An Initial Survey of Cell Types in the Central Nucleus of the Amygdala Using Single-Cell RNA-Sequencing to Understand Anxiety

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Why the Central Nucleus of the Amygdala?

Anxiety disorders are highly prevalent and more effective treatments are needed. Neuroimaging studies in humans and nonhuman primates have identified the central nucleus of the amygdala (Ce) as a core component of the neural circuit that underlies anxiety. Studies in nonhuman primates and rodents demonstrate that the Ce is essential for mounting a threat response.

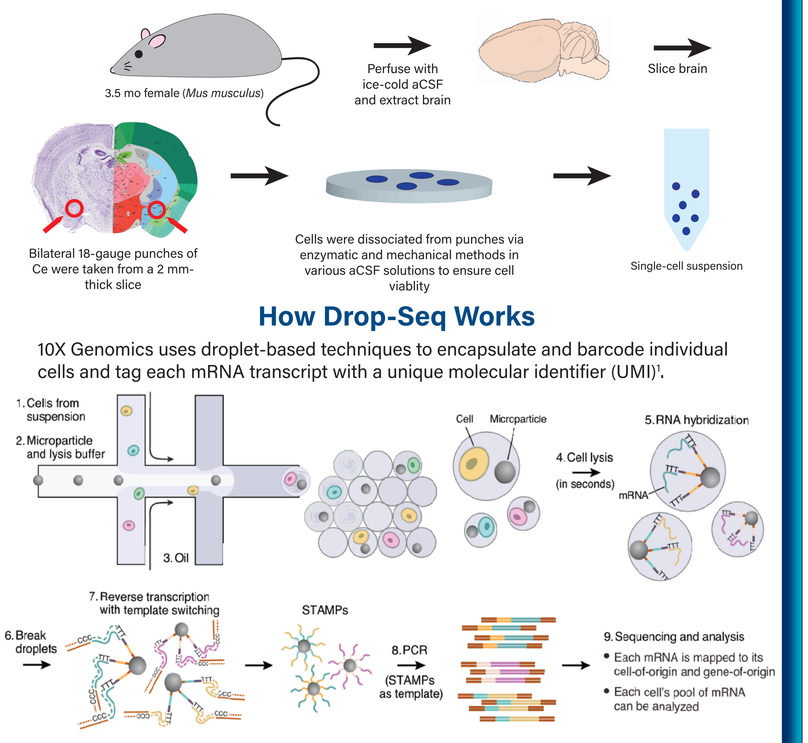
Why Cell Types?

Identifying cell types in the amygdala will contribute to our overall understanding of the neuronal basis of fear and anxiety and provide the foundation for identifying their role in threat processing. Ultimately, our goal is to identify unique molecular targets for distinct cell types within the extended amygdala that can aid in the development of novel treatment strategies to alleviate the suffering of patients with anxiety disorders.

Why Single-Cell RNA-Sequencing?

Understanding an individual cell's transcriptome can help in understanding dynamic cellular states and how gene expression can alter cellular function. Single-cell RNA-sequencing (scRNA-seq) is a powerful tool that allows us to identify individual cell transcriptomes in highly heterogeneous samples which is beneficial for high-throughput genetic analysis at single-cell resolution.

Cell Dissociation

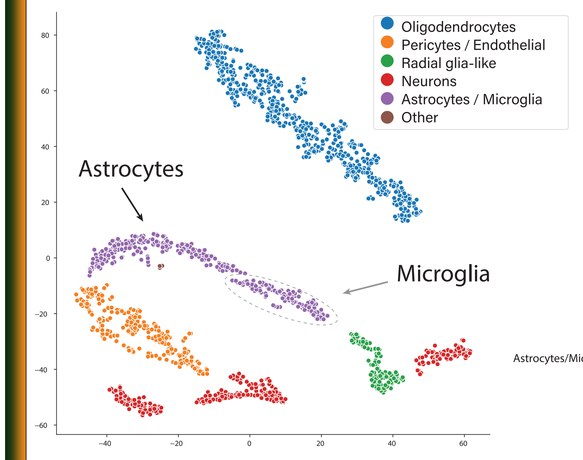


How Drop-Seq Works

10X Genomics uses droplet-based techniques to encapsulate and barcode individual cells and tag each mRNA transcript with a unique molecular identifier (UMI)[1].

Results: Determining Cell Types

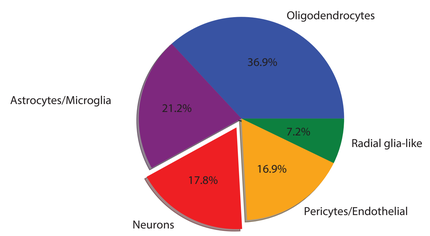
t-SNE of cells



Statistics Summary

Estimated number of cells: 1,542
Median UMI counts per cell: 2,718
Median genes per cell: 1,314
Mean reads per cell: 169,693
Reads mapped to genome: 88.3%

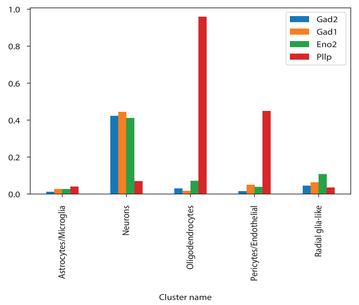
Estimated percentages of different cell types in Ce



What did we get?

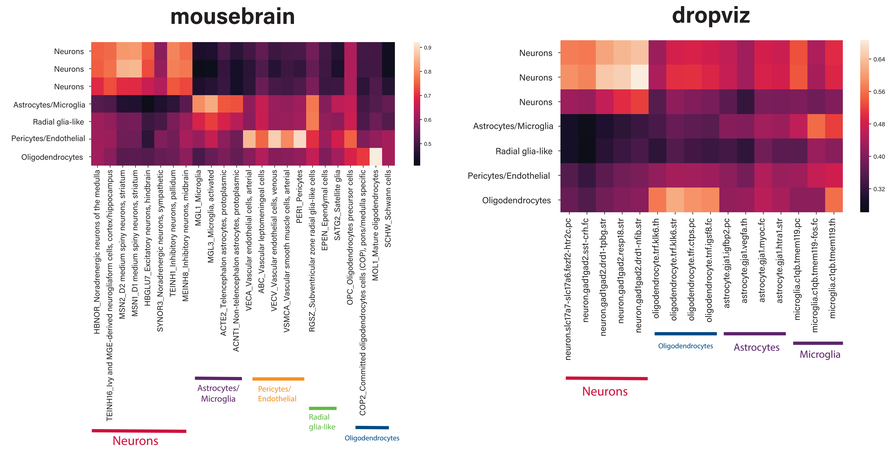
A total of 1,711 high-quality cells from the Ce were reported by the 10X Genomics pipeline. Dropping doublets from the filtered dataset resulted in 1,542 total cells. We created a gene correlation matrix from the filtered dataset then performed t-SNE clustering, resulting in 7 clusters. We then ran DBSCAN, a density-based clustering algorithm, on the resulting t-SNE. **Cell types** were determined based on **expression of known markers** and **correlation with established cell types.**

Expression of known cell-type markers in each cluster



Correlation with established cell types from mousebrain.org and dropviz.org

The heatmaps below show the correlation between our cell type clusters (y-axis) and known cell types from mousebrain.org[2] and dropviz.org[3] (x-axis).



Acknowledgements

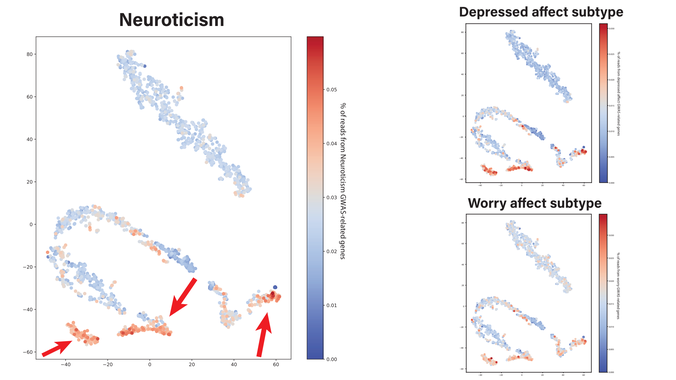
We would like to thank the staff of the UC Davis Genome Center, Biotechnologies & Expression Analysis Core and Bioinformatics Core, and especially Diana Burkart-Waco for her assistance.

References

1. Macosko, E.Z. et al. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell, 161, 1202-1214.
2. Zeisal, A. et al. (2018). Molecular architecture of the mouse nervous system. Cell, 174, 999-1014.
3. Saunders, A. et al. (2018). Molecular diversity and specializations among the cells of the adult mouse brain. Cell, 174, 1015-1030.
4. Nagel, M. et al. (2018). Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways. Nature Genetics, 50, 920-927.
5. Kim, J., Zhang, Muralidhar, S.,LeBlanc, S.A., Tonegawa, S. (2017). Basolateral to central amygdala neural circuits for appetitive behaviors. Neuron, 93, 1464-1479.

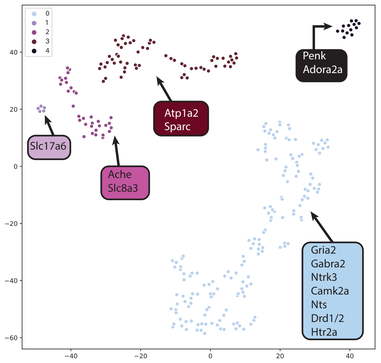
Results: Further Exploration

Neurons in the Ce have more transcripts from genes implicated in neuroticism from meta-analysis of GWAS data



The plots above show percentages of UMIs that are associated with neuroticism, depressed affect, and worry affect-related genes found in a meta-analysis of genome-wide association studies (GWAS) comprised of over 400,000 individuals[4]. As shown, there are higher percentages of UMIs associated with neuroticism, depressed affect, or worry affect in the **neuronal clusters** (indicated by red arrows) relative to the non-neuronal clusters.

t-SNE of neurons

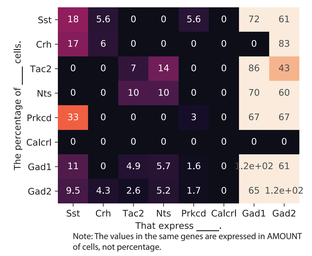


Looking at gene expression in neurons...

We dropped all non-neuronal clusters from our original t-SNE, then performed t-SNE clustering on 274 neurons resulting in 5 clusters. We performed Mann Whitney U tests on each cluster, comparing gene expression between clusters and found differential gene enrichment.

Overlap of peptide gene expression across neurons

In order to determine the percentage of overlap in peptide gene expression between neurons in our sample, we analyzed co-expression of genes previously found in Ce cell types[5]. To complete this matrix, we will enrich for neurons prior to our next round of sequencing, which will provide better representation of peptide gene expression.



Note: The values in the same genes are expressed in AMOUNT of cells, not percentage.

Discussion

The Ce is critically involved in threat responding. This initial study lends insight into the cell types within the mouse Ce. This approach provides the foundation for cross-species studies of Ce cell types. To better understand types of neurons within Ce, we plan to enrich for neurons via immunopanning. These data will be combined with data from nonhuman primates to help bridge the gap between rodent optogenetic studies and the distributed brain alterations characteristic of human psychopathology. As we better understand the how molecular processes within individual neurons contribute to their electrophysiological and morphological features, this approach promises to provide unprecedented insight into the function of primate brain systems. Ultimately, this work promises to further our understanding of the specific molecular and cellular features that contribute to stress-related psychopathology.