The gene expression landscape of the human locus coeruleus revealed by single-nucleus and spatially-resolved transcriptomics

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ABSTRACT

Norepinephrine (NE) neurons in the locus coeruleus (LC) project widely throughout the central nervous system, playing critical roles in arousal and mood, as well as various components of cognition including attention, learning, and memory. The LC-NE system is also implicated in multiple neurological and neuropsychiatric disorders. Importantly, LC-NE neurons are highly sensitive to degeneration in both Alzheimer's and Parkinson's disease. Despite the clinical importance of the brain region and the prominent role of LC-NE neurons in a variety of brain and behavioral functions, a detailed molecular characterization of the LC is lacking. Here, we used a combination of spatially-resolved transcriptomics and single-nucleus RNA-sequencing to characterize the molecular landscape of the LC region and the transcriptomic profile of LC-NE neurons in the human brain. We provide a freely accessible resource of these data in web-accessible formats.

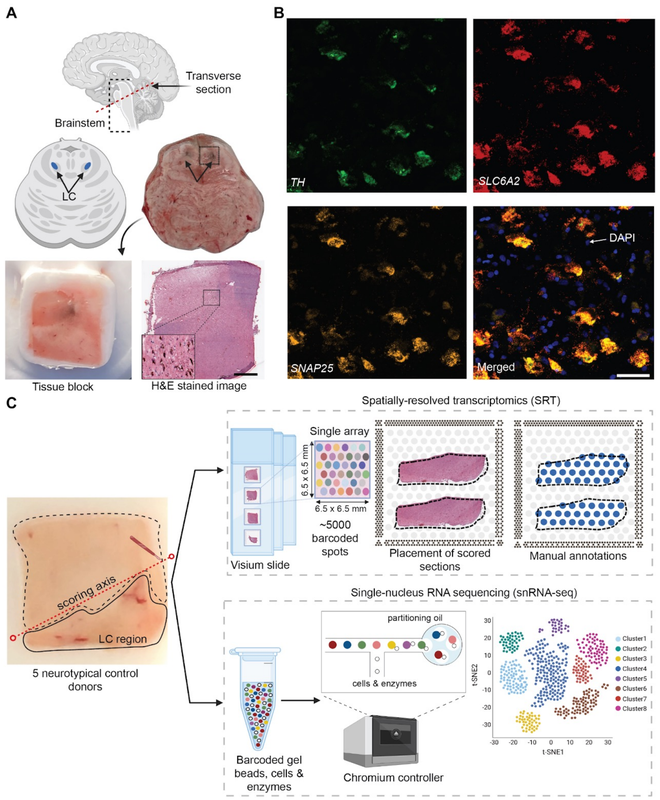
EXPERIMENTAL DESIGN AND QUALITY CONTROL

5 neurotypical adult (age 30-40) human brain donors

Spatially-resolved transcriptomics (SRT) using 10x Genomics Visium platform: 8 samples (Visium capture areas) from 4 donors after quality control

Single-nucleus RNA-sequencing (snRNA-seq) using 10x Genomics Chromium 3' platform: 20,191 nuclei from 3 donors after quality control

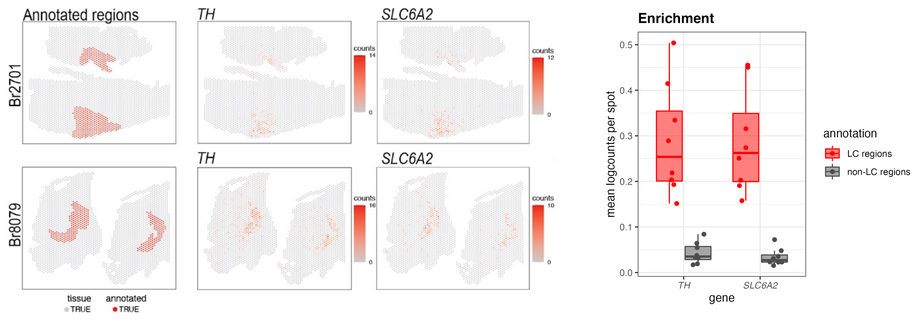
LC identified in SRT samples by neuroanatomical landmarks and presence of neuromelanin pigmented neurons, validated by probing for pan-neuronal and NE neuron-specific marker genes (*SNAP25*, *TH*, *SLC6A2*) by single-molecule fluorescence in situ hybridization (smFISH) using RNAscope



ANNOTATION OF LC REGIONS

Visium SRT data: LC regions identified by manual annotation of Visium spots based on pigmentation, cell size, and morphology from stained histology images, and validated by visualizing and quantifying expression of NE neuron marker genes (*TH*, *SLC6A2*) within annotated LC regions

13 annotated LC regions after quality control (1-3 tissue sections per Visium capture area to maximize use of Visium slides), 124 to 395 Visium spots per annotated LC region



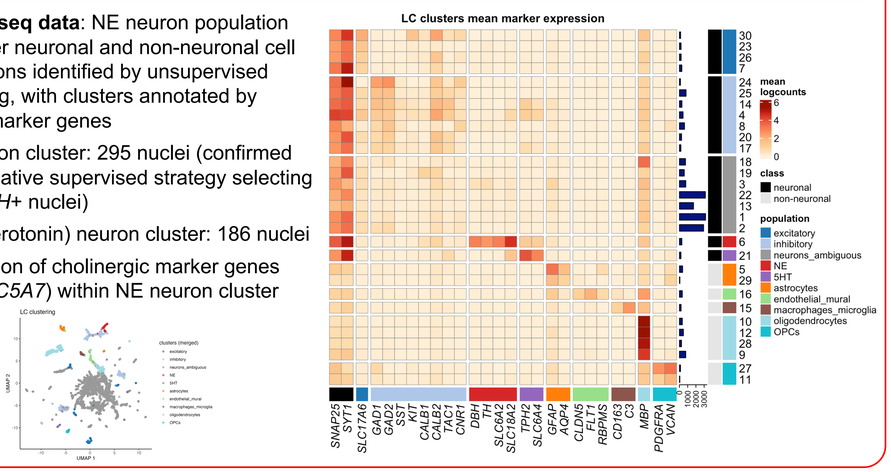
UNSUPERVISED CLUSTERING

snRNA-seq data: NE neuron population and other neuronal and non-neuronal cell populations identified by unsupervised clustering, with clusters annotated by known marker genes

NE neuron cluster: 295 nuclei (confirmed by alternative supervised strategy selecting *DBH+ TH+* nuclei)

5-HT (serotonin) neuron cluster: 186 nuclei

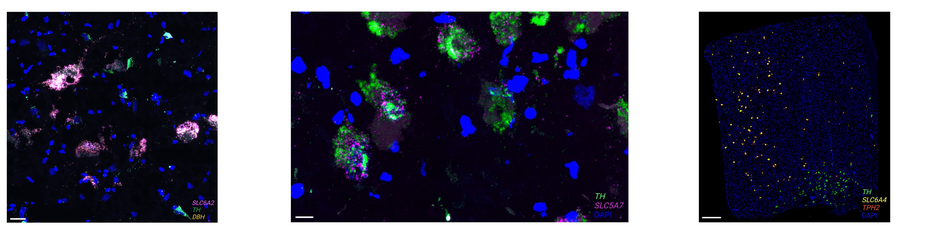
Expression of cholinergic marker genes (e.g. *SLC5A7*) within NE neuron cluster



VALIDATION BY RNASCOPE

Validation of results by smFISH RNAscope and high-magnification confocal imaging

Left: co-expression of NE neuron marker genes within individual cells (scale: 20 µm)
Middle: co-expression of NE neuron and cholinergic marker genes within individual cells (scale: 25 µm)
Right: distribution of expression of NE neuron and 5-HT neuron marker genes across span of tissue section used for Visium sample (scale: 500 µm)



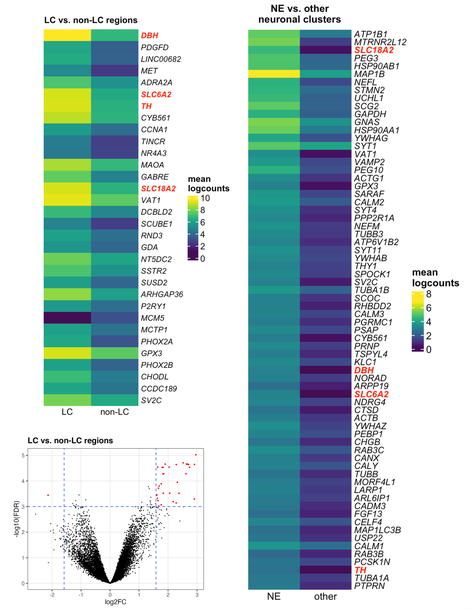
DIFFERENTIALLY EXPRESSED GENES

Identification of differentially expressed (DE) genes associated with pseudobulked (aggregated) data from manually annotated LC regions (**Visium SRT data**, left heatmap and volcano plot) and NE neuron cluster (**snRNA-seq data**, right heatmap)

Full results include 437 and 327 statistically significant DE genes in Visium SRT and snRNA-seq data respectively (see preprint)

Heatmaps display top DE genes ranked by false discovery rate (FDR), with known NE neuron marker genes highlighted (red)

Additional results for 5-HT neuron cluster (**snRNA-seq data**) (see preprint)



ADDITIONAL RESULTS

Comparison with previous studies in rodents using alternative platforms reveals partial conservation of NE neuron-associated genes across species

Identification and characterization of 5-HT (5-hydroxytryptamine, serotonin) neuron population as well as inhibitory neuronal sub-populations in snRNA-seq data

Expression of cholinergic marker genes within NE neuron cluster

Mitochondrial reads affect preprocessing analyses

Additional analyses including spatially-aware clustering, spatially variable genes, and SRT spot-level deconvolution of snRNA-seq populations

LINKS AND DATA RESOURCES

Preprint

- https://www.biorxiv.org/content/10.1101/2022.10.28.514241v1

Data resources

- Web-accessible Visium SRT data: https://libd.shinyapps.io/locus-c_Visium/
- Web-accessible snRNA-seq data: https://libd.shinyapps.io/locus-c_snRNA-seq/
- Downloadable data objects in R/Bioconductor formats: https://bioconductor.org/packages/WeberDivechaLCdata

Code repository

- Reproducible analysis workflows: https://github.com/lmweber/locus-c

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We extend our gratitude to the families and next of kin of the donors for their generosity in supporting and expanding knowledge of the human brain and neuropsychiatric disease.