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Comprehensive Single-Cell Transcriptomic Atlas of the Mouse Pons and Medulla

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The pons and medulla, key regions of the hindbrain within the brainstem, regulate a wide range of complex behaviors, ranging from motor control to autonomic regulation and reflexes. As the central hub of neural projections and gut-to-brain communication, the cellular diversity that supports these functions remains ambiguous. To address this, we integrated eight single-cell and single-nucleus RNA sequencing (sc/snRNA-seq) datasets from mouse brains. We constructed a large-scale single-cell atlas encompassing 318,522 single cells from different sub-regions of the pons and medulla. Using a rigorous metadata standardization and annotation approach, we identified 45 cell types that span major populations and exhibit subtype-specific variability. We observed high diversity among neuronal populations, while non-neuronal cells were dominated by glial and vascular cells, each with unique transcriptional profiles. This atlas serves as the foundational resource for exploring region-specific cellular diversity in the pons and medulla, enabling comparative analyses for future studies.

Background & Summary

The mammalian brain is a complex network of interacting neuronal and non-neuronal populations^{1,2}. It is characterized by the unique morphology of anatomical regions, each involving diverse cell types with specialized functions^{3–5}. Among these, the pons and medulla, key brainstem regions, regulate autonomic nervous activity, motor coordination, and modulation of sensory information^{6,7}. The pons, located above the medulla, act as a bi-directional communication center between the higher and lower brain to transmit the signals^{8,9}. Meanwhile, the medulla, which is located below the pons, connects the higher brain to the spinal cord and regulates vasomotor control functions^{10,11}. Recent studies highlighted their involvement in the progression of Lewy bodies from the gut to the brain, which is the hallmark of Parkinson's disease^{12,13}. However, very little is known about the molecular identification of neural components that are pivotal in understanding these neural circuit properties and disease pathogenesis.

With the development of high-throughput single-cell/single-nucleus RNA-sequencing (sc/sn-RNA-seq) technologies, we have observed a rapid increase in the number of datasets collected from mouse pons, medulla, and their subregions. However, a lack of a large-scale integrated dataset with standardized cell-level metadata has limited comprehensive cellular and molecular characterization. In this study, we have integrated eight sc/sn-RNA-seq datasets comprising 318,522 single cells from mouse pons and medulla subregions. We employed anchor-based mapping to minimize the biological and technical variations across each dataset^{14,15}. However, the majority of cells from the dorsal pons retained region-restricted signatures. We have standardized the metadata, harmonized the data, and identified the 45 cell types and subtypes across the pons and medulla subregions.

Finally, our large-scale integrated dataset can serve as a resource to explore the dynamics of gene expression underlying multi-synaptic communication in the pons and medulla subregions. Our study demonstrates the utility of large-scale integration to explore cellular diversity to understand the biology of brain functions.

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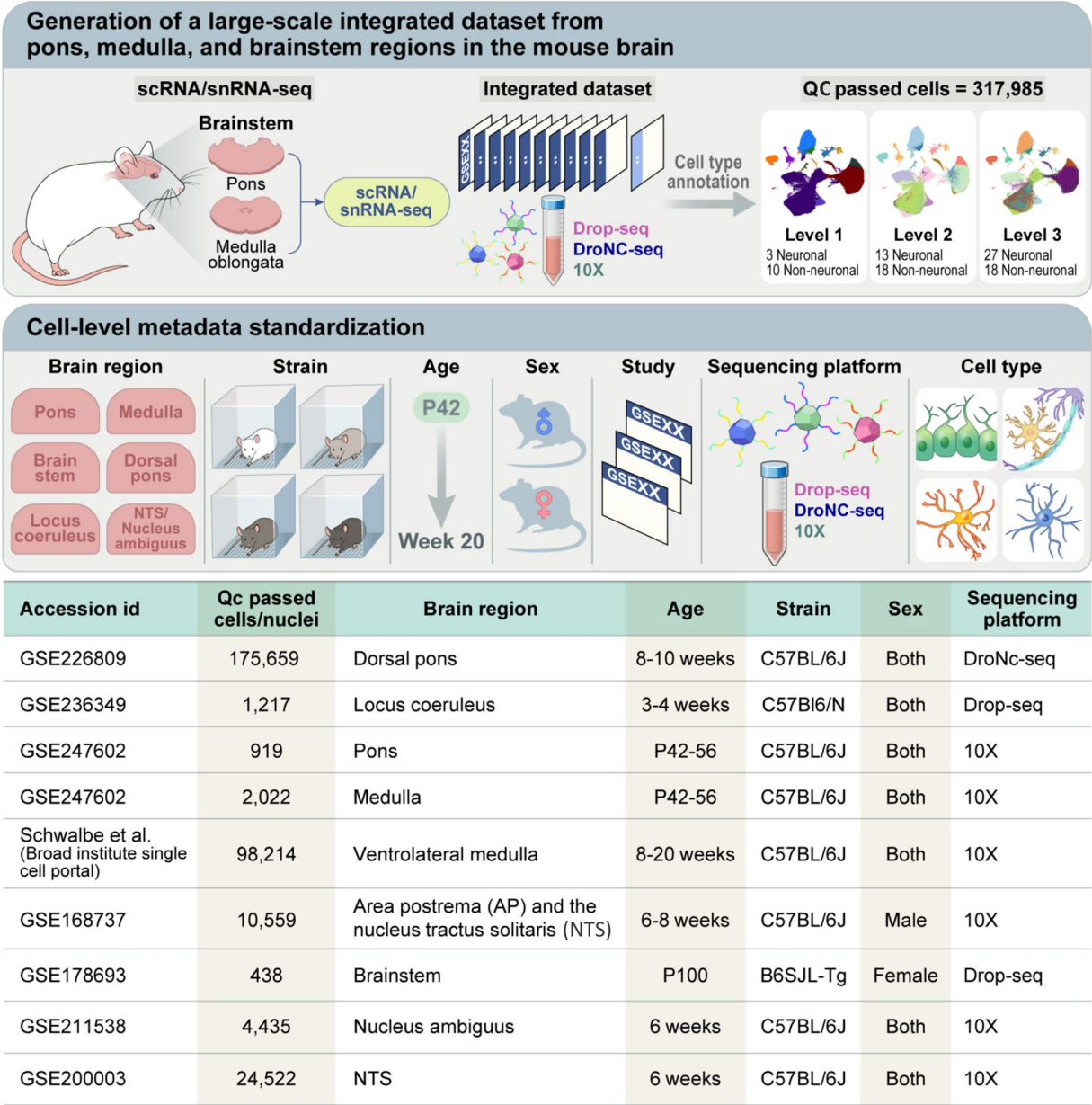


Fig. 1 Schematic representation of the generation of an integrated dataset of the pons and medulla.

Methods

Data collection. We collected eight independent sc/sn-RNA seq datasets from publicly available sources such as the Gene Expression Omnibus database (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>) under the accession numbers GSE226809¹⁶, GSE236349¹⁷, GSE247602¹⁸, GSE168737¹⁹, GSE178693²⁰, GSE211538²¹, GSE200003²¹ and the Broad Institute’s single-cell portal²². Each dataset represents a specific anatomical subregion of the pons, medulla, and brainstem, such as dorsal pons, locus coeruleus (LC), ventrolateral medulla (VLM), area postrema (AP), nucleus tractus solitarius (NTS), and nucleus ambiguus (NA). These datasets were obtained from mice of various strains, including C57BL/6J, C57Bl6/N, and B6SJL-Tg (Fig. 1). Each dataset was selected based on its anatomical relevance and compatibility with the integration workflow. Subsequently, we performed metadata standardization to ensure consistency for each parameter, such as age, strain, sex, and sequencing platform.

Preprocessing and quality control. Data preprocessing was primarily conducted using the R package “Seurat (v4.4.2)”. After merging the raw counts of each dataset, a threshold of 5% mitochondrial content was chosen based on the observed distribution, followed by feature counts over 3000, and less than 200 were filtered out (Supplementary Fig. 1a,b). Considering the quiescent nature of neurons, our filtering approach not only preserves the low transcript counts but also effectively removes poor-quality or damaged cells (Fig. 2a). Subsequently, we performed the dataset normalization using the “NormalizeData()” function, followed by the “FindVariableFeature()” function to identify the top variable genes. Next, the “ScaleData()” function was used

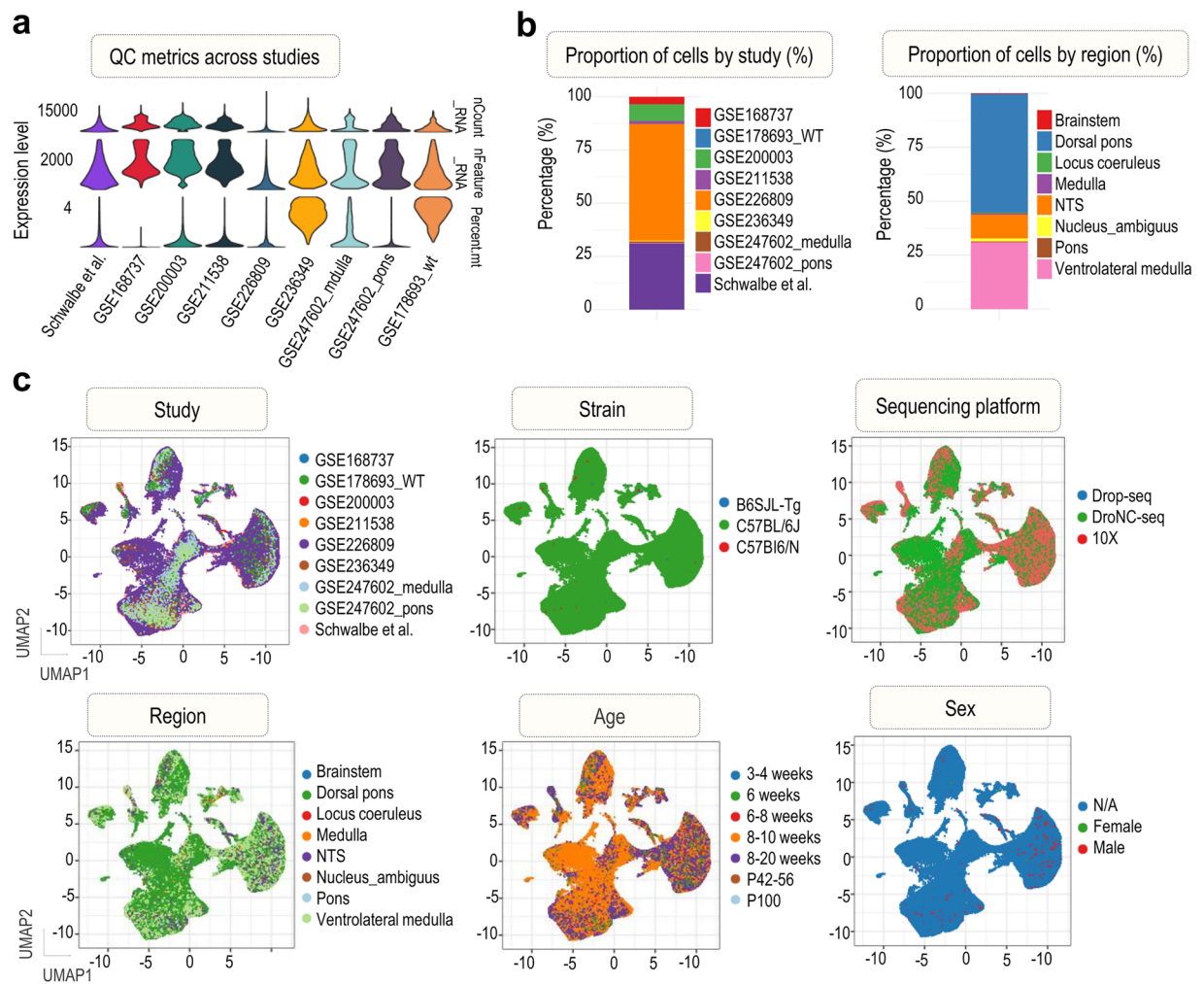


Fig. 2 Overview of data quality and proportion of cells in the final batch corrected integrated dataset.

(a) Violin plots showing the expression level of mitochondrial genes (percent.mt), number of unique genes (nFeature_RNA), and the total count of RNA molecules (nCount_RNA) for each study. The distribution of violin peaks reflects dataset-specific variability in sequencing depth and quality. (b) Barplots showing the proportion of cells derived from each study and mouse brain regions. Datasets GSE226809 (dorsal pons) and Schwalbe *et al.* (Ventrolateral medulla) contributed a high proportion of cell numbers from specific subregions. (c) UMAP plots display the batch-corrected integrated dataset, colored by each metadata standardization, including study, age, brain regions, sequencing platform, sex, and strain. The integration successfully harmonized the technical batch between metadata variables.

to standardize the expression across datasets. Principal component analyses (PCA) were performed, and the number of significant principal components was determined based on the elbow plot (Supplementary Fig. 1c,d).

Data integration and clustering. Next, the “FindIntegrationAnchors()” and “IntegrateData()” functions of Seurat were applied to the merged dataset to find the shared cell population across each dataset based on the transcriptomic similarity. This integration method computes the anchor genes based on their canonical correlation analyses (CCA) space. Finally, a total of 318,522 -QC passed cells were distributed evenly throughout each study and brain region (Fig. 2b). The dimension reduction was performed using Uniform manifold approximation and projection (UMAP) to display the data distribution across key batch-corrected metadata variables (Fig. 2c). In our integrated dataset, GSE226809 contributes most of the region-restricted cell types, specifically profiled from dorsal pontine tegmentum (dPnTg). This high representation likely reflects biological diversity rather than a technical artifact.

Following the integration, we performed clustering analyses to identify the cell-type-specific clusters based on their marker expression. To ensure clear granularity and avoid overlapping of comparable cells, we applied multiple resolutions and selected 0.2 resolution for downstream analyses (Supplementary Fig. 2a). Differential expression analyses were performed using the “FindAllMarkers()” function in Seurat. This function performed differential expression analyses based on the Wilcoxon rank sum test with a log fold change of 0.25 for significance.

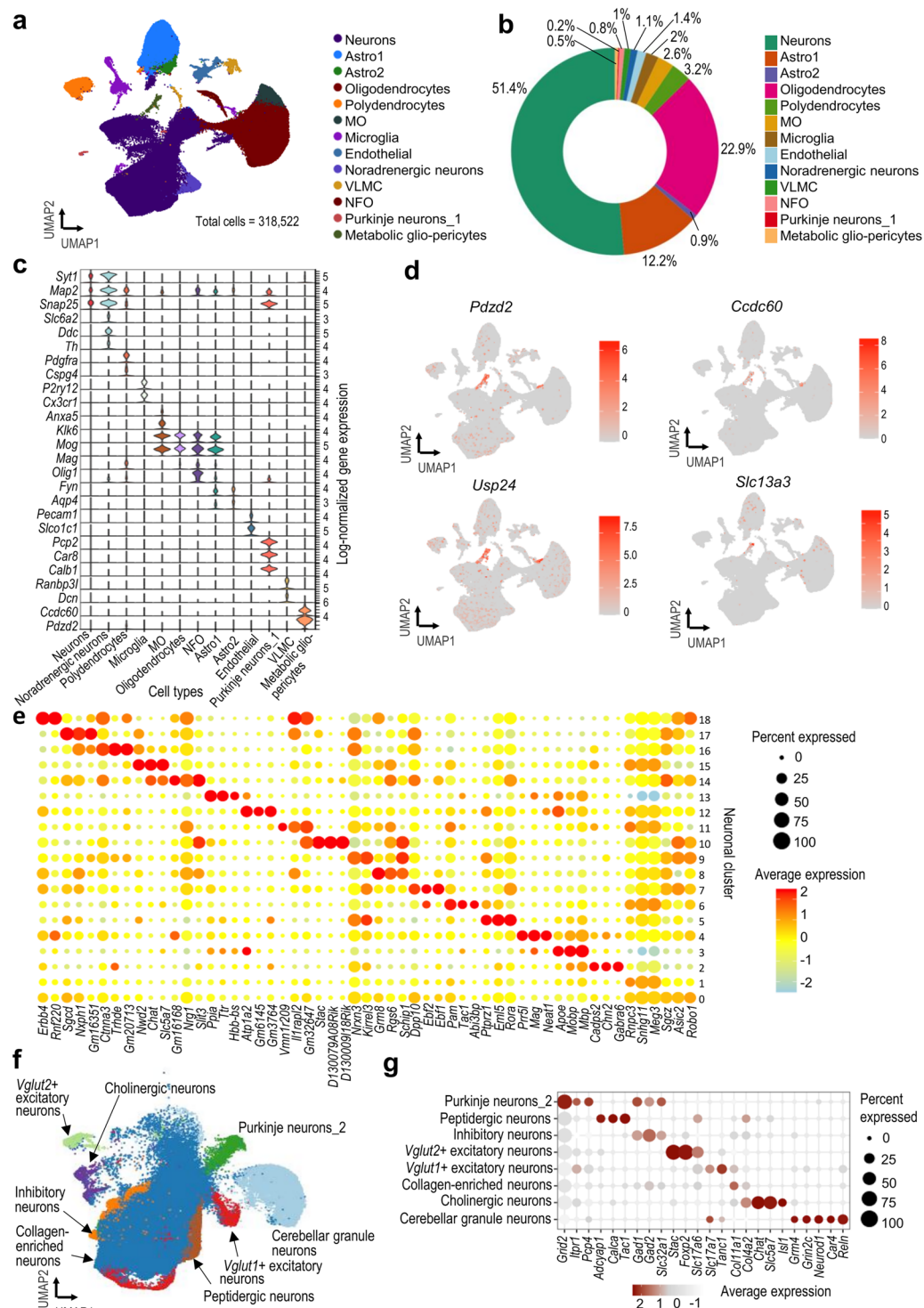


Fig. 3 Major cell type annotation and level 2 subtyping of the neuronal cluster in the integrated dataset. **(a)** UMAP plot showing the level 1 annotation of major cell types. **(b)** The pie chart quantifies the percentage distribution of the major cell types. **(c)** Violin plot showing the expression pattern of cell-type-specific markers used to define each cell type. Statistical significance of marker expression was determined using differential gene expression analysis. **(d)** The feature plots showing the expression of selected genes, revealing cluster-specific metabolic and signaling-related activity. **(e)** The dot plot exhibits a detailed view of the level 2 subtyping of neuronal clusters with their top markers. **(f,g)** UMAP showing the level 2 annotation of neuronal-specific cell types, **(g)** followed by their cell type-specific gene expression.

Identification of major cell types. Based on cell-type-specific markers, we have identified a total of 13 clusters, including three neuronal clusters for level 1 annotation (Fig. 3a,b). These cells include myelinating

oligodendrocytes (MO: *Mag*, *Mog*, *Klk6*)²³, newly formed oligodendrocytes (NFO: *Fyn*)²⁴, and polydendrocytes marked with the expression of *Pdgfra* and *Cspg4*²⁵ (Fig. 3c). We also identified two subtypes of astrocytes (Astro1, 2) expressing *Aqp4* at varying levels²⁶. A study revealed that astrocytes found in the brainstem are involved in modulating the respiratory rhythm and determining the exercise capacity²⁷. Three neuronal clusters were also identified using pan-neuronal genes (*Snap25*, *Map2*, and *Syt1*). Among these, Purkinje neurons_1 show distinguished expressions of *Pcp2*, *Car8*, and *Calb1*. These cells primarily originated from GSE226809, which sampled the region dorsal pontine tegmentum (dPnTg) adjacent to the cerebellum, where rare Purkinje neurons may have been captured due to the proximity of the cerebellar cortex. While noradrenergic neurons were marked with expression of *Ddc*, *Slc6a2*, and *Th*^{28–31}. In addition, we found other distinct clusters readily identifiable as microglia (*Cx3cr1*), endothelial cells (*Pecam1*), and vascular leptomeningeal cells (VLMC: *Dcn*)¹⁵. Microglia found in the brainstem have been shown to induce whisker map plasticity in the downstream thalamus circuit after peripheral injury³². Interestingly, we also found one cluster with specific expression of metabolic and perivascular-related genes such as *Slc13a3*, *Ccdc60*, *Pdzd2*, and *Usp24*^{33–35} (Fig. 3d). Furthermore, *Usp24* has been implicated in Parkinson's disease as part of the *PARK10* locus and was identified as a negative regulator of autophagy³⁶.

As the neurons constitute 51.4% of the total cell type population, we decided to subset the neurons from the integrated dataset for level 2 annotation. Next, we performed the second round of preprocessing using the Seurat package and selected 0.2 resolution for clustering to profile the cells based on their transcriptional similarity (Supplementary Fig. 2b, Fig. 3e). Based on dynamic expression patterns of marker genes, we have identified distinct neuronal sub-clusters, including *Vglut2* + excitatory neurons (*Foxp2*, *Slc17a6*)³⁷, Purkinje neurons_2 (*Grid2*, *Itpr1*, *Pcp4*)³⁸, inhibitory GABAergic neurons (*Gad1*, *Gad2*), *Vglut1* + excitatory neurons (*Slc17a7*), and peptide-expressing subsets such as CGRP⁺ neurons (*Calca*)³⁹ (Fig. 3f,g). We further examined the regional distribution of annotated neuronal subtypes. Interestingly, *Vglut2* + excitatory neurons were enriched in ventrolateral medulla, whereas *Vglut1* + neurons were more broadly distributed across other subregions of pons and medulla (Supplementary Fig. 2c). During the second round of neuronal clustering, we also observed a fraction of other types of cells, such as erythrocytes (*Hbb-bs*), myelinating oligo (oligodendrocytes) (*Mbp*), and astro3 (astrocytes) (*Aqp4/Slc1a2*) (Supplementary Fig. 2d). These cells were removed and excluded from the neuron-only atlas.

Characterization of neuronal subtypes. To further resolve the neuronal subtypes, we performed the level 3 annotation by sub-setting the excitatory and inhibitory neurons from the neuronal subpopulation of level 2. Again, we performed the third round of dimensional reduction and clustering using “FindVariablesFeatures()”, “ScaleData()”, “RunPCA()”, and “RunUMAP()” functions of the Seurat package. A high clustering resolution parameter was used to distinguish the fine population of excitatory and inhibitory neurons. Subsequently, differential expression analyses were performed to identify the markers specific to each subtype (Fig. 4a). Next, we also examined the expression pattern of neurotransmitters such as *Slc17a6*, *Slc17a7* for glutaminergic neurons, and *Gad1*, and *Gad2* for GABAergic neurons^{40–42}. Overall, each subtype can be identified with a unique combination of gene expression at different levels (Fig. 4b,c). Next, we performed the sub-clustering analyses again for collagen-enriched neurons and identified the dynamic gene expression pattern for each subcluster (Fig. 4d,e). Interestingly, we also found expression of adhesion and synaptic organization genes such as *Nrg3*, *Csmd3*, *Tenm2*, and *Ctnna3*^{43–46}. The gene ontology (GO) analyses were performed using the enrichGO function from the R package clusterProfiler (v4.12.6) to associate the collagen-enriched neural subcluster with neuronal migration, cell projection organization, and synapse assembly (Fig. 4g). Then, we mapped the cell barcodes of each neuronal subtype in level 3 to level 2 original dataset of neurons and drew the final UMAP of overall neuron subtypes (Fig. 4h).

Sub-typing of oligodendrocytes. To further classify the oligodendrocyte subpopulations, we followed the same steps used for neuronal subtyping. Following the standardized Seurat's workflow, we have performed the dimensional reduction and sub-clustering to identify the distinct population of oligodendrocytes (Fig. 5a). To validate the oligodendrocytes classification, we checked the expression of key markers such as *Enpp6*, *Sox10*, *Cnp*, and *Qk*, which highlighted the dynamic state of oligodendrocyte differentiation^{47–50} (Fig. 5b,c).

After subtyping neuronal and non-neuronal cell types, we mapped the barcodes of all identified subpopulations to the original Seurat object at level 1 annotation. This large-scale annotation ensures the integration of hierarchical classification. We have shown all major cell types and their subtypes altogether in our final UMAP (Fig. 5d,e).

Data Records

The final integrated dataset named “Final_Integrated_Pons_Medulla.rds.gz” has been deposited to the figshare repository, and it is publicly accessible (<https://doi.org/10.6084/m9.figshare.28342025.v4>)⁵¹. This dataset contains all the neuronal and non-neuronal cell types with cell-level metadata information and serves as the primary reference for this study. It includes the normalized expression data, which can be used to construct quality-control metrics.

In addition, we have also provided the separate files for neurons populations (PM_Neurons_level3.rds) and for excitatory (glutamatergic) and inhibitory (GABAergic) neurons (GABA_Gluta.rds) with level 2 and level 3 annotations. This file contains all neuron cell types with their subtypes and associated metadata. Both datasets are well structured and support the reusability for downstream analyses. In addition, differentially expressed genes (DEGs) analyses were performed detailing the transcriptional differences between clusters at the base level1 has been provided as “DEG_Level1_Pons_Medulla.csv” and for neurons only as “DEG_Level1_Neurons.csv”.

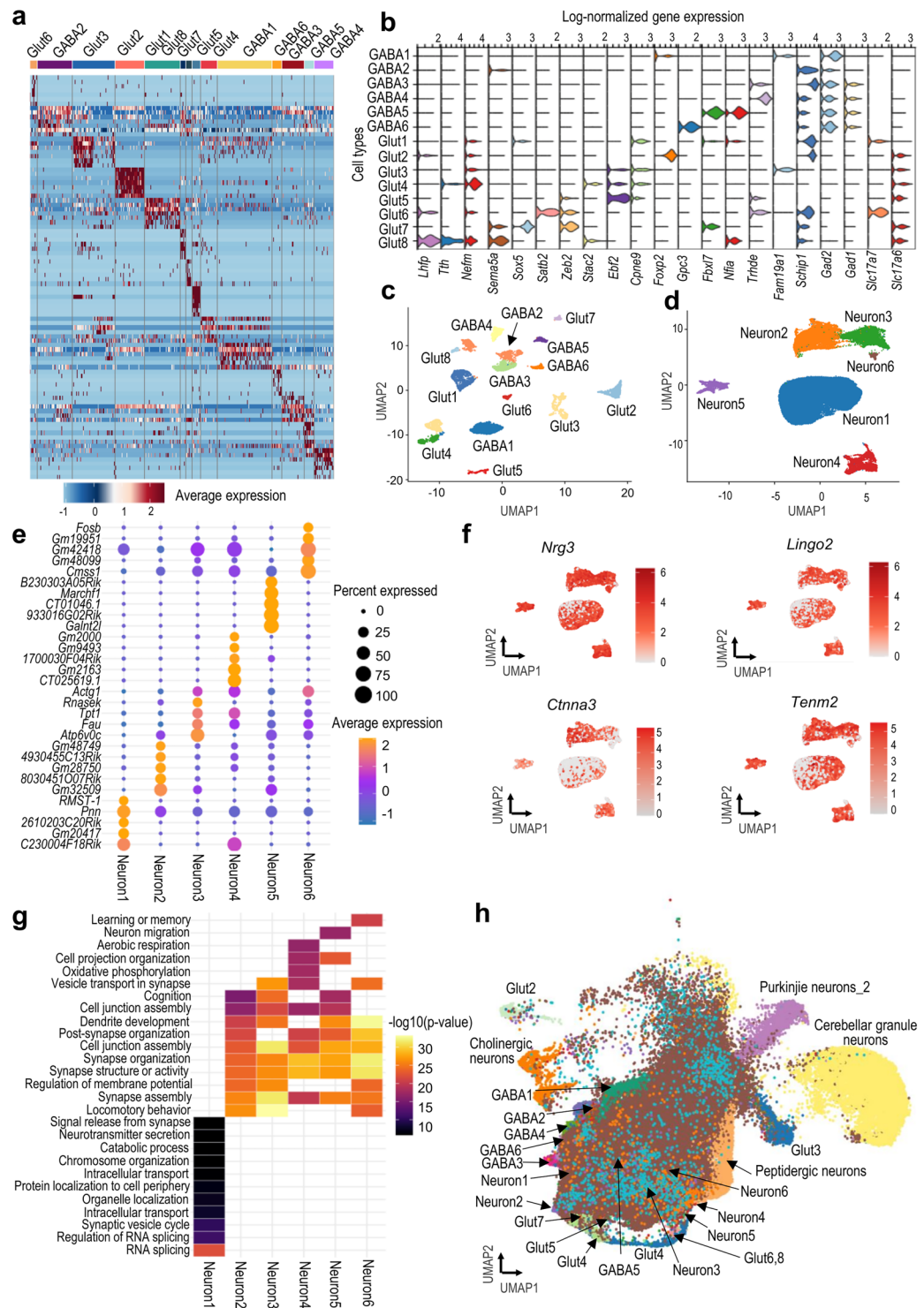


Fig. 4 Detailed sub-clustering of level 3 neuronal cells highlights subtype-specific variability. **(a,b)** Heatmap and violin plot showing the transcriptional diversity within level 3 subtyping of excitatory (glutamatergic; Glut) and inhibitory (GABAergic; GABA) neurons. **(c)** UMAP showing the subtypes annotation of GABA and Glut sub-clusters. **(d)** UMAP showing the annotation for subtypes of collagen-enriched neurons. **(e)** The dot plot highlights the top five markers for each subtype of collagen-enriched neurons; dot size reflects the proportion of cell expression in each marker, while color intensity indicates the expression levels. **(f)** The feature plots showing the expression level of adhesion and synaptic organization gene markers across the subtypes. **(g)** GO results showing the enriched biological processes associated with subtypes. **(h)** The final UMAP plot showing the comprehensive level 2 and 3 subtyping of neurons, revealing the diversity captured within these neuronal cells.

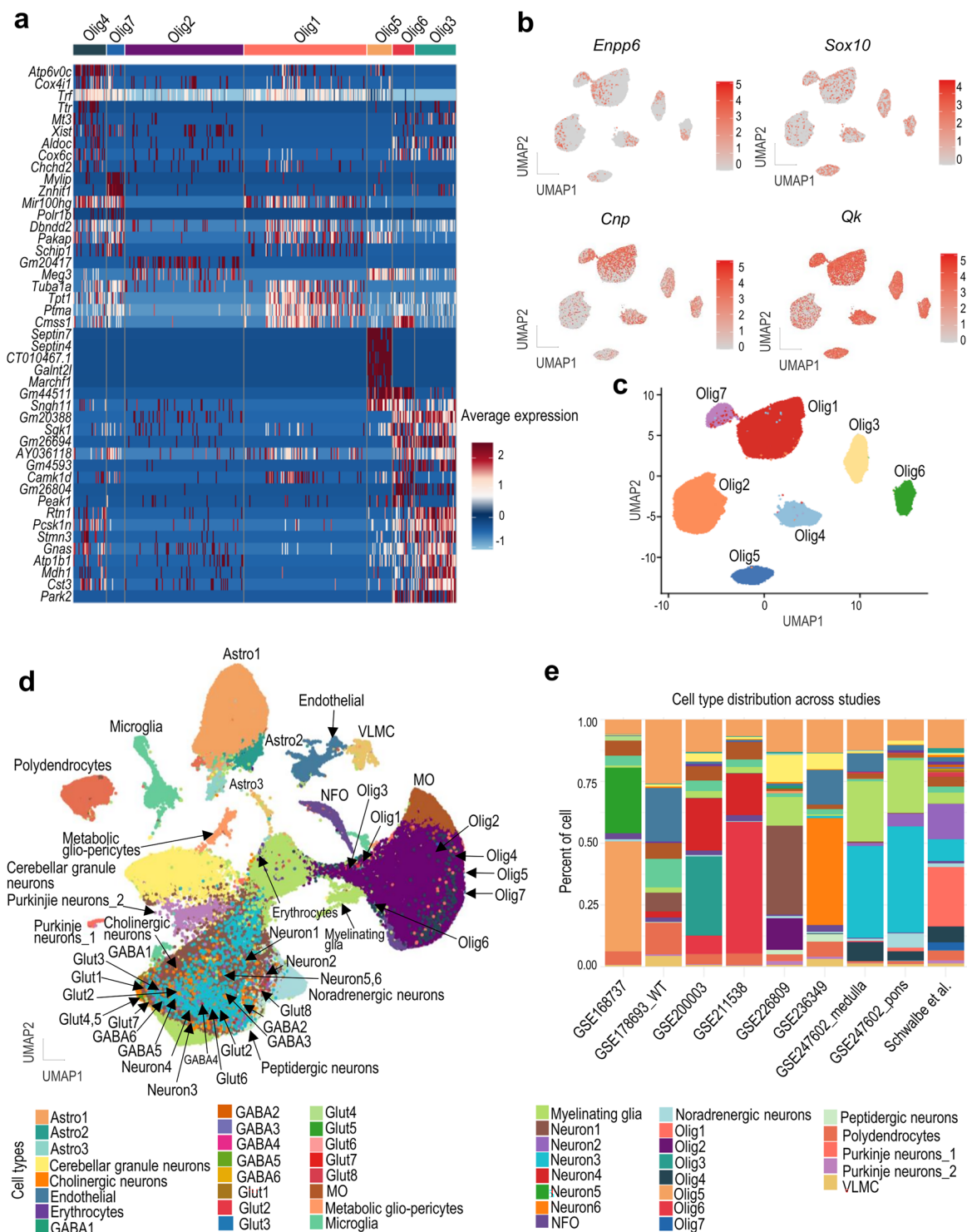


Fig. 5 Level 2 sub-clustering of oligodendrocytes and final annotated integrated dataset. **(a)** Heatmap showing the gene expression dynamics captured by each subtype of oligodendrocytes. **(b)** The feature plots showing the expression of selected genes' dynamic state of oligodendrocyte differentiation. **(c)** Final UMAP plot showing subtypes of neuronal and non-neuronal cell types and their subtypes, **(d)** followed by their proportion in each study.

Technical Validation

To ensure the reliability and accuracy of our integrated dataset, we performed comprehensive quality control (QC) analysis by accessing data integrity across various parameters (See Supplementary Figure S1 for QC metrics and filtering thresholds). Our systematic approach to refining our integrated dataset has not only minimized the technical noise but also retained biologically meaningful variation.

A key metric to assess data quality was the correlation between the number of detected genes (nFeature_RNA) and the total RNA counts (nCount_RNA). For example, before applying for the QC, the correlation score was 0.92, indicating a strong but not optimal relationship. However, following the removal of low-quality cells with excessive mitochondrial contents and extreme outliers, this score increased to 0.98, reflecting data consistency with reduced technical noise (Supplementary Fig. 3a,b). Further, to confirm the validation of data quality, we assessed the key QC metrics such as mitochondrial gene content (percent.mt), (nFeature_RNA), and (nCount_RNA) across each study (Fig. 2a). The consistent distribution of these metrics confirmed the effectiveness of our QC strategy.

Subsequently, to evaluate the efficiency of dataset integration and batch correction, we compared the UMAP and PCA plots before and after integration (Supplementary Fig. 3c,d). Before integration, cells were clustered primarily based on each study-specific variation, representing the batch-driven difference. However, after integration, clusters were aligned based on their transcriptional similarity rather than technical artifacts.

To further assess the integration quality, we computed the integration Local Inverse Simpson's Index (iLISI), which quantifies dataset mixing at the local neighborhood level using the *vegan* (v2.7-1) package in R. Most of the cells exhibited mixed-well cells, supporting the effective batch correction (blue color, Supplementary Fig. 3e). Clusters dominated by GSE226809 (grey) likely representing the region-specific cell types because this dataset was profiled from the dorsal pontine tegmentum (dPnTg). At the level 1 clustering, some clusters, such as 2, 5, and 16, also have a median value of iLISI near 1, consistent with their biological specificity (Supplementary Fig. 3f).

We also evaluated whether feature selection can influence integration by reintegrating the datasets with the union of highly variable genes (HVGs; 10,000) instead of the original 2000 HVGs. Our reintegrated dataset maintained consistent clustering as shown in the heatmap with one-to-one correspondence between cell type clusters from the original and reintegrated dataset (dark diagonal, Supplementary Fig. 3g). This also validates that the choice of feature selection did not affect the identity of cell types. As in the brainstem region, transcriptional changes are subtle, and we also expected moderate silhouette scores, but we want to show that clusters are still well defined (Supplementary Fig. 3h).

Furthermore, we adopted a hierarchical clustering approach to preserve the biological diversity of neuronal subtypes. Most of the clusters at level 1 and neuronal subtypes contain cells from multiple datasets (Supplementary Fig. 4a). Finally, to access the transcriptional profiles of each cell type, we examined the distribution of (percent.mt), (nFeature_RNA), and (nCount_RNA) across each cell type (Supplementary Fig. 4b).

To validate the biological relevance of our annotations, we benchmarked the integrated dataset against the whole-brain atlas by Yao *et al.*, using label transfer⁵². Interestingly, most of the cells mapped to our dataset belonged to the brainstem-related class and subclass annotations from Yao *et al.* Only small fractions of cells were assigned from other brain regions (e.g., midbrain (MB) Glut, hypothalamus (HY) GABA), likely due to differences in anatomical scope (Supplementary Fig. S5). The transferred labels, along with their prediction scores, are also included in our integrated dataset as metadata columns (predicted.Class, predicted.Subclass, predicted.Neurotransmitter). Together, these results support that our integrated dataset preserves region-specific transcriptomic profiles and underscores the robustness and reusability for future downstream analyses.

Usage Note

This large-scale integrated dataset serves as a valuable source to study gene expression dynamics and region-specific cell type diversity in the pons and medulla. The normalized expression with metadata standardization, this dataset can be explored to facilitate a wide range of downstream analyses such as comparative studies with other brain regions, label transfer to other datasets, or investigating specific cell types and their role in any neurodegenerative disease for future studies. We have provided preprocessing scripts and an analysis workflow in our GitHub repository to support reproducibility and enable users to replicate or extend the integration process. Users can download the final integrated dataset and neuronal subset from the Figshare repository, and instructions for loading these into R/Seurat are included in the README file.

Data availability

All datasets analyzed in this study are publicly available under the accession numbers cited in the method section. Final processed and integrated dataset with UMAP embeddings is available at the figshare repository: (<https://doi.org/10.6084/m9.figshare.28342025.v4>)⁵¹.

Code availability

The R script used for data integration, clustering, cell type annotations, and downstream analyses can be found on GitHub, along with a README file that provides step-by-step instructions for reproducibility: https://github.com/Junaid13913/Pons_Medulla_Atlas.

Received: 19 February 2025; Accepted: 6 October 2025;

Published online: 17 November 2025

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Acknowledgements

S.B.L. is supported by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HR22C1734) and the National Research Foundation (NRF) of Korea (RS-2020-NR049588, RS-2020-NR046270, 2022R1C1C1004756). E.J.L. is supported by the NRF of Korea (2022R1C1C1005741, RS-2023-00217595, RS-2025-02217836).

Author contributions

S.B.L. and E.J.L. conceptualized and designed the study. M.J., S.B.L. and E.J.L. analyzed and interpreted the data. M.J. and S.B.L. wrote the manuscript and finalized the figures. All authors edited and approved the final version.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41597-025-06093-3>.

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