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Cell type-specific effects of C9orf72 repeat expansion in the motor cortex of mouse models of fALS at single cell resolution.

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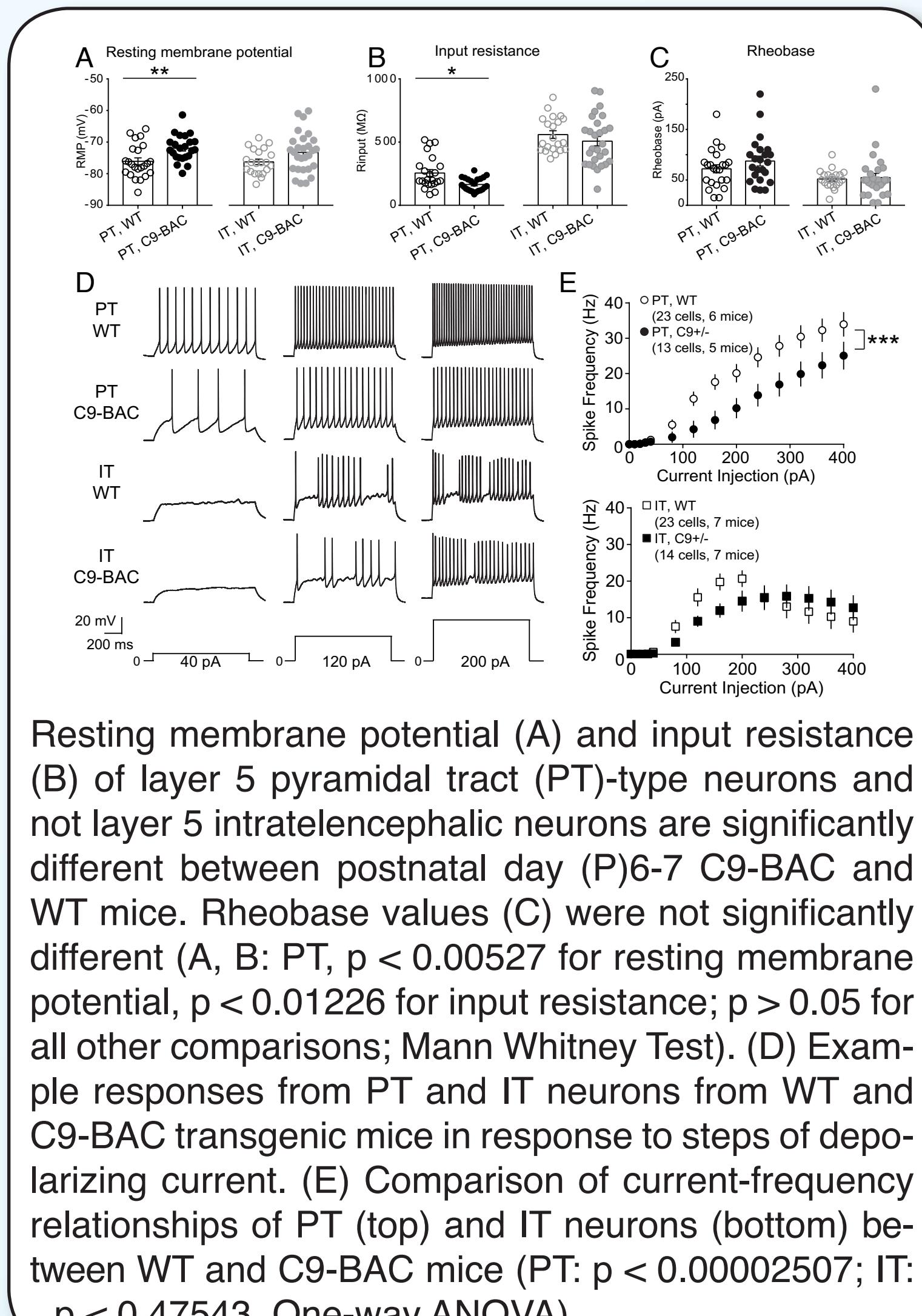
Abstract

Neurodegenerative disorders (ND) are characterized by neuronal loss often diverging based on the selective vulnerability of different neuron types. Amyotrophic Lateral Sclerosis (ALS) is a progressive and selective loss of motor neurons in the motor cortex and spinal cord. Several pathological features of ALS, including neuronal hyperexcitability, are observed across many different cortical cell types in mouse models. While bulk gene expression studies across models have revealed both distinct and common features among NDs, suggesting commonalities in the underlying pathological processes, these studies do not describe the distinct pathological responses of discrete cell types; a hallmark of selective vulnerability. Here we use single-cell RNA-Seq (scRNA-Seq) to examine the cell-type-specific transcriptional responses in the motor cortex of a mouse model of C9orf72 repeat expansion, a causal variant for familial ALS. We identify a subset of differentially expressed genes capable of modulating the observed electrophysiological response of layer 5 pyramidal tract (PT) neurons to C9orf72 repeat expansion. We further identify distinct and shared molecular pathological features across discrete cell types in the motor cortex. These gene expression differences represent both common cellular responses to the pathogenic C9orf72 repeat expansion as well as responses that are unique to subpopulations of cells, and can be used to enhance understanding of the selective vulnerability to this pathogenic repeat. Future work will involve recursive analysis of major cell types, and exploration of the cell type specific genetic architecture of C9orf72 disease. This analysis provides a template for single cell-resolution analysis of complex NDs as part of a larger, integrated study across time points and select models of ALS.

Experimental Questions

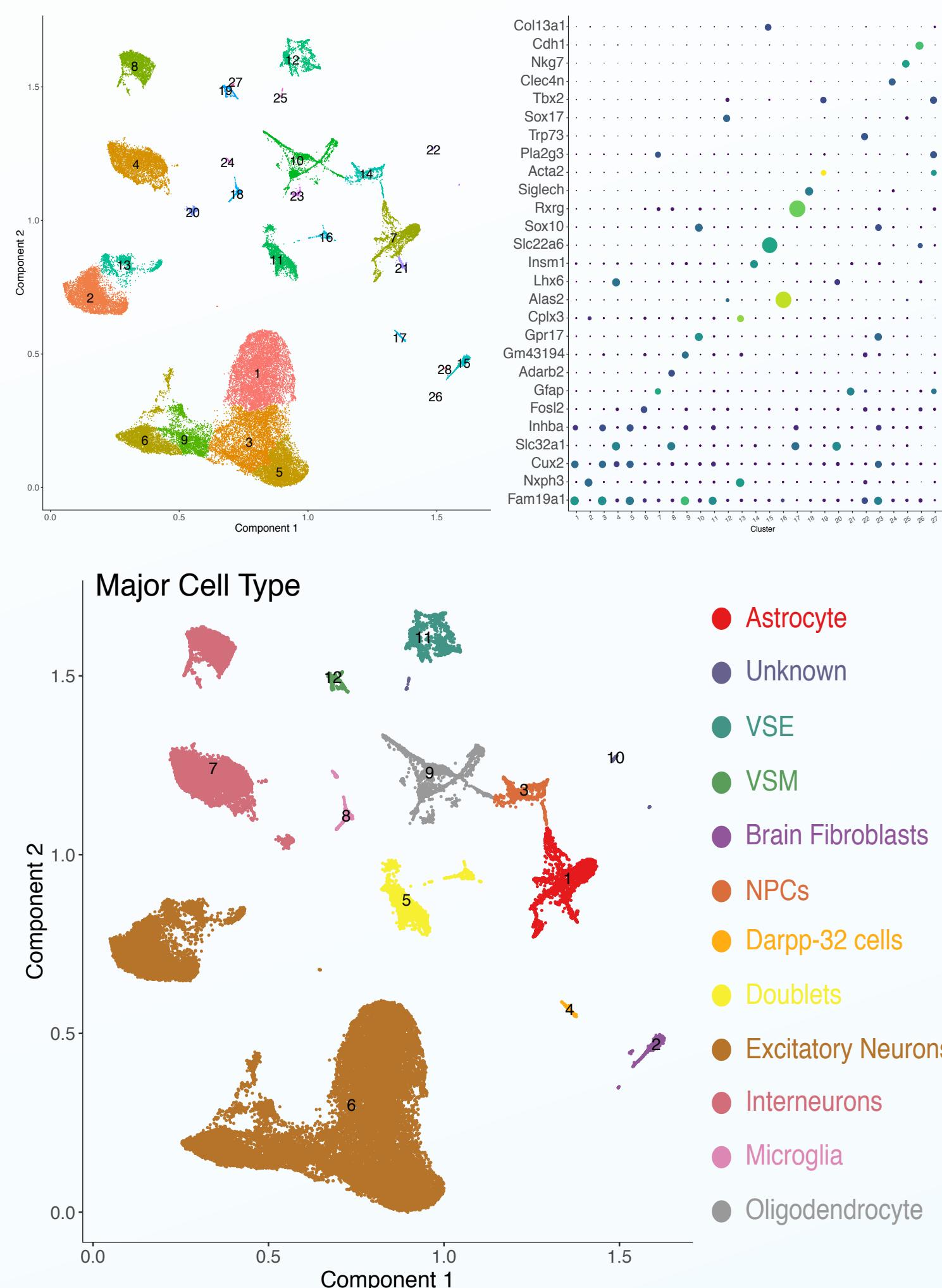
- Can we identify common and divergent effects of pathogenic C9orf72 expression in distinct mouse motor cortex cell types?
- Does C9orf72 repeat expansion affect M1 tissue composition in presymptomatic developmental stages?
- Is there a bias in differentially expressed gene burden across cell types?
- Can we identify DE genes that may contribute to PT hyperexcitability?
- What is the extent of nuclear pore gene expression heterogeneity in the motor cortex?

Cortical Hyperexcitability in a mouse model of C9orf72 disease



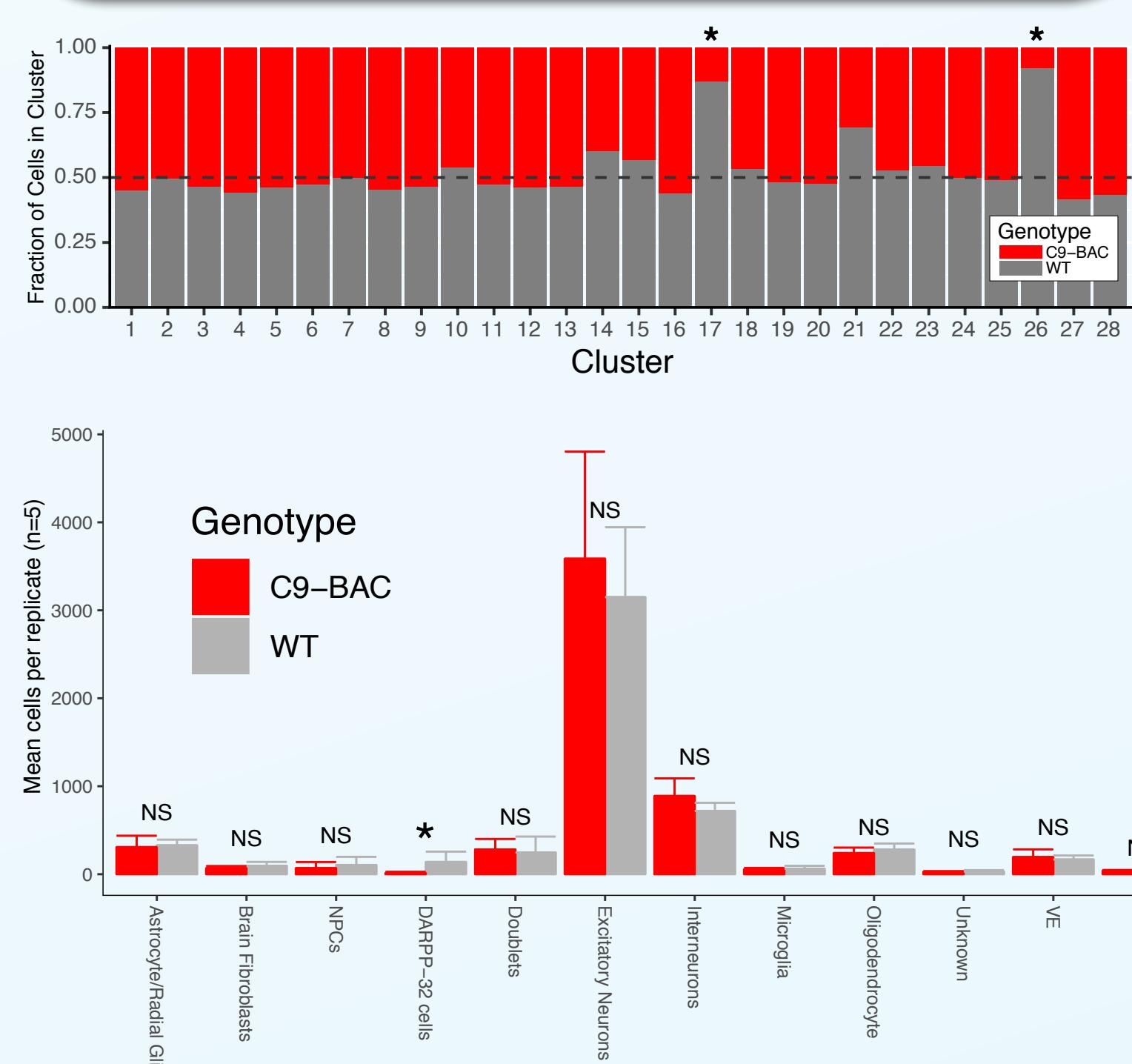
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Annotation of Major Cell Types



Dissected motor cortex from WT and C9-BAC repeat-expanded mice (Liu, et al 2016) were enzymatically dissociated ($n=5$) and processed using the 10x Genomics single cell RNA-Seq platform. Mice were balanced w.r.t. litter, sex, and genotype. 55,371 cells passed QC with a mean of 34,389 UMI per cell and a median of 2,341 genes detected per cell. A subset of high-variance genes (positive residuals to negative binomial fit of coefficient of variation \sim mean copies per cell) was selected, log-transformed, and used as input for PCA. The top 25 PCs were embedded in 2D via Uniform Manifold Approximation and Projection (UMAP). Louvain clustering of the 2D UMAP identified 28 distinct clusters. Clusters were associated with major cell types using a combination of manual annotation and identification of spatially autocorrelated genes via Moran's I test (Monocle3).

Differential Tissue Composition in C9-BAC mice



- All major cell types in M1 are represented
- Most with consistent proportions across genotype
- Two distinct clusters exhibit a significant reduction in the proportion of cells derived from p6-7 C9-BAC mice including the DARPP-32+ cellular cluster (Chi-Sq test for equal proportions; $p < 2.2e-16$)
- A significant reduction in the number of DARPP-32+ neurons in the C9-BAC mouse model at p6-7. (Student's T-test, $p < 0.0231$, $n=5$)

