Cortical glia in SOD1(G93A) mouse are subtly affected by ALS-like pathology

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Introduction

Glial cells, namely astrocytes, microglia, and oligodendrocytes, have been shown to actively participate in neurodegenerative diseases, playing a role in their progression and onset. Single-cell RNA sequencing (scRNA-seq) substantially contributed to discovery of various glial subpopulations, some of which emerge specifically in pathological environment, and respond by changing their transcriptional profile and function[1-3].

Here, we used scRNA-seq to analyze cortical glia of SOD1(G93A) mutant mouse, a widely used model of amyotrophic lateral sclerosis (ALS). We aimed to describe transcriptional changes occurring during the disease progression at four time points (1, 2, 3, and 4 months of age), and to identify cellular populations with a potential role in the ALS-like pathology.

Experimental Design

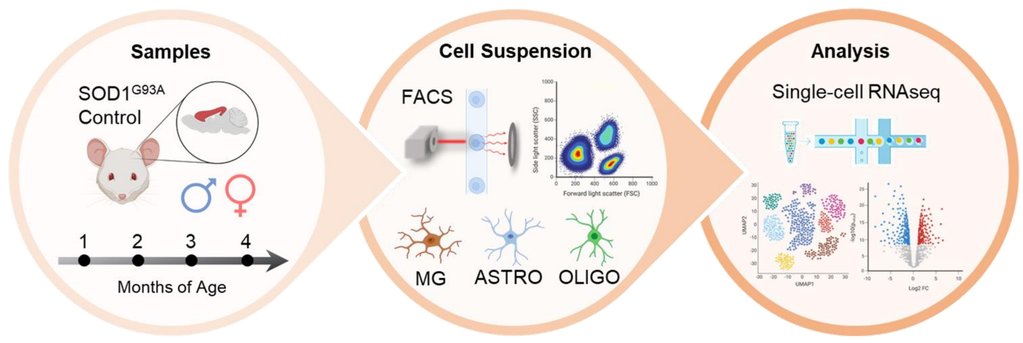


Fig. 1: The single-cell experiment included control and SOD1(G93A) mutant mice of both sexes at four time points. The single-cell suspension was enriched for microglia (MG), astrocytes (ASTRO), and oligodendrocytes (OLIGO), and was processed using the 10x Chromium platform. The sequencing data were analyzed using the Seurat R package[4].

Results

1. Motor skill impairment of SOD1 mice was accompanied by an insignificant loss of cortical motor neurons

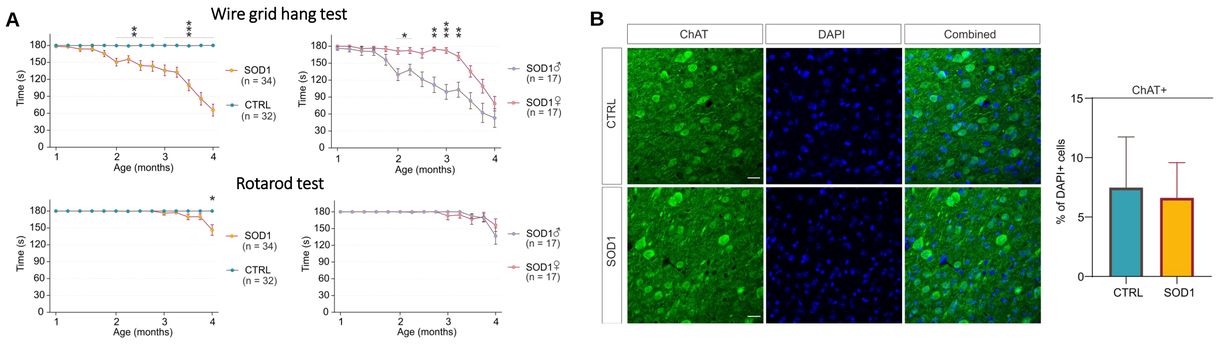
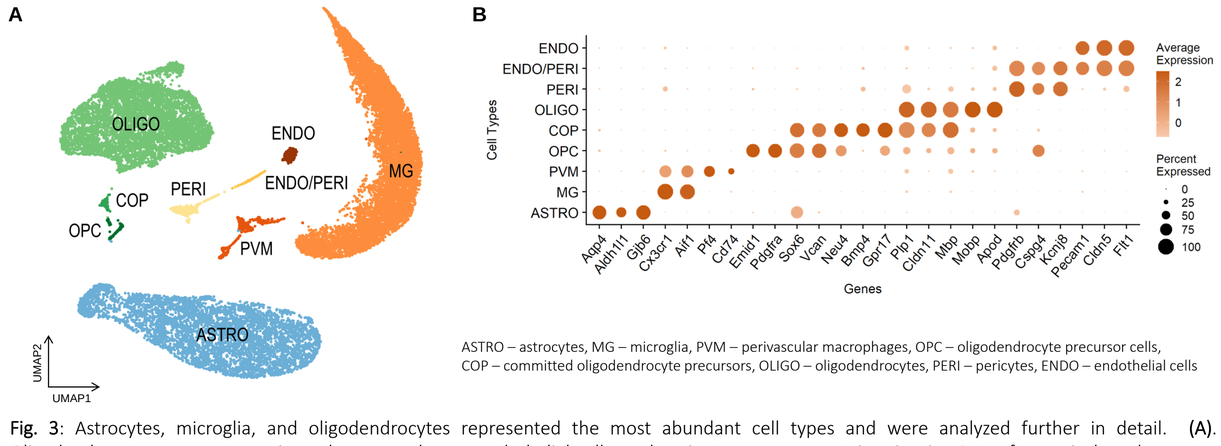


Fig. 2: The wire grid hang test revealed declining motor abilities of the SOD1(G93A) mice since two months of age **(A)**. Males showed the decline sooner than females, but both sexes reached a similar performance at four months, which was considered the end-stage. At four months, also the rotarod test showed a significant impairment of motor coordination. Although behavioral tests confirmed the ALS-like pathology, motor neuron (MN) staining with choline acetyl transferase (ChAT) antibody did not detect a significant difference in MN numbers in the cortex of SOD1 mice **(B)**.

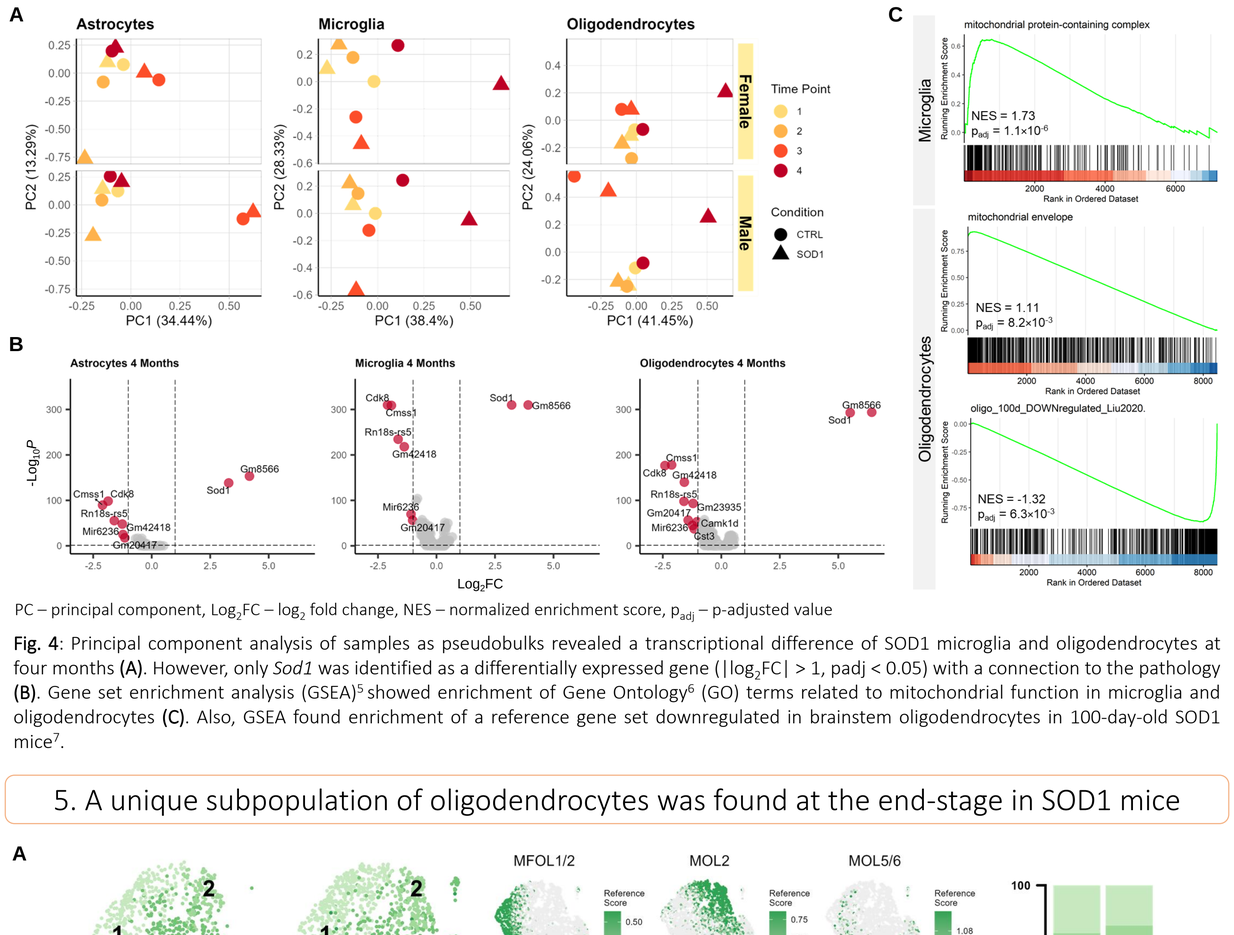
2. Eight major cell types were identified in the scRNA-seq data



ASTRO – astrocytes, MG – microglia, PVM – perivascular macrophages, OPC – oligodendrocyte precursor cells, COP – committed oligodendrocyte precursors, OLIGO – oligodendrocytes, PERI – pericytes, ENDO – endothelial cells

Fig. 3: Astrocytes, microglia, and oligodendrocytes represented the most abundant cell types and were analyzed further in detail. **(A)**. Oligodendrocyte precursors, perivascular macrophages, endothelial cells, and pericytes were present in minority. Sets of canonical marker genes were used for annotation of the clusters **(B)**.

3. Mitochondria-related genes were dysregulated in microglia and oligodendrocytes at the end-stage of the disease



PC – principal component, Log_2FC – log_2 fold change, NES – normalized enrichment score, p_{adj} – p-adjusted value

Fig. 4: Principal component analysis of samples as pseudobulks revealed a transcriptional difference of SOD1 microglia and oligodendrocytes at four months **(A)**. However, only *Sod1* was identified as a differentially expressed gene ($|log_2FC| > 1$, padj < 0.05) with a connection to the pathology **(B)**. Gene set enrichment analysis (GSEA)[5] showed enrichment of Gene Ontology[6] (GO) terms related to mitochondrial function in microglia and oligodendrocytes **(C)**. Also, GSEA found enrichment of a reference gene set downregulated in brainstem oligodendrocytes in 100-day-old SOD1 mice[7].

4. Control and SOD1 astrocytes and microglia comprised of identical subpopulations

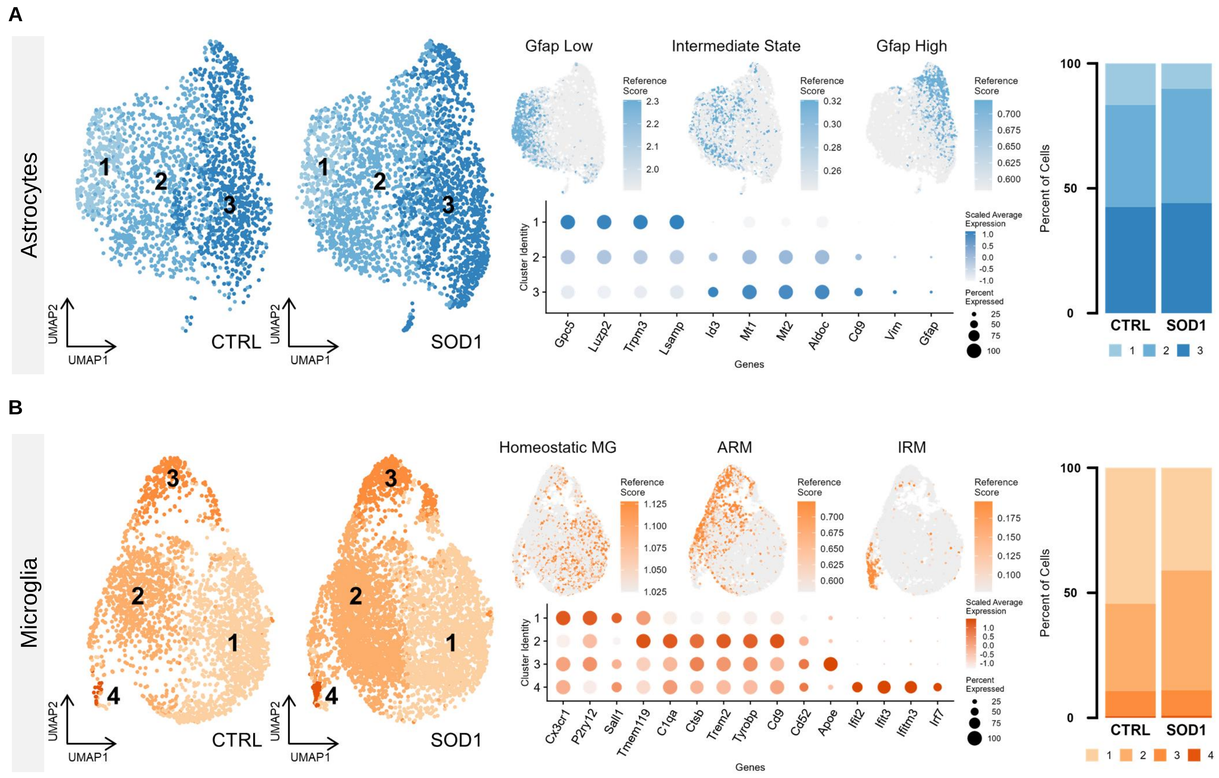


Fig. 5: Overlap with available reference data sets helped to identify Gfap Low and Gfap High-like subpopulations of astrocytes, defined by expression of activation markers[2] **(A)**, and subpopulations of microglia including homeostatic, activated response microglia (ARM), and interferon response microglia (IRM)[8] **(B)**. Intermediate state of astrocytes and activated microglia were more abundant in the SOD1 samples, however, no cluster was present uniquely in one of the conditions.

5. A unique subpopulation of oligodendrocytes was found at the end-stage in SOD1 mice

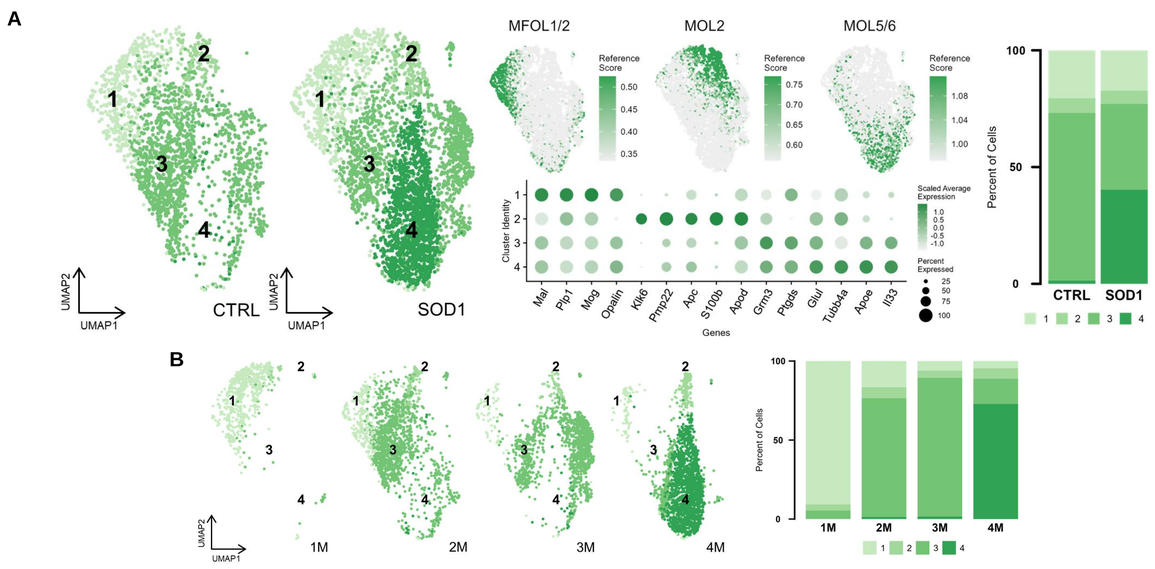
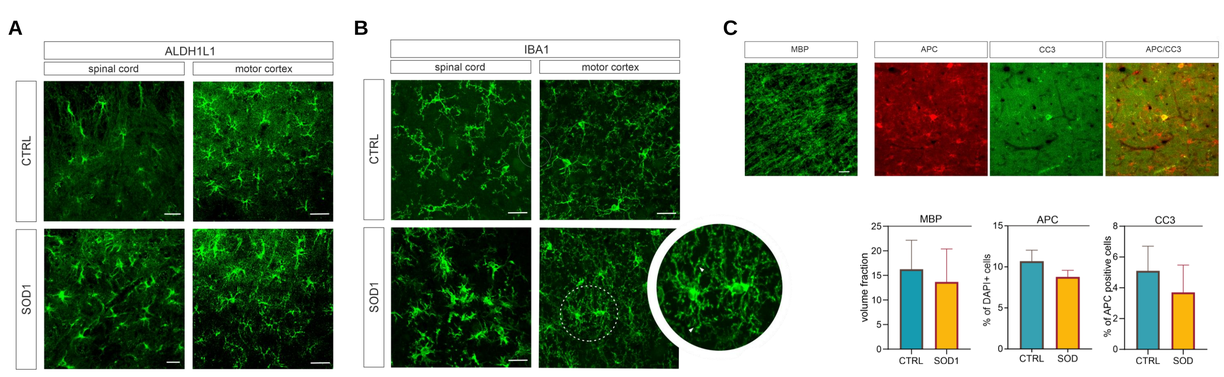


Fig. 6: Oligodendrocyte subpopulations were also characterized using a reference data set[9] **(A)**. They showed higher diversity across time points **(B)**. At one month, myelin forming oligodendrocytes (MFOL1/2) were the most abundant cluster. Mature oligodendrocytes (MOL) prevailed at later time points in both conditions. On the other hand, cluster 4 expressing *Il33* and *Apoe* genes was enriched at four months, only in the SOD1 samples.

6. Immunostaining of cortical microglia revealed signs of their starting activation



ALDH1L1 – aldehyde dehydrogenase 1 family, member L1, IBA1 – ionized calcium-binding adapter molecule 1, MBP – myelin basic protein, APC – adenomatous polyposis coli, CC3 – cleaved caspase 3

Fig. 7: ALDH1L1 immunostaining of cortical astrocytes showed no activation-related changes in cellular morphology, compared to hypertrophic spinal astrocytes in the SOD1 samples at four months **(A)**. Microglia staining with IBA1 revealed mild signs of starting activation represented by *bulbous termini* **(B)**. SOD1 oligodendrocytes did not show significantly increased rate of apoptosis (CC3 staining) or demyelination (MBP and APC staining), suggesting these processes were not responsible for emergence of the disease-specific subpopulation in the scRNA-seq data **(C)**.

Conclusions

- Microglia and oligodendrocytes appear to be the only affected cortical glia in the SOD1(G93A) mouse model, both showing dysregulation of mitochondrial function, which has been often discussed in the context of ALS[10].
- Microglia also mildly respond to pathological stimuli by increased activation.
- *Il33* and *Apoe* genes defining the SOD1-specific oligodendrocyte subpopulation have been previously associated with disease-related oligodendrocytes[11,12]. The unchanged rates of oligodendrocyte apoptosis and demyelination indicate an active role of this subpopulation in the ongoing pathology.
- The overall minor transcriptional alterations in the cortex of SOD1(G93A) mouse do not agree with extensive changes in ALS patients' cortex[13,14]. Therefore, we do not recommend the SOD1(G93A) mouse model for studying the cortical pathology in ALS.

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