Single-cell transcriptomic changes in non-neuronal cells in the aging mouse brain

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1. Introduction

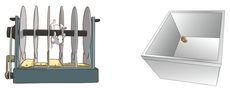
•Aging is the leading risk factor for most major chronic diseases in humans, including neurodegeneration

•The mamalian brain is highly heterogenous and composed of diverse neuronal and non-neuronal cell types. How these cell types changes with age remains to be fully understood

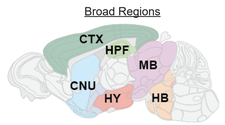
•We have profiled broad regions of the adult and aged brain using single-cell RNA sequencing (scRNA seq) and spatial RNA profiling in an effort to characterize how and which cell types in the brain are most sensitive to aging. In this poster, we highlight transcriptional changes in non-neuronal cells

2. Experimental Design and Methods Summary

I. Age out and monitor mice for *healthy* aging and behavior



II. Dissect brain regions



III. Isolate cells and make libraries

50% Neuron enriched sampling

50% Unbiased sampling

IV. Single-cell RNA seq.

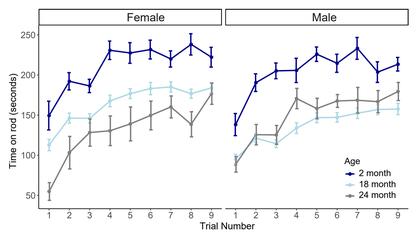
10x GENOMICS

V. *In situ* spatial RNA profiling

resolve biosciences

3. Rotarod performance in healthy aged animals

Figure 1. Each mouse was monitored for healthy aging and tested for performance on Rotarod prior to experimental use. Briefly, each mouse underwent 9x 5 minute trials balancing on a rotating rod. Plotted are mean (± standard error) time spent on the rod by age group and sex. Sample size per sex and age group: n_{2mo}=15-16; n_{18mo}= 53-56; n_{24mo}=11-13



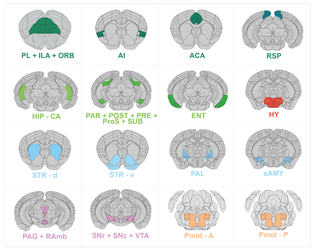
4. Single-cell RNA seq. dataset summary: over 1.2 million aged mouse brain cells profiled

A.

Total number of scRNA seq. cells

Broad Region	Region Acronym	Aged		Adult	
		Female	Male	Female	Male
Isocortex	ACA	37,617	37,719	18,208	50,364
	AI-CLA	45,101	41,355	23,073	48,603
	PL-ILA-ORB	42,968	41,365	8,965	49,173
Hippocampal formation	RSP	34,261	35,943	29,240	56,017
	ENT	48,777	70,454	34,604	52,564
	HIP - CA	28,958	36,294	48,449	41,271
	PAR-POST-PRE-SUB-ProS	27,883	32,180	38,643	49,760
Hypothalamus	HY - MEZ-PVZ-PVR	34,112	85,594	21,380	35,127
Cerebral nuclei	CNU - PAL	29,908	35,504	47,670	16,680
	STR - sAMY	29,094	47,122	41,622	40,305
	STR - STRd	37,668	59,135	43,137	20,615
	STR - STRv	46,981	43,348	18,712	44,952
Hindbrain	PONS - Pmot-Psat ant	33,285	29,756	27,704	17,325
	PONS - Pmot-Psat post	34,195	22,391	29,137	22,597
Midbrain	MB - PAG-RAmb	41,910	44,093	35,062	49,642
	MB - VTA-SN	17,722	23,140	29,104	26,941
	Total by sex/age:	570,440	685,393	494,710	621,936
	Total by age:	1,255,833 (53%)		1,116,646 (47%)	
	Total:	2,372,479			

B.



C.

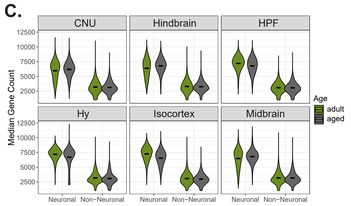


Figure 2. (A) Total numbers of high quality cells collected for scRNA seq, broken down by region, age, and sex. (B) Diagram of fine regions colored by broad region. See Summary section for full abbreviations. (C) Median gene counts, a measure of quality for scRNA seq data, does not differ substantially between adult and aged cells, suggesting data quality is comparable between age groups.

5. A wide range of cell types are detected in single cells collected from adult and aged mouse brains using single-cell RNA sequencing

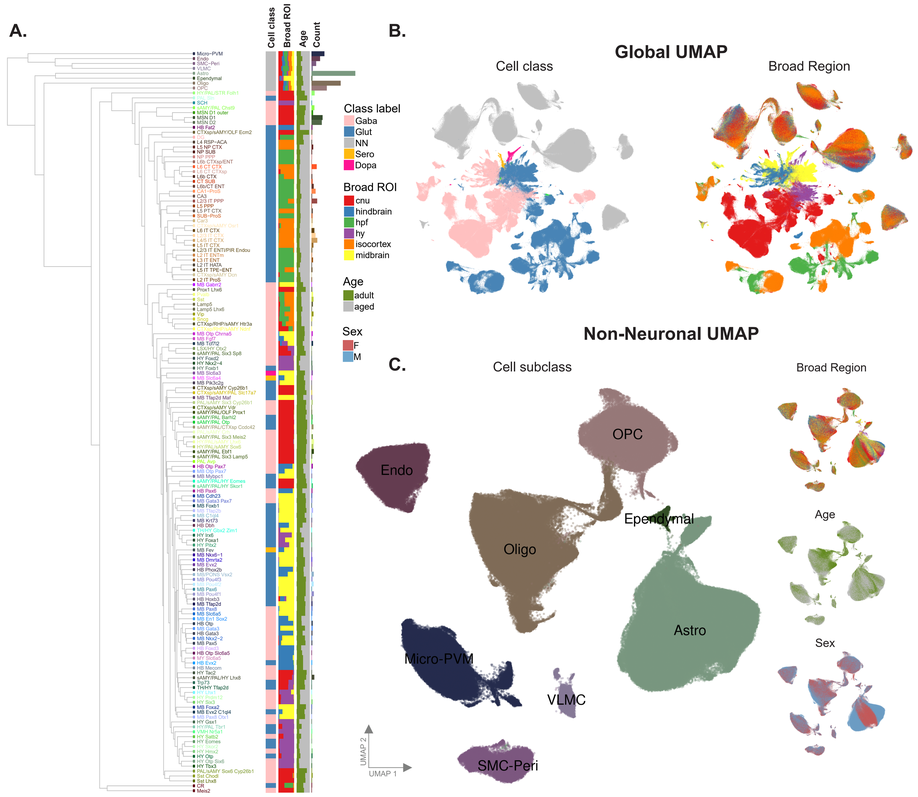
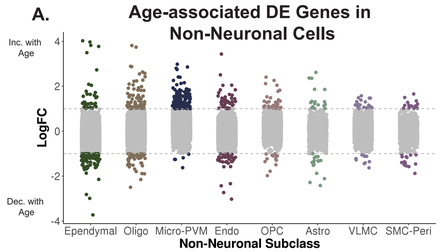
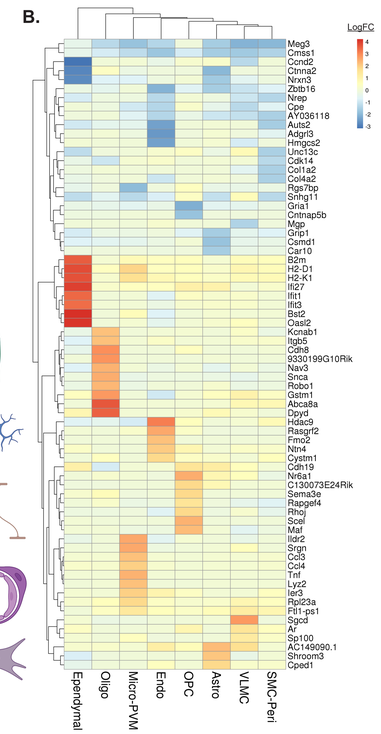


Figure 3. (A) Dendrogram of 151 distinct cell subclasses detected across cells collected from single-cell RNA sequencing data from adult (2 month) and aged (18 month) mouse brains. Vertical bar plots represent identity and fractions of cells according to cell class, broad region, age, number of cells collected. **(B)** UMAP representation of all cells in the dataset colored by class and broad region. **(C)** UMAP representation of only non-neuronal cells colored by subclass, broad region, age, and sex. See Figure 2A for total numbers of high quality single cells profiled

6. Gene expression changes in non-neuronal cells in the brain show sub-class specific signature of aging

Figure 4. (A) Dot plot of genes that are differentially expressed (DE) with age across non-neuronal subclasses. Each point represents one gene. Significant genes are in color (P_{adjust} < 0.001 & abs(logFC) > 1). **(B)** Heatmap representation of the top 10 most significant age-associated genes from each non-neuronal subclass. Colors in each cell represent the logFC value. Notably, most genes that increase with age appear to be subclass-specific, thereby representing aging signatures unique to each type of cell. In contrast, some genes decrease across all subclasses with age (such as *Meg3* and *Cmss1*), potentially representing conserved age-associated transcriptional program. **(C)** Table of top Gene Ontology (GO) terms associated with DE genes from select non-neuronal subclasses.





C.

GO Term	P (adjusted)	Subclass
defense response to virus	1.86E-08	Ependymal
response to virus	1.86E-08	Ependymal
response to interferon-beta	4.02E-06	Ependymal
negative regulation of cell projection organization	2.25E-05	Ependymal
positive regulation of leukocyte differentiation	1.23E-04	Micro-PVM
regulation of adaptive immune response	1.51E-04	Micro-PVM
regulation of inflammatory response	4.41E-04	Micro-PVM
positive regulation of T cell activation	9.48E-04	Micro-PVM
synaptic membrane	1.88E-06	Oligo
myelin sheath	1.67E-03	Oligo
cortical actin cytoskeleton	1.67E-03	Oligo
dendrite development	2.39E-03	Oligo
positive regulation of cell projection organization	2.39E-03	Oligo
complex of collagen trimers	2.98E-10	SMC-Peri
collagen-activated tyrosine kinase receptor signaling pathway	1.25E-04	SMC-Peri
circadian behavior	3.64E-03	SMC-Peri
circadian rhythm	5.22E-03	SMC-Peri
collagen-containing extracellular matrix	4.02E-05	VLMC
biomineralization	2.83E-03	VLMC
morphogenesis of a branching structure	5.64E-03	VLMC

7. *In situ* RNA profiling confirms increase in Abca8a expression in aged oligodendrocytes

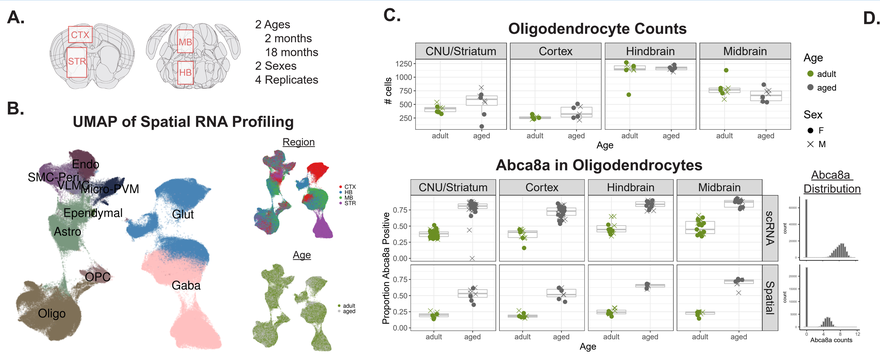


Figure 5. (A) Diagram of approximate regions profiled using Resolve Molecular Cartography spatial RNA profiling. **(B)** UMAP representation of cells detected from Resolve data. **(C)** Abca8a is one gene found to increase in oligodendrocytes with age. While the number of oligodendrocytes does not change much with age across regions (upper panel), the proportion of Abca8a$^+$ (non-zero expression) cells changes significantly with age (lower panel). **(D)** Example of DAPI-stained (greyscale) images from an adult and aged female midbrain sample overlaid with oligodendrocyte marker genes (Opalin, Sox10, Apod, Enpp6, Nkx6-2; yellow) and Abca8a (magenta) as detected by the Resolve molecular cartography platform. Number of high quality Resolve cells after filtering: n=215,824

8. Summary & Next Steps

•We have generated the largest extant sc-RNA seq dataset of aged mouse brain cells in an effort to characterize healthy brain aging

•Distinctive gene ontology terms were enriched among the differentially expressed genes in non-neuronal cells, suggesting unique aging signatures across different non-neuronal cell types.

•In aged oligodendrocytes, we see an increase in the proportion of cells that express the transcript Abca8a, an ATP Binding Cassette Subfamily gene that is involved in lipid metabolism and formation and maintenance of myelin. This increase is confirmed with *in situ* spatial RNA profiling.

•Future efforts include continued investigation into transcriptional changes in neuronal populations with age

Region abbrevations: prelimbic area + infralimbic area + Orbital area (PL + ILA + ORB), anterior insular area (AI), anterior cingulate area (ACA), retrosplenial area (RSP), hippocampal region (HIP - CA), parasubiculum + postsubiculum + presubiculum + prosubiculum + subiculum (PAR + POST PRE + ProS + SUB), entorhinal area (ENT), hypothalamus (HY), striatum - dorsal and ventral (STR - d, STR - v), pallidum (PAL), striatum-like amygdalar nuclei (sAMY), periaqueductal gray + midbrain raphe nuclei (PAG + RAmb), substantia nigra, reticular & compact part + ventral tegmental area (SNr + SNc + VTA), pons motor related - anterior and posterior (Pmot - A, Pmost - P)

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