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Genetic approaches to access cell types in mammalian nervous systems

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Understanding brain circuit organization and function requires systematic dissection of its cellular components. With vast cell number and diversity, mammalian nervous systems present a daunting challenge for achieving specific and comprehensive cell type access — prerequisite to circuit analysis. Genetic approaches in the mouse have relied on germline engineering to access marker-defined cell populations. Combinatorial strategies that engage marker intersection, anatomy and projection pattern (e.g. antero-grade and retro-grade viral vectors), and developmental lineage substantially increase the specificity of cell type targeting. While increasing number of mouse cell types are becoming experimentally accessible, comprehensive coverage requires larger coordinated efforts with strategic infrastructural and fiscal planning. CRISPR-based genome editing may enable cell type access in other species, but issues of time, cost and ethics remain, especially for primates. Novel approaches that bypass the germline, such as somatic cell engineering and cell surface-based gene delivery, may reduce the barrier of genetic access to mammalian cell types.

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Introduction

Understanding the organization, function and assembly of neural circuits requires systematic dissection of their basic elements, groups of nerve cells that share similar anatomical and physiological properties, that is, cell types. To reliably identify these cell types — individual nodes of the brain circuit connectome, and to measure and manipulate their activity in the context of behavior, we need a comprehensive toolkit that provides experimental access to a large set of these circuit elements. Indeed, a dream of many neuroscientists is to be able to readily identify, monitor and manipulate every cell type in the circuits that they investigate.

The broad challenges of cell type access are several-fold [1]. The first and foremost is *specificity* at an appropriate granularity. Precise targeting of a cell type in neural circuit analysis is, in a sense, analogous to successful cloning of a gene in genetics — it will provide the clarity in answers that cannot be obtained otherwise. Studying an unknown mixture of cells with non-specific tools is not only uninformative but often generates confusion. The second is *comprehensiveness*. It is highly desirable to be able access most if not all cell types in a given neural circuit to discover its precise organization and operation. The third is *systematic* coverage. With a vast number of cell types across brain regions, it is necessary to build broad collections of tools to cover many of these brain systems. The fourth is *robustness* of toolgenes (i.e. markers, sensors, transducers, among others) that allow easy and effective observation and manipulation.

Not surprisingly, the ease of accessing cell types is often correlated with the complexity of the nervous system and brain region in genetic model organisms (Table 1). In the round worm *C. elegans* with 302 neurons of exactly 118 types, thousands of cell type transgenic lines are available and each neuron type is covered on average by ~32 transgenic lines [2]. In *Drosophila melanogaster* with a total of $\sim 10^5$ neurons in the adult brain, over 2×10^4 driver lines have been generated to cover cell populations in most brain regions, with increasingly number of intersectional lines that target highly specific cell types [3]. And there are ample examples where every cell type in a brain circuit is recognized and targeted [4]. Together these cell type tools bestow unparalleled experimental versatility and have transformed the study of worm and fly neurobiology.

With over 10^8 neurons of vexing diversity and an unknown number of types in the mouse and most mammalian brains (Table 1), the challenge of cell type access is not only technical but also conceptual — the very definition of neuron type in many brain regions is often contentious [1,5,6]. Recent advances in single cell analysis, especially single cell genomics, present unprecedented opportunities for understanding, discovering, and accessing cell types in the mammalian brain.

Table 1

Comparison of cell type tools across several model organisms.

Species	Neurons	Neuron types	Cell type drivers	Drivers/cell type or population[a]
C. elegans	302	118	$>10^3$	~30 (4–150)
Drosophila	$\sim10^5$	Likely thousands	$\sim4 \times 10^4$	Many cell types have multiple drivers
Mouse	$\sim10^8$	Unknown	$\sim5 \times 10^2$	1 and none for most
Rat	$\sim10^8$	Unknown	$\sim2 \times 10^1$	None for most
Macaque	$\sim10^{10}$	Unknown	None yet	None
Marmoset	$\sim10^9$	Unknown	None yet	None

[a] The numbers include those that are specific to a cell type and those that are expressed in a cell type/population as well as in other cell types/populations.

Furthermore, programmable nucleases-based site-specific genome editing techniques, such as clustered regularly inter-spaced short palindromic repeats-Cas9 (CRISPR-Cas9), transcription activator-like effector nuclease (TALEN), and zinc-finger nucleases (ZFNs), facilitates germline and somatic engineering in broader mammalian species for cell access, especially in primate brains with orders of magnitudes more neurons ($\sim10^9$ in marmoset and $\sim10^{10}$ in rhesus macaque). Here we highlight recent progress in cell type tools in the mouse, consider the requirement and prospect of more broad and comprehensive cell access in this genetic model organism, discuss the opportunities and challenges in other mammalian species, and call for innovation of novel approaches in parallel to germline engineering.

Cell transcriptomes provide unprecedented opportunities for discovering and targeting cell types

Molecular markers are the starting point for genetic access to cell populations defined by gene expression. Until recently, cell type markers in the mammalian brain were very sparse and were mostly discovered in a serendipitous and piecemeal manner. This situation has fundamentally changed in the past two years, with innovations in massively parallel single cell and single nuclei RNA sequencing (scRNAseq, snRNAseq). Thousands to millions of single cells are sequenced in multiple brain regions and species [7•,8,9•,10•,11•,12,13•]. Unsupervised statistical clustering have identified increasing number of 'transcriptional types' with distinct expression profiles, many may serve as single or combinatorial markers.

Furthermore, new generation of mRNA *in situ* techniques such as multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) [14•] and *in situ* transcriptome profiling (seq-FISH) [15•,16••] enable cellular resolution spatial detection of dozens to hundreds mRNAs. These methods promise to reveal the precise spatial localization of transcriptional cell clusters, an important step toward identifying cell types. Together, the rapid accumulation of markers for transcriptional cell types and their spatial distribution pattern provide unprecedented opportunities for genetic access. Indeed, an increasingly restrictive bottleneck is the generation of recombinase driver lines and their characterization. It should be made clear though that progress in single cell transcriptomics by itself does not and cannot address the fundamental issue of how to define a cell type, a problem that can only be addressed by multi-faceted analyses of orthogonal cell features, which require reliable experimental access.

Toward an overarching and mechanistic definition of cardinal neuron types

Nerve cells are, in a quite real sense, individual microorganisms living in a highly connected brain cell society. They manifest multi-modal and multi-dimensional phenotypes that are extraordinarily difficult to describe and measure. These include morphology, connectivity pattern, physiological properties, gene expression profiles, developmental history, and ultimately circuit function. A fundamental conceptual and technical challenge is establishing an overarching and mechanistic framework of cell type identity that integrates multi-modal cell phenotypes. Combining genetic targeting, high-resolution single cell transcriptomics and computational analysis, a recent study discovered that the transcriptional architecture of synaptic communication delineates cortical GABAergic neuron identities [17••] (Figure 1). This architecture comprises six categories of ~40 gene families including cell adhesion molecules, transmitter-modulator receptors, ion channels, signaling proteins, neuropeptides and vesicular release components, and transcription factors. Combinatorial expression of select members across families shapes a multi-layered molecular scaffold along cell membrane that may customize synaptic connectivity patterns and input–output signaling properties. Transcriptional signatures of synaptic communication may integrate anatomical, physiological, functional and developmental genetic features that together define neuronal identity. This discovery provides an overarching and mechanistic framework for cell type definition, discovery, and cataloging. Future studies will evaluate whether this synaptic communication scheme apply to the definition of other neuron types, especially projection neurons whose input–output connectivity constitutes basic circuit elements of information processing and relay in global networks and brain systems [18]. Several methods begin to link

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