts in SVZ of both young and old mice • OPCs and activated NSCs are transcriptionally impacted the most of all SVZ cell types	Liu et al.[155]
	5 weeks of voluntary wheel running in young and old mice	• Oligodendrocytes experienced greatest rejuvenation by chronological aging clocks • Activated NSCs and neuroblasts experienced greatest rejuvenation by functional aging clocks	Buckley et al.[38]
	5 weeks of voluntary wheel running in young and old mice	• Cells of the brain vasculature experienced greatest (region-specific) rejuvenation by spatial aging clocks	Sun et al.[19]
	12 months of voluntary wheel running in young and old mice	• Astrocytes and excitatory neurons experienced greatest transcriptional rejuvenation in the brain	Sun et al.[173]
	3 weeks of voluntary wheel running in old mice	• Decreased T cell proportion in hippocampus • Microglia experienced the greatest transcriptional rejuvenation in hippocampus and transitioned to more homeostatic states	Chauquet et al.[174]
Blood factors	4–5 weeks of heterochronic parabiosis	• Endothelial cells were among the most rejuvenated cell types in the brain • Restored expression levels of genes associated with mitochondrial activity, oxidative stress response, and metabolism • Reduced gene expression associated with cell senescence	Ximerakis et al.[175]
	5 weeks of heterochronic parabiosis	• Activated NSCs experienced greatest rejuvenation by chronological aging clocks • Neuroblasts experienced greatest rejuvenation by functional aging clocks	Buckley et al.[38]
	5 weeks of heterochronic parabiosis	• Relatively low transcriptional response to heterochronic parabiosis in brain compared to other organs	Pálovics et al.[176]
	5–6 weeks of heterochronic parabiosis	• Relatively low transcriptional response to heterochronic parabiosis in brain compared to other organs	Ma et al.[177]
Partial reprogramming	whole-body partial reprogramming and SVZ-targeted partial reprogramming	• Increased proportion of neuroblasts in the SVZ	Xu et al.[178]
	whole-body partial reprogramming	• NSCs and neuroblasts experienced greatest rejuvenation by spatial aging clocks	Sun et al.[19]
GLP-1R agonist	about 1 month of daily intraperitoneal exenatide injections	• Astrocytes and OPCs experienced the greatest transcriptional rejuvenation	Li et al.[179]

Summary of select key single-cell omics studies of different brain rejuvenation interventions and associated findings.

of this snRNA-seq atlas showed that the central nervous system is more impacted by exercise in old mice than young counterparts (which is different than what is observed in other organs).[173] Cells with the greatest transcriptional rejuvenation by exercise in aged mice include ependymal and meningeal cells, endothelial cells and pericytes, and oligodendrocytes in the spinal cord, and astrocytes and excitatory neurons in the brain.[173]

Spatial aging clocks applied to spatial transcriptomics data of aged mice subjected to 5 weeks of voluntary exercise revealed substantial transcriptional rejuvenation across several cell types.[19] The most impacted cell types include brain vasculature cells in the cortex, striatum, and corpus callosum but not in the lateral ventricles. The rejuvenating effects of exercise on the brain vasculature may be due to their proximity to circulating blood factors, which transfer some of the beneficial effects of exercise.[26]

Exercise can also transcriptionally rejuvenate cells in the SVZ niche.[38,155] scRNA-seq of the SVZ niche from young and old mice subjected to voluntary exercise conditions for 5 weeks showed restoration of some cell-type proportions by exercise.[155] Notably, exercise boosts the proportion of activated NSCs and neuroblasts in both young and old mice,[155] suggesting that this intervention can improve neurogenesis, consistent with previous studies.[186,187] At the transcriptomic level, the cell

types whose transcriptomes are most restored by exercise include activated NSCs.[155] The transcriptional rejuvenation of activated NSCs may partially contribute to the striking increase in neurogenesis induced by exercise.

Cell-type-specific transcriptomic aging clocks based on single-cell transcriptomes in the mouse SVZ were also used to quantify the effects of exercise on different cell types.[38] This analysis revealed that oligodendrocytes experience the largest rejuvenation from exercise.[38] Activated NSCs and NPCs also experienced rejuvenation, particularly when aging clocks based on "function" (i.e., proliferation index of the niche) were used.[38] This rejuvenating effect of exercise on NSCs was also observed using CellBiAge, machine learning models based on binarization.[188] Thus, exercise rejuvenates several cell types, including activated NSCs.

Finally, another scRNA-seq study showed that exercise exerts a rejuvenating effect on the old hippocampus.[174] In this study, young and old mice are either in sedentary conditions or subjected (for old mice only) to 21 days of voluntary exercise, followed by 14 days of rest.[174] scRNA-seq analysis reveals that T cell number is significantly reduced by exercise in the aged hippocampus, and these results are confirmed with immunostaining.[174] Interestingly, the transcriptional effects of exercise are the most pronounced on microglia.[174] A contributor of the exercise-induced changes in microglia may be the reduction of infiltrating T cells. Finally, pharmacological depletion of microglia revealed that these cells are necessary for exercise-induced hippocampal neurogenesis but not for exercise-induced improvement on spatial learning and memory tasks.[174] These data suggest that the effect of exercise on microglia may mediate some but not all of the rejuvenating effects of exercise on the brain.

Collectively, these single-cell studies outline rejuvenating changes in response to exercise across multiple cell types, both in cell proportion and in transcriptional regulation. These studies implemented diverse exercise durations (e.g., 3 weeks, 5 weeks, and 12 months), profiled different brain regions, and used different approaches for acquiring single-cell transcriptomic data (scRNA-seq, snRNA-seq, and spatial transcriptomics). These biological and technical differences may underlie the differences in the cell types most impacted by exercise. Additionally, single-cell omics studies of exercise primarily focused on mice and on voluntary exercise regimens (most often using running wheels). Further single-cell omics studies of the effect of exercise on the human brain, in the context of endurance vs. resistance training, will be necessary to better understand the cell-intrinsic effectors of exercise.

Circulating blood factors

The introduction of factors from the blood of young animals to old animals has rejuvenating effects on multiple organs,[189] including the brain.[24,26,190] A prominent mode of introducing young blood factors into the circulation of old animals is via "heterochronic parabiosis," a procedure where the circulatory systems of an old and young animal are surgically joined.[189,191] We will use the terms "young blood" and "old blood" to describe the introduction of young blood into an old animal or old blood into a young animal, respectively (though other factors may contribute). We will focus on insights gleaned from single-cell and spatial transcriptomic studies.

To assess the effect of blood factors on individual cells, a study generated a single-cell transcriptomic atlas for the brain of young and old mice in both heterochronic parabiosis and isochronic parabiosis (control) conditions for 4–5 weeks.[175] This study showed that endothelial cells are most impacted by young blood and old blood and exhibit the greatest number of genes whose age-dependent expression is reversed by young blood.[175] Interestingly, genes involved in heat shock response are upregulated with age and reversed by young blood in endothelial cells, raising the possibility that young blood may improve the resilience of the brain vasculature.[175] Increased gene signatures for cellular senescence occur during aging in most cell types, and this is reduced by young blood in the aged brain.[175] Across multiple cell types, genes involved in mitochondrial activity, oxidative stress response, and metabolism broadly, which are downregulated with age, are restored by young blood in aged mice.[175] Interestingly, the strong rejuvenation of endothelial cells by young blood is reminiscent of that by exercise,[19] consistent with a role for blood factors in transferring the effects of exercise.[192,193]

To quantify the rejuvenating effect of parabiosis on neurogenic regions of the brain, another study applied cell-type-specific aging clocks to scRNA-seq profiles generated from adult SVZ niches from young and old male mice in heterochronic and isochronic (control) conditions.[38] These aging clocks revealed that activated NSCs are substantially "rejuvenated" transcriptionally by young blood in old mice across two independent parabiosis experiments.[38] However, unlike in other regions of the brain,[175] the endothelial cells in the SVZ are only slightly rejuvenated transcriptionally by young blood.[38] This may represent a region-specific response similar to what has been observed for exercise.[19]

To investigate the effects of young plasma (instead of young blood) on the aged brain, one study profiled the brains of aged male mice receiving recurring injections of young mouse plasma using region-resolved bulk RNA-seq.[36] The SVZ neurogenic niche is the region of the brain that is most impacted by young mouse plasma,[36] consistent with the strong rejuvenation of different cell types (notably activated NSCs) by young blood.[38]

By contrast, several multi-tissue single-cell transcriptomic atlases of heterochronic parabiosis have shown relatively low transcriptional responses to young blood or old blood in the brain compared with other organs.[176,177] Differences in results could be linked to technical differences (i.e., parabiosis surgeries with different protocols, distinct ages for the young and old parabionts or blood donors, etc.). Notably, there could be differences in the cell-recovery bias due to single-cell or single-nuclei isolation, and it will be interesting to use technologies that do not depend on dissociation (e.g., spatial transcriptomics) to identify the main source of differences.

Collectively, these studies suggest that young blood can have strong rejuvenating effects on several cells in the brain, notably endothelial cells across the whole brain and activated NSCs in the SVZ neurogenic niche. Young blood has also been shown to rejuvenate several aspects of the hippocampal neurogenic niche during aging.[126] Cells that are close to blood capillaries

(activated NSCs) or directly in contact with blood (endothelial cells) may experience the strongest impact of young blood interventions, and it will be interesting to further understand how different cells and distinct regions of the old brain respond to young blood factors.

Partial reprogramming

In vivo partial reprogramming, generally performed by transient expression of the Yamanaka reprogramming factors (OSKM: OCT4, SOX2, KLF4, and c-MYC) or a subset of them, *in vivo*, has emerged as a promising rejuvenation intervention.[194–196] In the brain, *in vivo* expression of OSKM improves neurogenesis,[178,197] reverses cognitive deficits of aging,[197] and improves recovery from brain injury.[198] *In vivo* expression of only one factor (c-MYC) is sufficient to rejuvenate aged OPCs,[199] and expression of three factors (OSK: OCT4, SOX2, and KLF4) is sufficient to restore vision loss.[200] Here, we focus on the insight provided by single-cell transcriptomic studies.

Interestingly, scRNA-seq of the SVZ niche from young and aged adult mice with or without partial reprogramming by inducible expression of all four Yamanaka reprogramming factors showed that the neuroblast proportion is restored to more youthful levels by partial reprogramming in aged mice.[178] Inducing the four reprogramming factors in the SVZ niche itself (rather than in the whole body) is sufficient to restore the neuroblast populations back to a more youthful level.[178] Immunocytochemistry experiments confirm the increase in neuroblasts and new neurons by partial reprogramming.[178] At the transcriptomic level, partial reprogramming rejuvenates gene signatures of aging in several cell types, including activated NSCs, NPCs, neuroblasts, and endothelial and mural cells.[178] However, partial reprogramming also exacerbates gene signatures of aging in other cell types such as microglia, astrocytes, and quiescent NSCs.[178] These results suggest that partial reprogramming restores neurogenesis by promoting the generation of neuroblasts, perhaps rejuvenating aspects of the aged transcriptome in these cells.

To further examine the effect of *in vivo* partial reprogramming in other brain regions, spatially resolved single-cell transcriptomic data have been collected from coronal brain sections from old mice subjected to partial reprogramming by inducible expression of all four Yamanaka factors.[19] Spatial aging clocks confirm that partial reprogramming improves NSCs and neuroblasts but has surprisingly muted or even pro-aging effects on other regions and cell types in the brain.[19] These studies suggest that partial reprogramming rejuvenates the progenitor cell lineage of the SVZ, yet may have detrimental effects elsewhere in the brain.

Given the beneficial effect of partial reprogramming on cell types involved in neurogenesis,[178,197] targeted partial reprogramming may represent a therapeutic strategy for countering the age-related decline in neurogenesis and in neurodegenerative diseases.[201,202] However, further research is needed to improve the safety of *in vivo* partial reprogramming interventions, including for intestinal function[203] and tumorigenesis.

Other rejuvenating strategies

Single-cell omics could be helpful to systematically evaluate the brain effects of other promising rejuvenation interventions, including senescent cell clearance,[79] rapamycin (mTOR inhibitor) treatment,[108,204] Klotho administration,[205] or interleukin-11 inhibition.[206]

Interestingly, glucagon-like peptide-1 (GLP-1) receptor agonists, including pharmaceuticals used as diabetes and weight loss drugs, have attracted attention as potential anti-aging therapies.[207] GLP-1 receptor agonists have been shown to have neuroprotective effects, primarily in the context of mouse neurodegenerative disease models.[208] In one study, old mice that received the GLP-1 receptor agonist exenatide showed transcriptional rejuvenation in multiple glial and neurovascular cell types by scRNA-seq, with the largest effects in astrocytes and OPCs.[179] This study did not analyze neurons, so it will be interesting to investigate whether the apparent benefits of GLP-1 receptor agonists for learning and memory[208] are mediated by these non-neuronal cells. More generally, the use of single-cell transcriptomic studies coupled with aging clocks could help compare the efficacy of different rejuvenation strategies.

THE SPECIFIC CASE OF REGENERATIVE CELL TYPES FOR REJUVENATION

As NSCs have regenerative capacity and can be involved in repair upon injury, identifying NSC-specific mechanisms of rejuvenation would be particularly valuable for the aging brain. As highlighted above, rejuvenation strategies such as young blood and exercise (systemic) as well as partial reprogramming (cell-intrinsic) restore youthful gene expression signatures in the neurogenic lineage and improve neurogenesis. Interestingly, many studies have identified additional strategies to rejuvenate NSCs and boost neurogenesis in old mice. They include modulating protein aggregation, epigenomic regulators, cell cycle function, and glucose metabolism, among others.[104,108,109,192,209–217] Thus, single-cell transcriptomic studies should be crucial in identifying important target pathways of these interventions, which could in turn be modulated to increase neurogenesis with age. For example, single-cell transcriptomics revealed differences in expression of metabolic genes between quiescent and activated cells.[218] Quiescent NSCs exhibit increased expression of mitochondrial pyruvate carrier 1, *Mpc1*, at the RNA level compared with activated NSCs.[216] Consistently, depleting *Mpc1 in vivo* in NSCs increases the number of proliferating NSCs and newborn neurons in the DG of young and middle-aged mice.[216] In addition, *in vitro* and *in vivo* CRISPR screens revealed that knocking out the glucose transporter gene *Slc2a4 (GLUT4)* results in increased NSC activation *in vitro* and increased SVZ neurogenesis in old mice.[217] It will be interesting to test the effect of metabolic interventions on multiple cell types in the neurogenic niches using unbiased, single-cell-based approaches. Moreover, coupling genetic or pharmacological strategies to transcriptomic studies (e.g., Perturb-seq) should provide new insights into NSC rejuvenation.

Beneficial effects in NSCs have the potential to affect the tissue more globally given their impact on the production of new cells and perhaps cell-non-autonomous effect on other cells. Several studies show that targeting the DG with rejuvenating strategies results in increased neurogenesis, measured by *in vivo* labeling and microscopy, and improved cognitive function in old mice[109,219]

(though the boost in neurogenesis and cognition are not always directly correlated). As described above, NSCs and neuroblasts may have a beneficial effect on neighboring cell types[19] (see "potential influence of NSCs on other cells"). As NSCs and their daughter cells differentiate into neurons, they could also rejuvenate new neurons. Future investigations will be needed to characterize how rejuvenating NSCs could influence neighboring cell types or progeny, thereby amplifying the effect of the intervention.

TOWARD COMBINATORIAL REJUVENATION INTERVENTIONS FOR THE BRAIN

Single-cell transcriptomic studies of aging and rejuvenation generally focus on a single rejuvenation intervention. Combining multiple rejuvenation interventions could achieve synergistic and additive rejuvenation effects. In the brain, combinations of two different interventions (e.g., physical exercise and diet) result in amplified hippocampal neurogenesis[220–223] and further improvements in cognition.[223,224] Exploration of the rich combinatorial rejuvenation intervention space, perhaps through developing new methodologies to search for optimal combinations of interventions, may open promising avenues for brain rejuvenation.

One approach for identifying promising combinations of rejuvenation interventions is to leverage single-cell omics technologies to determine the amount of overlap between different interventions at the level of cell types. For example, cell-type-specific transcriptomic aging clocks trained to predict age have been used to compare the transcriptional effects of heterochronic parabiosis and exercise on predicted age across different cell types.[38] Exercise rejuvenates oligodendrocytes the most in old mice.[38] By contrast, young blood rejuvenates activated NSCs, quiescent NSCs, and microglia the most in old mice.[38] These results suggest that exercise and heterochronic parabiosis rejuvenate different cell types and thus have high potential for synergy when deployed in combination (perhaps through the injection of young blood factors into exercising mice). Comparison of cell-type-specific signatures of age-related processes (e.g., neurogenesis, neuroinflammation, etc.) may provide fine-grained understanding of the overlap between interventions to help advise the design of combinatorial interventions for rejuvenating the brain.

Similarly, spatial aging clocks can augment the comparison of rejuvenation interventions across both different brain regions and different cell types.[19] These spatial aging clocks reveal that exercise primarily rejuvenates cells of the brain vasculature and that this rejuvenation occurs in multiple brain regions, except for the lateral ventricles.[19] Meanwhile, spatial aging clocks show that partial reprogramming selectively rejuvenates NSCs and neuroblasts in the lateral ventricles but can be slightly detrimental to other cell types in other brain regions.[19] Combining partial reprogramming with exercise will be particularly interesting given their orthogonal rejuvenation effects on both different subsets of cell types and different brain regions.[19]

Extending single-cell transcriptomics to profile the molecular changes occurring in combinatorial rejuvenation interventions should provide new insight into how these synergistic or additive rejuvenation effects manifest—whether the interventions target different cell types and biological pathways or amplify the same underlying rejuvenation mechanism. Given the complexity of combinatorial rejuvenation interventions, computational frameworks for optimizing combinations of interventions using data from model organisms may be particularly fruitful, especially given the low availability of human brain samples with rejuvenation interventions.

LINK BETWEEN BRAIN AGING AND INJURY AND DISEASE

Age is the greatest risk factor for many diseases that affect the brain, including neurodegenerative diseases such as Alzheimer's or Parkinson's disease.[1] In addition, in the context of injury such as traumatic brain injury or stroke, the brain's ability to recover generally declines with age,[225,226] although data for stroke are mixed.[227] Single-cell transcriptomics approaches have allowed for a comparison between aged healthy and diseased brains and proposed explanations for the increased susceptibility of the old brain to disease. They have also provided cell-type-specific insight into the decreased recovery upon injury in old individuals.

Potential commonalities between aging and disease states in the brain are underscored by the fact that in mice, scRNA-seq studies show that similar microglia subpopulations are enriched in old wild-type brains and young brains from disease models, such as Alzheimer's models.[50,228] In humans, microglia subpopulations enriched in healthy tissue from older patients show transcriptional similarities with subpopulations enriched in glioma tissue, including elevated expression of the pro-inflammatory cytokine gene *SPP1*.[41] These commonalities may reflect inflammatory states both in aging and disease.

Crucially, single-cell transcriptomics has also revealed how disease states differ from healthy aging: for example, snRNA-seq has identified transcriptomic states specific to brain samples from human Alzheimer's disease patients, both in individual cell types and in multi-cell-type "communities."[229–231] A snRNA-seq study of the aged human leptomeninges highlighted BAMs and fibroblasts among the cells with the most transcriptomic differences between healthy controls and Alzheimer's patients.[232] scRNA-seq and snRNA-seq have also identified diverse changes—including some that are region-specific—distinguishing cells of the healthy human brain vasculature, such as endothelial cells, from their counterparts in Alzheimer's disease and other pathological states.[233–235] In several cases, cells from diseased individuals share similarities with old cells but are "older than old." Indeed, spatial aging clocks reveal substantial acceleration of transcriptional aging of multiple cell types, particularly glia, in Alzheimer's disease as well as in response to multiple sclerosis injury.[19]

Given the strong impact of aging on disease risk, deeper characterization of disease models at old ages should be informative. It is encouraging to see that in a study that included different ages up to 24 months in both wild-type and Alzheimer's disease model mice, scRNA-seq identified disease-associated oligodendrocytes as an oligodendrocyte subpopulation that becomes progressively more prevalent both with age and disease progression.[236] More generally, work in which disease models

are analyzed at different ages at single-cell and spatial resolution (e.g., Kilfeather et al.[73] for Parkinson's disease) represents a promising development for the future.

Regarding response to injury, in mice, scRNA-seq suggested that old endothelial cells may be less able to promote angiogenesis post-stroke than their young counterparts and that old OPCs have a decreased ability to form new oligodendrocytes post-stroke compared with their young counterparts, which could be validated by staining for newborn oligodendrocytes.[237]

Integration of spatial and single-cell omics data[148,238] may provide higher quality characterization of cell-cell interactions underlying brain aging and disease. Single-cell transcriptomics will also be useful to unbiasedly evaluate treatments for diseases in animal models and to test the possibility that anti-aging interventions (possibly in combination with disease-specific treatments) could slow disease progression.

HOW TO MODEL BRAIN AGING

Single-cell omics studies have characterized many aging- and disease-related changes, but in most cases, the functional impact of these changes, and their mode of action, is not understood. Thus, there is a need to develop additional *in vivo* and *in vitro* models for brain aging.

In vivo, most functional studies on brain aging have so far relied on the mouse. Yet, the mouse is relatively long-lived, which can make understanding the interaction between genetic interventions and age low-throughput. The African turquoise killifish has been developed as a new model to study vertebrate brain aging at scale.[239] The killifish has a median lifespan of 4–6 months[240] but displays many phenotypes associated with mammalian brain aging and disease, including neuronal degeneration, increased inflammation, decreased neurogenesis, and cognitive decline.[239] Rejuvenation strategies, including dietary restriction and senescent cell clearance, have also been successfully used in the killifish.[241–243] scRNA-seq has recently profiled age-related changes in the killifish brain, which shares many key brain cell types with humans.[48] Consistent with mammalian data, killifish neurons experience fewer changes with age than non-neuronal brain cells.[48] In addition, inflammatory gene expression increases in multiple cell types of the killifish brain with age,[48] and expression of neurogenic transcripts in neuronal progenitors declines with age.[48] Genetic perturbation techniques, including CRISPR knockout[244] and knockin[245,246] have been developed for killifish, making it more feasible to functionally test the impact of aging-related changes in different brain cells on a rapid timescale.

In vitro brain aging models have been helpful to mechanistically understand age-related changes from many cell types. These include organotypic brain slices, cell culture models, and brain organoids. Organotypic SVZ whole mounts from young and old mice have revealed impaired NSC migration and dynamics with age.[112] Additionally, *in vitro* co-culture models can be designed to recapitulate aging phenotypes. For example, co-culture experiments with NSCs from young and old mice and T cells isolated from the spleen showed that T cell activation could negatively impact NSC proliferation.[53] Human *in vitro* systems, such as brain organoids, are critical to model human brain aging, which occurs over many decades.[247] Brain organoids can also help to study the genetic factors involved in normal aging, either by using patient-derived cells or when coupled to CRISPR editing.[247,248] Patient-derived brain organoids use patient fibroblasts that have been reprogrammed to a pluripotent state then differentiated to resemble the composition of a specified brain region. However, inducing pluripotency removes many features of aging and results in relatively immature cell types.[247] Therefore, there is a need to develop *in vitro* systems that do not rely on reprogramming. Notably, inhibiting neddylation in wild-type pluripotent stem cell-derived neurons recapitulates multiple aging hallmarks.[249] Furthermore, using direct reprogramming and bypassing pluripotency, 2D culture systems derived from human donors can recapitulate aging phenotypes.[250,251] *In vitro* models of brain aging and neurodegenerative diseases will be critical to directly test the cellular impact of transcriptomic changes with age. However, more work needs to be done to characterize how well *in vitro* models recapitulate *in vivo* phenotypes.

In summary, diverse model systems, both *in vivo* and *in vitro*, will be necessary to unravel the complex interplay between different cell types that drives vertebrate brain aging and impacts the success of rejuvenation interventions.

CONCLUSIONS AND OUTLOOK

This review has focused on vertebrate aging, with an emphasis on mouse and human models. The animal kingdom is diverse, and other specific model systems could be key to understand convergent pathways of aging. We have highlighted how recently published single-cell technologies have provided insight into the mosaic landscape of the aging brain. These approaches have given new clues into the cell-type-specific effects of aging and characterized aging at an unbiased, high-dimensional level. Single-cell technologies are powerful for hypothesis generation and identification of candidate pathways that shape brain aging. However, to understand the functional decline of the brain with age, single-cell technologies will need to be coupled to classical genetic, cell biology, and neuroscience techniques. High-throughput discovery with single-cell omics technology requires high-throughput validation of discoveries, and *in vitro* models of brain aging have emerged as promising tools to accomplish this task. In the future, establishing behavioral correlates of cell-type-specific patterns of aging will link discoveries from omics studies to functional decline in the aging brain.

Brain cell types all age differently, and aging is not just a cell-intrinsic feature. To understand how brain aging occurs at a holistic level, more work needs to be done to determine how cell-cell interactions shape the aging of the brain itself. Even rare cell types, like invading T cells, have drastic effects on nearby cell types, particularly glia. Glia could in turn have critical effects on neurons. A better understanding of the interactions and pathways identified by single-cell studies could be used to develop cell-intrinsic and -extrinsic rejuvenation techniques to improve brain function with age.

Well-known rejuvenating interventions such as caloric restriction, exercise, and young blood have been further assessed with

new single-cell technologies. Individual rejuvenation strategies have a unique fingerprint on the aging brain, and different cell types and brain regions respond in drastically different ways to various interventions. Recent innovations in rejuvenating strategies have identified partial reprogramming and GLP-1 receptor agonists as promising avenues for countering brain aging. It will be interesting to understand how new rejuvenation strategies affect the brain using high-throughput single-cell techniques to determine how different cell types in the old brain are impacted, leveraging model organisms. Furthermore, coupling genetic knockout with single-cell transcriptomics will represent a powerful method for understanding causal drivers of brain aging at scale. In the future, more work will need to be done to understand the synergistic effects of multiple interventions on diverse brain cell types, perhaps aided by aging clock models.

This review is primarily focused on single-cell transcriptomics, but other types of single-cell strategies, such as single-cell epigenomics and whole-genome sequencing, and eventually single-cell metabolomics, proteomics, and lipidomics, will provide a more complete understanding of brain aging and the effect of rejuvenation interventions. An exciting new direction in the single-cell omics field is the development of spatially resolved technologies, which provide a critical dimension to understanding cell-cell interactions and spatial proximity effects. Single-cell technologies are a rapidly growing field, and as they become cheaper to deploy, they will bring a richer view of brain aging.

Aging is the largest underlying factor for neurodegenerative diseases. As extensive single-cell studies have focused on neurodegenerative disease samples, it will be critical to understand the overlap between aging and disease. An important question is how rejuvenating interventions or interventions that counter aging improve disease outcome. Specific knowledge of the sensitivity of different cell types to aging and to specific disease risk factor (e.g., genetic factors), as well as malleability in response to rejuvenation interventions, will be critical to developing therapeutics in the future. Additionally, aging leads to decreased injury repair. As the brain can be subjected to devastating injuries throughout a lifespan (stroke, trauma), future studies that apply single-cell-based rejuvenating approaches to injury and repair could lead to new therapeutic strategies to improve brain health.

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AUTHOR CONTRIBUTIONS

E.D.S. wrote the introduction and sections on "single-cell omics technologies used to study brain aging," "neuronal cell types," "infiltrating and peripheral immune cell types," "interactions between cell types during aging," "dietary interventions," "exercise," "circulating blood factors," "partial reprogramming," and "toward combinatorial rejuvenation interventions for the brain" and generated Table 2 and Figures 1 and 3. R.N. wrote sections on "glial cell types," "cell types of the brain's borders," "other rejuvenating strategies," and "link between brain aging and injury and disease" and generated Tables 1 and 3 and Figures 4 and 2 together with A.N.P. A.N.P. wrote the conclusion and sections on "NSCs and progenitor cells," "the specific case of regenerative cell types rejuvenation," and "how to model brain aging" and generated Figure 2 together with R.N. A.B. helped with the overall structure of the review and with revising all sections. All authors contributed to the writing of other sections.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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