COLLEGE OF MEDICINE / DEPARTMENT OF NEUROSCIENCE

To DE or not DE: Molecular Signatures for Synapse and Circuit Formation in the Neocortex

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Background

- A prevailing challenge in neurobiology is understanding how diverse neuronal cell types form unique circuits necessary for emergent functional computations.
- Cellular adhesion molecules (CAMs) continue to be explored for their role in synaptic specificity –a neuron's selection of synaptic partners from a variety of often similar choices – and thus mediating circuit organization.
- The advent of high-throughput sequencing technology has advanced computational genomic strategies to profile cells and assess their defining features.
- Major glutamatergic (excitatory) subclasses in the anterior lateral motor cortex (ALM) and primary visual cortex (VISp) have unique global transcriptomic signatures[1]. This suggests that common subclasses may engage in unique local circuits, dependent on cortical region.
- Conversely, GABAergic (inhibitory) subclasses are found uniformly across the cortex[1], suggesting a region-independent integration into local circuits.
- We mined the Allen Institute for Brain Science's single-cell RNA-sequencing (sc-RNA seq) database of mouse cortical neurons to identify molecular signatures of synapse and circuit formation among major neuronal subclasses across the cortex.

Aim

Analyzing differential expression (DE) of CAMs and their regulatory genes, we investigated the extent genetic markers for synapse and circuit formation are distinguished among major classes of GABAergic and glutamatergic neurons across two functionally distinct cortical regions: the ALM and VISp.

Methods

Differential Expression Analysis:

- Merged Allen Institute's sc-RNA seq ALM and VISp datasets, subsetting for GABAergic and glutamatergic cells.
- R package Seurat determined shared and differential gene expression among neuronal subclasses defined by the Allen Institute's metadata.
- Gene ontology (GO) software PANTHER 16.0 classified DE genes by selected terms: "Cell-Cell Adhesion" (CCA), "Regulation of Cell-Cell Adhesion" (RCCA), and "Regulation of Trans-Synaptic Signaling" (RTSS).

Clinical Analysis:

- Using the software ClinVar Miner, variants were selected in ClinVar that are associated with neurological disorders and identified through clinical testing with a review status of at least "single submitter - criteria provided."

Collection and enrichment of neuronal subtypes for sc-RNA seq

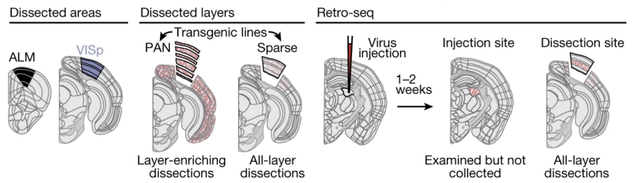


Figure 1: *Transgenically or retrogradely labelled cells and unlabelled cells were isolated in layer-enriching or all-layer microdissections from the ALM or VISp of adult mice (congenic C57BL/6J)[1]*

Comparing global transcriptomic signatures across brain regions and major neuronal cell types

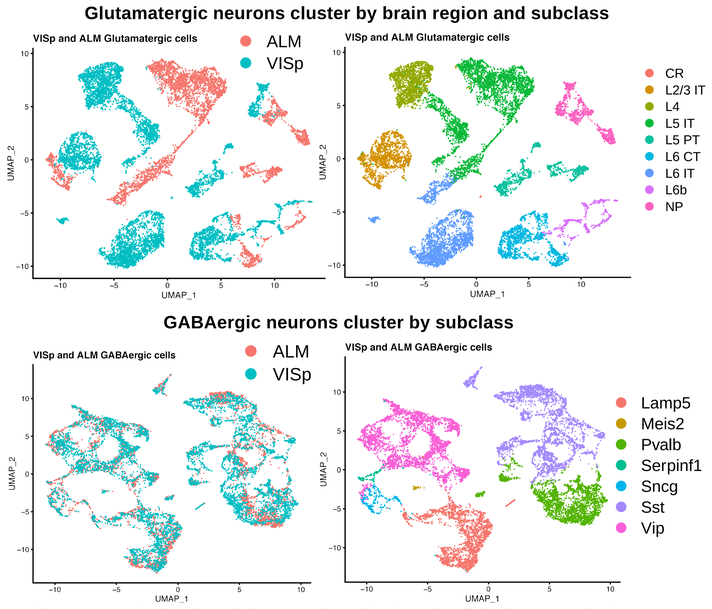
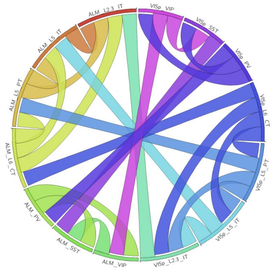


Figure 2: *UMAP clustering of glutamatergic and GABAergic cells in the ALM and VISp, labeled by brain regions (left pane) and subclasses (right pane)*

Differential expression tests between select neuronal subclasses

Figure 3: *Circos plot illustrating differential expression tests conducted for a given glutamatergic or GABAergic subclass between cortical regions and different subclasses within a region*



Null hypothesis – There are no differences between the proportions of differentially expressed genes among major neuronal subclasses across brain regions (depicted in equal thickness of ribbons)

Results

Molecular signatures of circuit architecture are subclass-dependent and brain region-independent

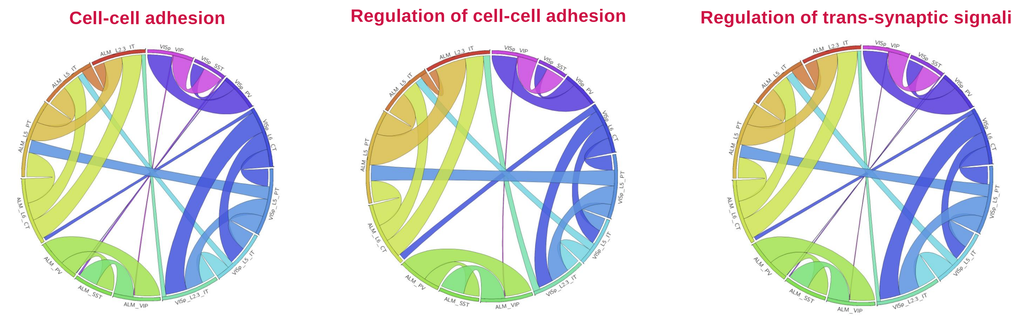


Figure 4: *Circos plots for each GO term with ribbon thickness denoting relative percentages of the number of DE genes over the total number of expressed genes (shared genes + DE genes) within each subclass comparison. No RCCA genes were DE between brain regions for SST and PV cells*

Subclass-specific candidate mechanisms are revealed in patients with neurological disorders

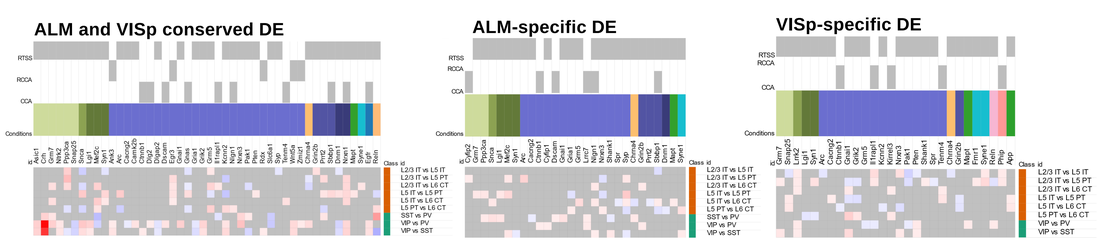


Figure 5: *Variants associated with neurological disorders and identified during clinical testing with comprehensive review were selected to identify clinically relevant CAM-related genes that are DE between neuronal subclasses. Using the software Morpheus from the Broad institute, heat maps were generated based on pooled data from DE tests across brain regions, separated by genes exhibiting a conserved fold change direction of regulation (up/down) in subclasses in both brain regions or genes uniquely DE in subclasses from one brain region.*

Conclusions

- Glutamatergic and GABAergic neuronal subclasses display brain region-independent conservation of gene expression associated with circuit formation. Notable differences between L5 PT cells between regions likely due to projection targets.
- Evidence supporting the presence of subclass-specific canonical wiring principles guiding the organization of circuit motifs.
- With over half of the clinically relevant DE genes sequenced in both glutamatergic and GABAergic cells, this suggests common contributors to perturbations in excitatory and inhibitory signaling.
- The striking observation that many of the DE genes are related to neurological disorders provides candidate mechanisms for investigation of how aberrations in neural circuit components are pronounced in disease states.

References

1 Tasic, B., Yao, Z., Graybuck, L.T. et al. Shared and distinct transcriptomic cell types across neocortical areas. Nature 563, 72–78 (2018). https://doi.org/10.1038/s41586-018-0654-5

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