**Understanding the Cellular and Transcriptomic Diversity of Neurons in the Paraventricular Nucleus of the Thalamus**

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**Introduction**

Neurons in the brain form trillions of connections to establish brain circuits and achieve various cognitive functions. These connections are not formed randomly. Instead, specific cell types establish connections guided by molecular cues to ensure the circuits are wired properly for their functions. Therefore, to understand brain function in health and disease, it is important to know the cell types in the brain to understand the brain’s architecture. This is especially important for brain regions responsible for higher level cognitive functions such as memory, motivation, emotion, etc. One brain region that emerged recently as an important node for these higher-level cognitive functions is the paraventricular nucleus of the thalamus (PVT), which is located at the midline of the thalamus. Recent studies in mouse and primates show that this region is important for emotional processing, memory consolidation, and contribute to the pleasure feeling of drug addiction. Despite its importance, little is known about the cellular and molecular makeup of this brain region. Using published single-cell transcriptomics dataset, I was able to classify cell types in the PVT and found anatomical organizations of these distinct cell clusters.

**Results**

From the analysis, I was able to identify 16 cell clusters. With previously published thalamus cell type markers, these cell types are annotated. In total, I identified 8 different neuronal subtypes, as well as glial cell types such as astrocytes, oligodendrocytes, microglia, pericytes, and oligodendrocyte precursors. The distribution of these cell types in the dimensionally reduced graph correctly reflected their relative relationships. For instance, neurons are clustered together and are far apart from astrocytes clusters. These clusters also have distinct molecular markers. For instance, one neuron cluster, cluster 0, has the gene Maob as the most differentially expressed gene. Maob is known to express in neurons that express dopamine receptors, and previous studies have identified dopamine receptor neurons in the PVT. The expression of Maob is also highly restrictive and only locates in specific clusters in the dimension reduced plot. With these molecular markers, I dived further into the anatomical organizations of the PVT. Previous studies have hinted that the anterior and posterior parts of the PVT could have different functions. With the identified molecular markers, I used the Allen brain atlas to look at their distribution in the PVT. For neuronal subtypes that express Maob (dopamine receptor expressing neurons) and Calb1 expressing neurons (inhibitory neurons), their distributions are largely segregated. Dopamine receptor neurons are mostly located in posterior PVT, and Calb1-expressing inhibitory neurons are in the anterior PVT. This suggests that previous ideas about functionally segregated PVT regions are possible with different cell type distributions across the nucleus.

**Methods**

The single-cell sequencing dataset and thalamic cell type labels are acquired from previous publications. To perform single cell sequencing analysis, I used the python package Scanpy. The first step to analyze the data is to clean the dataset by removing cells that express too little genes, and genes that are expressed in too few cells. These low-quality data is probably from experimental variations and could skew the results. Then, mitochondrial genes are removed from the dataset due to their uniformly high expression across cell types. Transcript counts and cell number counts are then plotted to make sure there is no abnormal distributions which could contribute variance in cell clustering. Then, the high dimension dataset is reduced using PCA, and the first 20 dimensions are selected for clustering as they covered most variance of the dataset. The dimension reduced dataset is then plotted on a UMAP, where each individual dot is a cell type, and the relative distance between clusters show their relationship. The top 10 most differentially expressed genes are also extracted from each clusters, and their relative abundance is plotted on the UMAP. With the differential gene expression information, one could then search through the Allen institute fluorescent in situ hybridization database to uncover the cell type distributions across PVT.

**Discussion**

This analysis shows that there is diverse cell types in the PVT, and many neuronal cluster could be modulated by different neuromodulators. There are neuromodulator receptors such as dopamine, vasopressin, and oxytocin expressed in the PVT. This information could mean that the PVT is heavily modulated by various neuromodulation systems, and serves various functions. With the identified cell type markers, it is also interesting to look at the cell type and functional segregation between different anatomical subregions of PVT. This resonates with previous ideas that different subregions in the PVT could serve different functions by forming connections between different cell types. This analysis is the first step to understand such molecular and functional diversity and could potentially guide future behavior and physiological studies of different subregions and cell types in the PVT.

**Citations**

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