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Cerebral cortical neuron diversity and development at single-cell resolution

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Over a century of efforts to categorize the astonishing diversity of cortical neurons has relied on criteria of morphology, electrophysiology, ontology, and the expression of a few transcripts and proteins. The rapid development of single-cell RNA sequencing (scRNA-seq) adds genome-wide gene expression patterns to this list of criteria, and promises to reveal new insights into the transitions that establish neuronal identity during development, differentiation, activity, and disease. Comparing single neuron data to reference atlases constructed from hundreds of thousands of single-cell transcriptomes will be critical to understanding these transitions and the molecular mechanisms that drive them. We review early efforts, and discuss future challenges and opportunities, in applying scRNA-seq to the elucidation of neuronal subtypes and their development.

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Introduction

The classification of cell types in the cerebral cortex has challenged the greatest minds in the history of neuroscience, and so perhaps it is no surprise that we do not quite have it figured out yet. Ramon y Cajal and other early histologists described the two major cortical neuronal types — those with long, distantly projecting axons and those with short, locally projecting axons — and documented their many morphological variations [1]. Brodmann, Campbell, Vogt and others used the distribution of morphological types to subdivide the cortex into cytoarchitectonic areas which we now understand have important functional correlates [2–4]. Yet, whereas classical neuroscientists reached consensus around the neuronal types in brain structures like the cerebellum over a century ago, the effort to develop a comprehensive neuronal 'parts list' for the cortex has lagged. Electrophysiological and circuit analyses arrived in the mid-twentieth century with new tools and the idea that morphological and functional classes of neurons might somehow correspond, though the labor-intensive nature of combining electrophysiology and morphology has limited the ability to integrate form and function. The revolution in molecular biology of the late 20th century allowed an integration of developmental lineage, inferred from the expression of a few marker genes [5], yet still it is not clear whether these criteria can define a clean, non-overlapping 'periodic table' of cortical neuronal types, or whether instead the classification of cortical neurons is inherently less precise than in other brain areas, with a mix of some sharply defined classes and other, fuzzier categories [6,7]. This review will focus on the relevance of single-cell transcriptomics to the classification of cortical neuron subtypes by genome-wide gene expression, and explore the unique perspective afforded by scRNA-seq on the dynamic processes of cortical neurogenesis and differentiation.

Transcriptomic classification of neuronal cell types

Pioneering single-cell microarray and qRT-PCR studies elucidated progenitor and neuronal subtypes in the mouse brainstem [8], olfactory system [9], retina [10,11], inner ear [12], and embryonic cortex [13,14], as well as developing human and ferret cortex [15]. Now, single-cell RNA-seq has opened the floodgates for deep transcriptomic analysis of CNS cell types [16,17••,18–26] (see also recent review by [27]). Although some early scRNA-seq studies have tested specific hypotheses — for example, confirming the 'one neuron-one receptor rule,' that each individual primary olfactory neuron expresses one and only one olfactory receptor gene [28–31] — most have aimed to elaborate on the molecular identities of classically defined neuronal types, discover new types, and begin to establish definitive brain cell type taxonomies (Table 1). These studies employ a generalizable two-stage approach to scRNA-seq-based cell type classification. In the first stage of analysis, single-cell transcriptomes are grouped through

Table 1

Summary of experimental methods and main results for selected scRNA-seq studies classifying cell types in the mammalian brain. UMI, unique molecular identifiers; ERCC, spike-in synthetic control RNAs.

Reference	Cell selection and isolation method	cDNA type	Number of cells used (total cells sequenced)	Source of cells assayed	Sequencing depth (avg or median reads per cell)	Numbers of genes detected (average per cell and/or cumulative) and used for classification	Cell types identified/ classified	Other notes
Kodama [8]	Manual dissection and cell picking	qRT-PCR (3-Prime end)	167 (208)	Mouse medial vestibular nucleus	N/A	59 hand-picked genes	6 neuronal types: 3 excitatory, 3 inhibitory	Further subdivisions likely, but classification correlates with known morphological and functional subtypes
Saraiva [29]	FACS selection followed by Fluidigim C1	Full-length (SMARTer) with ERCC	21 (58)	Mouse olfactory sensory neurons	4.4 Million	4717 detected per cell; 13 582 total; 509 genes found to be differentially expressed between individual neurons	18 known cell types, one confirmed new cell type	Confirmed the 'one-neuron-one-receptor' hypothesis
Uoskin [17••]	Manual dissection and automated cell picking	5-Prime (STRT) with UMI	622 (799)	Mouse dorsal root ganglion	1.1 Million	3574 ± 2010 detected per cell; 12 750 used to ID initial 4 neuronal subgroups & non-neuronal cells; 11 658 used for final iterative clustering of 11 subtypes	11 neuronal subtypes	68 'outliers or unresolved ID' (8.5% of all cells sequenced)
Darmanis [35]	Fluidigm C1	Full-length (SMARTer)	466 (482)	Human adult temporal lobe and fetal cortex	2.8 Million	~4000 detected per cell	7 neuronal types: 2 excitatory, 5 inhibitory; one fetal progenitor class	16 (3.3%) cells excluded for low reads (<400k)
Tasic [33••]	Manual dissection followed by single-cell FACS into microtiter plates	Full-length (SMARTer) with ERCC	1679 (1739)	Mouse (8 wks) primary visual cortex	8.7 Million	7278 detected per cell; 13 878 used for classification	49 'core' cell types: 19 excitatory, 23 inhibitory, 7 non-neuronal; plus 'intermediate' cells w/mixed identity between two or more 'core' types	255 (15.2%) good cells are of 'intermediate' neuronal subtype
Zeisel [32••]	Manual dissection ± FACS selection followed by Fluidigm C1	5-Prime (STRT) with UMI	3005 (3315)	Mouse (3–5 wks) somatosensory cortex & hippocampus	0.5 Million	~4500 detected per cell; 15k total detected; top 5000 by variance used for classification	47 cell types: 7 excitatory neuron, 16 interneuron, 2 astrocyte, 6 oligodendrocyte, and 2 immune classes	310 outlier/poor quality cells (9.4%)