Analysis of neuronal cell type specific gene expression patterns in the developing cerebral cortex.

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Abstract

Neuronal connectivity patterns determine brain function. The distinct morphologies of neurons in the brain help facilitate neuronal connectivity. To evaluate the molecular mechanisms that control distinct neuronal architecture, we analyzed the neuronal type specific expression patterns of genes that may impact neuronal architecture in the developing cerebral cortex.

Introduction

- Patterns of neuronal connectivity constrain and predispose brain function. Elaboration of distinct morphologies in the developing brain by appropriately positioned neurons establishes the foundation for functional neuronal connectivity. This essential relationship is most robust in the cerebral cortex, the brain region that generates the most complex behaviors.
- Cortical neurons in general can be categorized into two major classes: spiny excitatory (glutamatergic) projection neurons and non-spiny inhibitory (GABAergic) interneurons. The emergence of distinct projection neuron and inhibitory neuron architecture is fundamental to the formation of neuronal connectome (Figure 1).

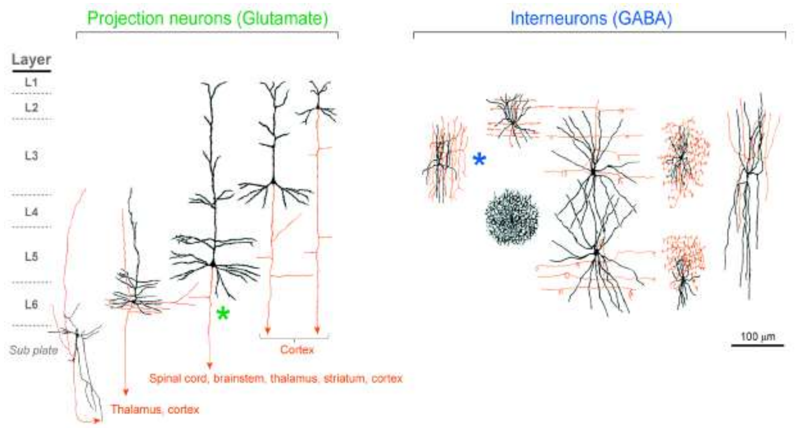


Figure 1. Distinct architecture and connectivity of projection neurons and interneurons. Cortical neurons (shown here for primates) are categorized into two major classes: spiny excitatory (glutamatergic) projection neurons (left panel) and non-spiny inhibitory (GABAergic) interneurons (right panel). Adjacent projection neurons and interneurons display marked differences in the morphology of their dendrites (black) and in the targets of their axonal projections (red). The diversity in morphology and the resultant patterns of connectivity leads to differences in cortical circuitry.

- We want to discern how these neurons that are positioned adjacent to each other within the developing cerebral cortex elaborate distinctly different morphologies. For example, a cortical projection neuron in primates can extend several feet of axons to connect with neurons in the spinal cord, whereas an adjacent interneuron synapsing with it restricts its axonal extension to a range of hundreds of micrometers. Neuronal architecture in essence determines patterns of neuronal connectome. But the molecular logic of neuronal architecture and how it impacts circuit formation are undeciphered.

Methods

- To explore if such mechanisms underlie the differences in the emergence of interneuron and projection neuron morphologies in an unbiased manner, we did the following: (1) analyze publicly available data from RNAseq studies of different classes of CNS neurons for gene expression in Interneurons (INs) and Projector Neurons (PNs) during late embryonic/ early neonatal stages when neuronal architecture is elaborated (Rosenberg et al., 2018, doi:10.1126/science.aam8999; Heavner et al., 2020, https://doi.org/10.1073/pnas.2008013117; Mayer et al., 2018, doi:10.1038/nature25999; Fecher et al., 2019; https://doi.org/10.1038/s41593-019-0479-z)
- Through this screen we created a list of genes that are preferentially expressed in INs and PNs (Fig 1).
- As control, we also identified genes that are selectively expressed in non neural cells (all different types of glia), widely expressed in all different cell types of the brain, and barely expressed in any cell's types of the brain.
- We then analysed Allen scRNASeq, Mousebrain.org databases for the expression of these genes in cortical INs or PNs.

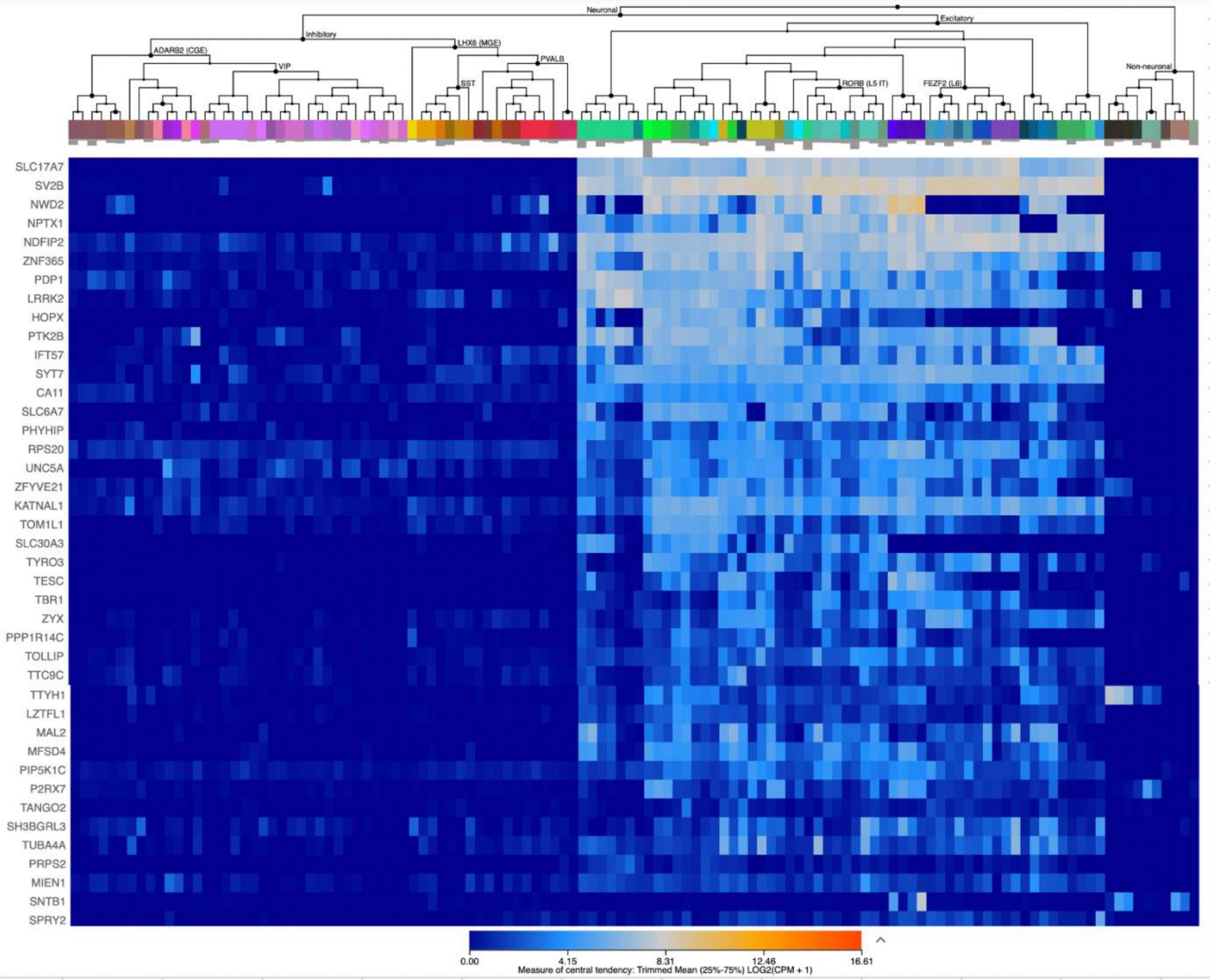
Fig 1. Neuron genes (partial list of 75 genes of a total of 1612 genes)

Projection Neuron Genes	Interneuron Genes	Glial Genes
Myl4	Lhx8	Fam107a
Nxph3	Lhx6	Mlc1
Fezf2	Gbx1	Gfap
Tshz2	Elfn1	Ntsr2
Lypd1	Cacna2d2	Hopx
Cplx3	A230065H16Rik	Ppp1r3g
Nxph3	Zic1	Mfge8
Nr4a2	Cacna2d2	Cldn10
Hs3st4	Arhgap6	S1pr1
3110035E14Rik	Scn5a	Fjx1
Rprm	Ecel1	Atp1a2
3110035E14Rik	Gal	Cobll1
Nxph3	Peg10	Col15a1
Tbr1	Irs4	Enpp2
Hs3st4	Gpr139	Eps8
Fezf2	Vip	Gfap
Nov	Crh	Glul
Coro6	Tac2	Itih5
Kcng1	Cxcl14	Mobp
Lamp5	Adra1b	Opalin
Rxfp1	Vip	Pdgfrb
Fermt1	Myl1	Plp1
Nr4a2	Pthlh	Pon2
Cbln4	Cbln2	Ptn
Cemip	Npas1	S100b
Tshz2	Tac2	Slc1a3
Neurod6	Npas1	Slc2a1
Igfbp6	Rgs12	Slc7a11
Dkk3	Rpp25	St18
Kcns1	Dlx1	Tmem63a
A830009L08Rik	Htr3a	Ugt8
Igfbp6	Npas1	Vcan
Lamp5	Gm8797	Abcg2
Calb1	Tac2	Cldn11
Gm12371	Calb2	Mog
Krt12	Vip	P2ry12
Dkkl1	Htr3a	Ptgds
A830036E02Rik	Myl1	Slc7a1
Rorb	Tac2	Fam107b
Tnnc1	Npas1	Fa2h
Dkkl1	Vip	Etnppl
Tshz2	Htr3a	Clmn
Fezf2	Krt73	Dbi
Npnt	Gm8797	Pax6
Lypd1	Npas1	Tpd52l1
Hs3st2	Yjefn3	Opalin
Scube1	Htr3a	Mal
Myl4	Npas1	Ppp1r14a
Dkkl1	Gm8797	Mog
Pde1a	Krt73	Ninj2
Igfbp6	Col19a1	Cnksr3
Rasl10a	Cnr1	Rras2
Hs3st2	Adarb2	Tmem2
Satb2	Npas1	Il23a
Gm11549	Yjefn3	Tmem163
Nr4a2	Krt73	Tnni1
Oprk1	5330429C05Rik	Cldn14
B3gat2	Egln3	Mag
Sstr2	Adarb2	Mobp
Rgs12	Cnr1	Casr
Wfs1	Ibsp	Adamts4
Vwc2l	Ndnf	Plp1
Lhx2	Cnr1	Mcam
Tacstd2	Ntng1	Mbp
Prkcg	Rgs8	Gm26834
Cbln1	Cplx3	Elovl7
Htr1a	Teddm3	Kif19a
Gpr101	Reln	Nipal4
Nwd2	Cxcl14	Mog
Nr4a2	Ndnf	Myrf
Scube1	Npy	Onecut2
Fam19a2	Cplx3	Cldn11
Reln	Pnoc	Bfsp2
A830036E02Rik	Kit	Ugt8a
Pkib	Lamp5	Cnp

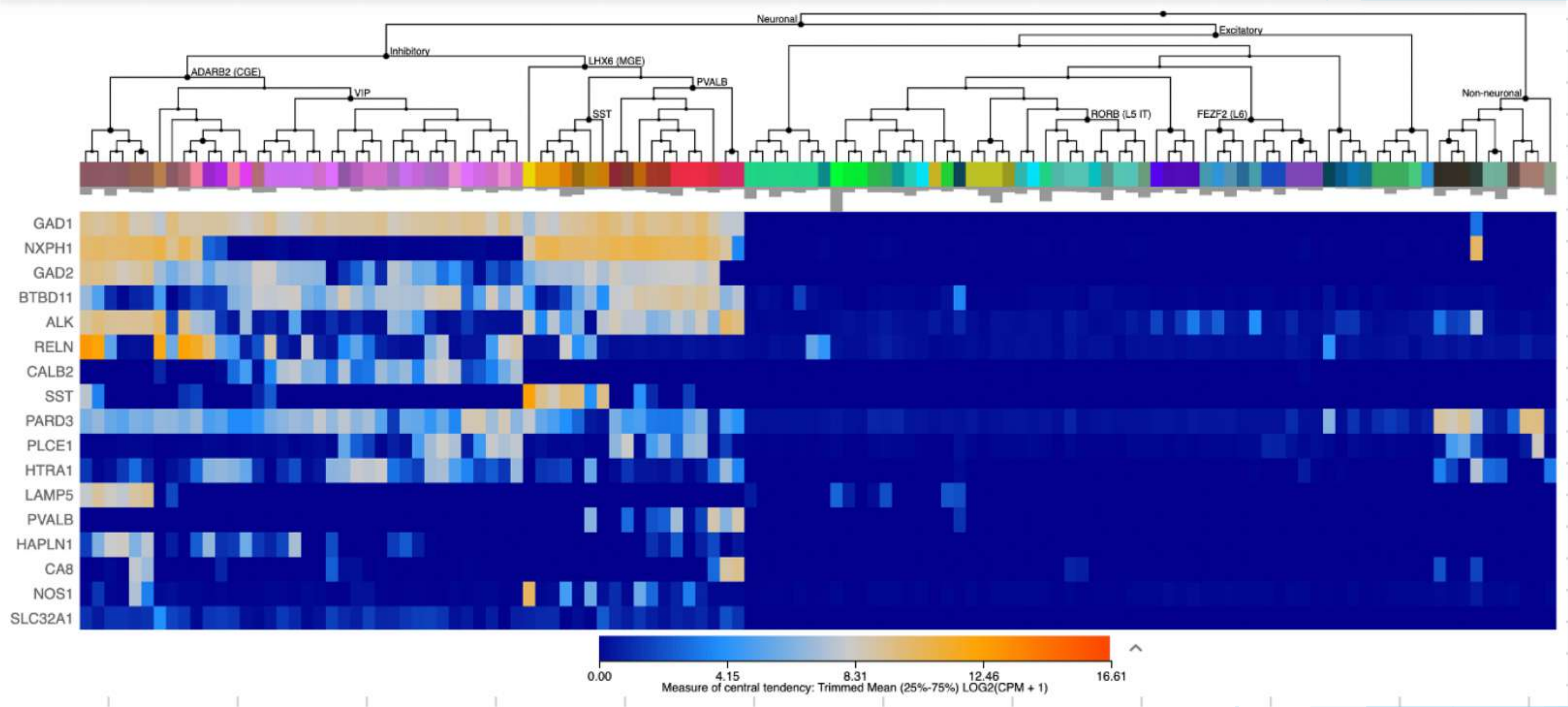
Results

- Using the single cell RNA-sequencing data in Allen Brain Atlas, we were able to generate the gene expression maps for various projector neuron and interneuron genes. Attached below are some of the Human brain gene expression maps.

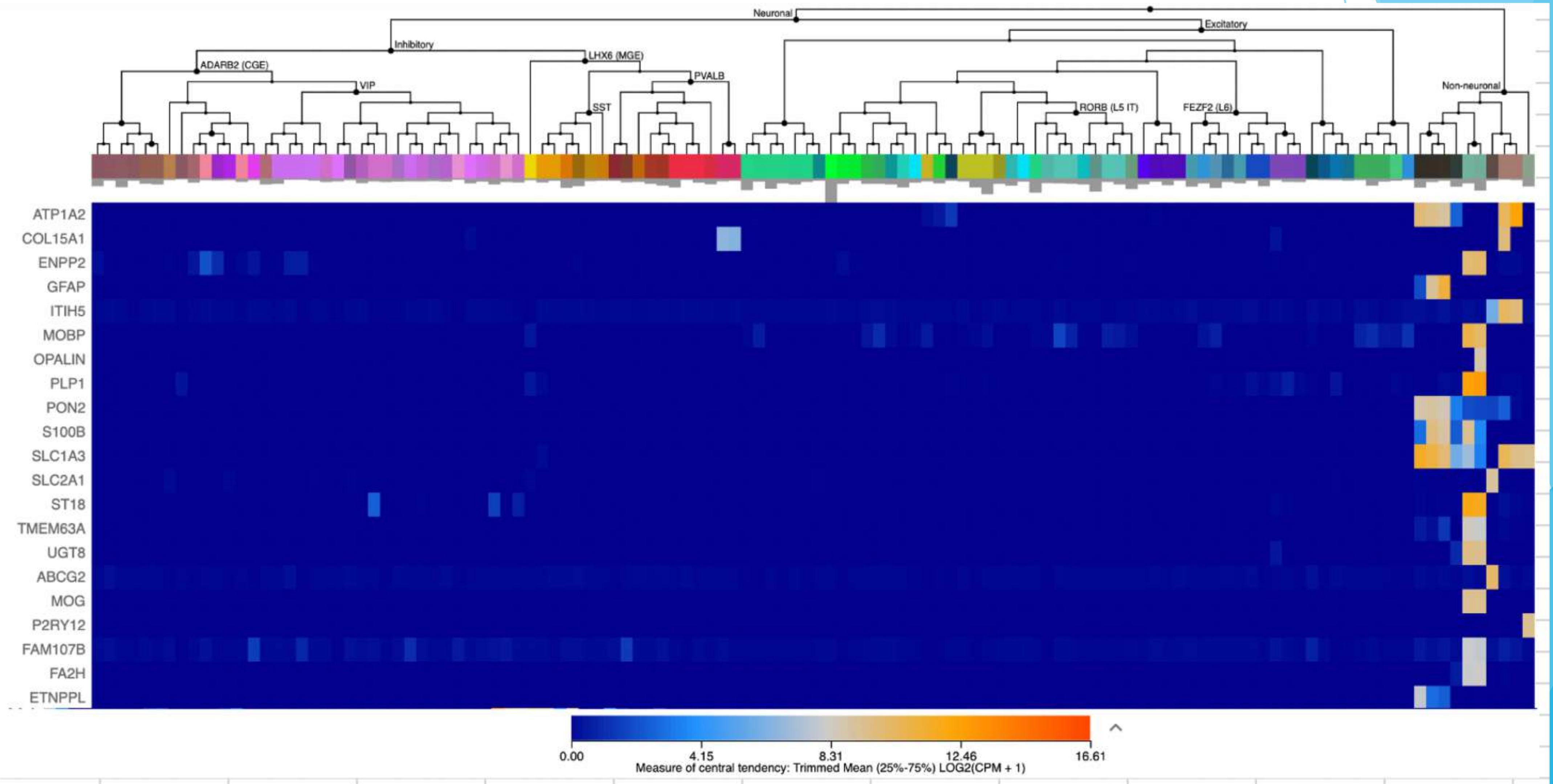
- Here in this gene expression map, we can see that the genes such as SLC17A7, SV2B, NPTX1, and others are preferentially expressed in projection neurons.



This is a gene expression map of some of the interneuron genes.



This is a gene expression map of some of the non neural genes.



Discussion

- Distinct neuronal morphologies in the brain enable its functionality. The neuronal architecture and connectivity patterns ultimately enable the function and fate of neuronal circuits in the brain. By studying the genes that influence projector neurons and interneurons, I was able to analyze the expression patterns of genes that may influence the neuronal architecture during brain formation.
- In this data, Single cell RNA-Sequencing data from developing human cerebral cortex was used to analyze gene expression of interneurons and the projector neurons in the brain.

- The distinct cell type specific expression patterns of the genes in the gene expression maps may indicate if they can affect neuronal architecture and connectivity.
- The impact of these candidate genes in neuronal architecture and neuronal connectivity in the brain could be analyzed in functional studies.
- Knowing these genes and their neuronal expression patterns will help us examine how mutations in these genes expression may ultimately affect the neuronal architecture in humans and mice.
- In the future, we will perform biological pathway analysis (GO term) based on this gene list to identify biological pathways that are selectively active in the developing INs and PNs as a way to identify metabolic or membrane growth/addition pathways that may underlie the elaboration of distinct IN/PN architecture.

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