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Review

What is a cell type and how to define it?

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SUMMARY

Cell types are the basic functional units of an organism. Cell types exhibit diverse phenotypic properties at multiple levels, making them challenging to define, categorize, and understand. This review provides an overview of the basic principles of cell types rooted in evolution and development and discusses approaches to characterize and classify cell types and investigate how they contribute to the organism's function, using the mammalian brain as a primary example. I propose a roadmap toward a conceptual framework and knowledge base of cell types that will enable a deeper understanding of the dynamic changes of cellular function under healthy and diseased conditions.

INTRODUCTION

A cell is the basic unit of all living organisms (except for viruses) (Mazzarello, 1999). The evolution from unicellular to increasingly complex multicellular organisms involves multiplication of individual cells as well as groups of cells and diversification of the function of cells. As such, billions of years of evolutionary process leads to the vast array of species whose diverse biological attributes are built upon their cellular compositions that exhibit similarities and differences both between species and among different organs within an individual organism (e.g., an animal or a plant). Thus, understanding the organization and function of cells within an organism lays the essential foundation for understanding how an organism works. Similarly, comparing the organization and function of cells between species allows understanding of functional diversity across species.

Studies over the past century have revealed that cells within an organism can be grouped into types—cells within a type exhibit similar structure and function that are distinct from cells in other types (Arendt, 2008). Categorizing cells into types greatly reduces the complexity of investigating the organization and function of cells, especially for large organisms with billions to trillions of cells in the body, e.g., mammals. Researchers have measured a wide range of cellular properties and used these measurements to classify cell types (Petilla Interneuron Nomenclature Group et al., 2008; Regev et al., 2017; Zeng and Sanes, 2017). However, there has not been a consistent and standard definition of cell types, although it is critical for reproducible investigation. It is often unclear if cell types defined by different phenotypic features agree with each other nor which feature is the *right* one to define cell types. Furthermore, lacking a systematic approach and effort, we do not know if all the cell types in an organism have been identified and where gaps may be.

Recent advent of single-cell transcriptomics with its unprecedented depth and scalability is revolutionizing the way we understand cell types. It has been used to define cell types in a variety of species, tissue organs, and brain regions (Armand et al., 2021; Svensson et al., 2020; Tanay and Sebé-Pedrós, 2021). However, despite many illuminating studies, it remains an open question to what extent transcriptomic clusters represent true cell types and what level of granularity is appropriate for defining cell types. Nonetheless, over the past few years, tremendous progress has been made and many new insights have been generated around these questions. In this review, I will mainly use the mammalian brain as an example (but also refer to other organs or species) to address key questions pertaining to the conceptual and operational definition of cell types.

APPROACHES TO CHARACTERIZE CELL TYPES

Cell types in the brain and the body exhibit diverse properties in many modalities—molecular, morphological, physiological, and functional. Numerous studies in these different modalities in the brain over the past century, dating back to Ramón y Cajal and his contemporaries, have converged on a consistent high-level picture of cell type organization across brain regions (Fishell and Heintz, 2013; Huang and Paul, 2019; Markram et al., 2004; Masland, 2012; Mukamel and Ngai, 2019; Nelson et al., 2006; Petilla Interneuron Nomenclature Group et al., 2008; Sanes and Masland, 2015; Seung and Sümbül, 2014; Somogyi and Klausberger, 2005; Yuste et al., 2020; Zeng and Sanes, 2017). At the same time, cellular properties at individual cell level are highly heterogeneous, and variations in different modalities do not necessarily exhibit high degrees of concordance, making it often impossible to define exactly what is a cell type and draw clear boundaries between *types*. In many cases, lacking a way to reproducibly label a cell type (typically using a molecular genetic approach) presents a major hurdle to relate different studies and findings to each other.

To untangle this complexity, it is necessary to adopt approaches that provide comprehensive, unbiased, quantitative, and standardizable measurements and are scalable to densely sample a sufficient number of cells within a brain region or tissue organ as well as across the entire brain and body to eventually reach *completeness*, and then perform data-driven computational clustering and

analysis to obtain cell type classification. The Petilla convention to define criteria for defining cortical interneuron types represents a significant community effort to specify such approaches (Petilla Interneuron Nomenclature Group et al., 2008). Given that physiological properties can take many different forms under different conditions and functional properties are unknown or poorly defined for many types of cells, as well as the fact that these two modalities are better examined *in vivo*, it is challenging to scale up the physiological and functional approaches, such as *in vivo* electrode recording or functional imaging, in a comprehensive and unbiased manner as a primary way to define cell types. On the other hand, molecular and anatomical approaches are more suited for this purpose (Figure 1A). Molecular approaches include the profiling of RNA transcripts (transcriptomics), chromatin modifications (epigenomics), and proteins (proteomics). Anatomical approaches include the characterization of the spatial distribution, morphology, and connectivity of individual cells. Currently, single-cell transcriptomics and connectomics (i.e., delineating the patterns of interconnections between individual neurons) are two primary approaches that have the potential to meet the completeness requirement. Both approaches are now being realized in simpler model organisms including *C. elegans* (Taylor et al., 2021; White et al., 1986; Witvliet et al., 2021) and *Drosophila* (Hulse et al., 2021; Li et al., 2022; Scheffer et al., 2020; Zheng et al., 2018), whereas in mammals, transcriptomics is currently feasible and connectomics is still in development (Abbott et al., 2020).

Transcriptomics by single-cell or single-nucleus RNA-sequencing (scRNA-seq or snRNA-seq) is now the most widely used approach to generate cell type taxonomies or atlases from many species, tissue organs, and brain regions, due to its comprehensiveness and high dimensionality (i.e., profiling thousands of expressed genes per cell in a largely unbiased manner) as well as its high scalability (to hundreds of thousands or millions of cells). Transcriptomic cell atlases at the whole organism level have been generated for *Drosophila* (Li et al., 2022), Ciona (Cao et al., 2019a), and the nervous system of *C. elegans* (Taylor et al., 2021). The Human Cell Atlas community effort aims to create cell atlases for all organs in the human body (Lindeboom et al., 2021; Regev et al., 2017). The BRAIN Initiative cell census effort has the goal of creating high-resolution whole-brain cell type atlases for mouse, human, and non-human primates (Brain Initiative Cell Census Network, 2021; Ecker et al., 2017; Ngai, 2022). A variety of transcriptomic cell atlases have been generated in mouse from many different regions of the nervous system, such as cortex, hippocampus, striatum, thalamus, hypothalamus, cerebellum, spinal cord, and retina (Cembrowski et al., 2018; Hashikawa et al., 2020; Kozareva et al., 2021; Macosko et al., 2015; Marques et al., 2016; Phillips et al., 2019; Pool et al., 2020; Poulin et al., 2014; Ren et al., 2019; Romanov et al., 2017; Russ et al., 2021; Sathyamurthy et al., 2018; Saunders et al., 2018; Shekhar et al., 2016; Stanley et al., 2020; Tasic et al., 2018; Van Hove et al., 2019; Yao et al., 2021a, 2021b; Zeisel et al., 2015, 2018), as well as body organs (Han et al., 2018a; Jaitin et al., 2014; Tabula Muris Consortium et al., 2018), and increasingly more in human and non-human primates (Bakken et al., 2021; Darmanis et al., 2015; Drokhlyansky et al., 2020; Garcia et al., 2022; Han et al., 2022; Hodge et al., 2019; Kamath et al., 2022; Lake et al., 2016; Masuda et al., 2019; Tabula Sapiens Consortium et al., 2022; Winkler et al., 2022; Yang et al., 2022).

Single-cell epigenomics, such as single-nucleus ATAC-seq (to characterize chromatin accessibility) or DNA methylation-sequencing, has also been used to generate cell type atlases for different brain regions that are consistent with transcriptomic cell atlases and further reveal cell type-specific gene and chromatin regulatory landscapes (Cusanovich et al., 2018; Lake et al., 2018; Li et al., 2021; Liu et al., 2021; Luo et al., 2017; Preissl et al., 2018; Yao et al., 2021a). Spatially resolved transcriptomics, including a variety of techniques based on *in situ* imaging, *in situ* capture, or *in situ* sequencing (Close et al., 2021; Larsson et al., 2021; Lein et al., 2017; Moses and Pachter, 2022; Rao et al., 2021; Zhuang, 2021), is a powerful approach combining molecular and spatial characterization at single-cell or near-single-cell level, revealing spatial relationships between cell types in both local environment and global architecture (Chen et al., 2021; Moffitt et al., 2018; Ortiz et al., 2020; Rao et al., 2021; Wang et al., 2021; Zhang et al., 2021a). Other attributes in the transcriptomes, such as alternatively spliced variants, can provide further information and help to refine cell types (Booeshaghi et al., 2021). An area awaiting critical technology development is single-cell proteomics (Cho et al., 2022; Slavov, 2021), as the expression and subcellular distribution of proteins provides a crucial link between gene expression and cellular structure and function, and it may not have lock-step correlation with the transcriptome of the same cell type.

A cell's morphology (i.e., shape) and connectivity (especially for neurons) has been regarded as the most defining feature of brain cell types since the era of Cajal, although its place may be overtaken by transcriptome (for reasons detailed below). A cell's morphology can be reconstructed from high-resolution light microscopy (LM) datasets (coupled with colorimetric or fluorescent sparse labeling) (Gao et al., 2022; Jenett et al., 2012; Peng et al., 2021; Winnubst et al., 2019; Wolff and Rubin, 2018) or electron microscopy (EM) datasets (Hulse et al., 2021; Scheffer et al., 2020; Seung and Sümbül, 2014; Zheng et al., 2018). Connections among individual neurons can be identified using approaches such as EM (Gour et al., 2021; Helmstaedter et al., 2013; Hildebrand et al., 2017; Hulse et al., 2021; Morgan et al., 2016; Scheffer et al., 2020; Schneider-Mizell et al., 2021; Turner et al., 2022; Witvliet et al., 2021), single-neuron *trans*-synaptic tracing (Schwarz and Remy, 2019), and barcoded connectomics (Chen et al., 2019; Clark et al., 2021; Gergues et al., 2020; Han et al., 2018b; Kebschull et al., 2016; Sun et al., 2021). Again, for definitive cell type classification, a fully representative, rather than partial and biased, set of morphological and connectional features is required. In this regard, with the acquisition of whole-brain EM connectomic and LM morphological datasets in *Drosophila*, refined cell type classification in the brain of this species has been primarily driven by morphology and connectivity (Hulse et al., 2021; Jenett et al., 2012; Scheffer et al., 2020; Wolff and Rubin, 2018).

Most critically, these various approaches need to be integrated to achieve a coherent understanding of cell types and their function and to resolve issues such as which approach(es) (e.g., between transcriptomics and connectomics) can define cell types more clearly. The most common type of integration is to relate transcriptomic profiles with other modalities.

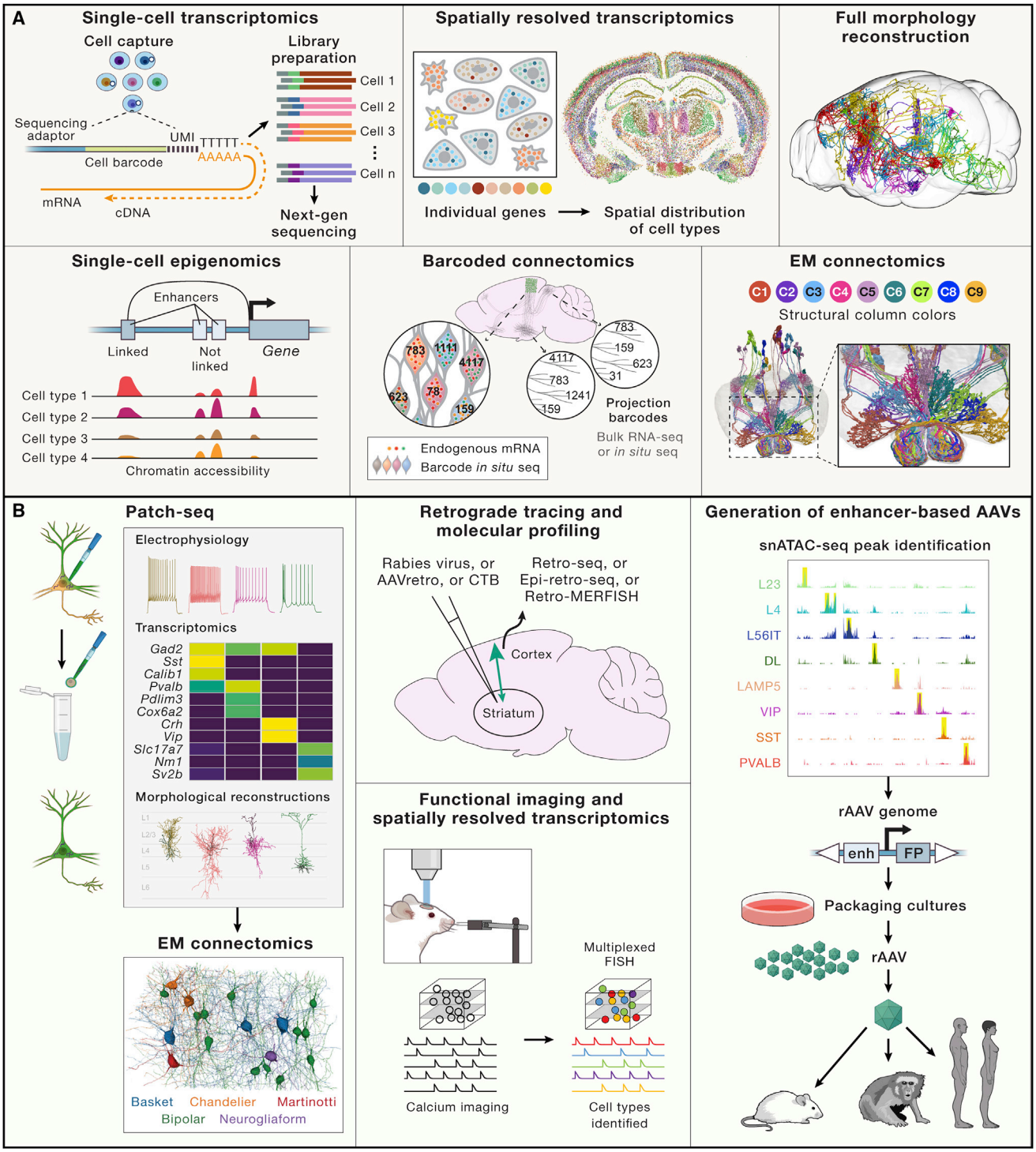

Figure 1. Approaches to characterize cell types

(A) Molecular and anatomical approaches as primary ways of single-cell characterization include single-cell transcriptomics by sc/snRNA-seq, single-cell epigenomics exampled by snATAC-seq, spatially resolved transcriptomics exampled by MERFISH, full morphology reconstruction exampled by MouseLight (image adapted from Winnubst et al., 2019), EM connectomics (image adapted from Hulse et al., 2021), and barcoded connectomics exampled by BARseq (image adapted from Chen et al., 2019).

(B) Cross-modality integrated approaches include Patch-seq (image adapted from Lee et al., 2021), retrograde tracing followed by molecular profiling, functional imaging followed by spatially resolved transcriptomics, using Patch-seq data as a Rosetta stone to assign molecular identities to neurons reconstructed from EM dataset (image adapted from Turner et al., 2022), and generation of enhancer based viral vectors (image adapted from Mich et al., 2021).

Technically, there are three ways to achieve such integration (Figure 1B).

- First, conduct multimodal characterization from the same cell using approaches such as single-cell multi-omics (Zhu et al., 2020), Patch-seq which collects electrophysiological, morphological and transcriptomic data from a single patched cell (Berg et al., 2021; Cadwell et al., 2016; Fuzik et al., 2016; Gouwens et al., 2020; Lee et al., 2021; Muñoz-Manchado et al., 2018; Scala et al., 2021), retrograde connectivity tracing coupled with single-cell molecular profiling (e.g., Retro-seq, Epi-retro-seq, or Retro-MERFISH) (Kim et al., 2020; Tasic et al., 2018; Zhang et al., 2021a, 2021b), or *in vivo* calcium imaging followed by multiplexed FISH (Bugeon et al., 2022; Condylis et al., 2022; Lovett-Barron et al., 2020; von Buchholtz et al., 2021; Xu et al., 2020).
- Second, perform label transfer between independently collected datasets through "Rosetta stone" features, e.g., integration between single-cell transcriptomic and epigenomic datasets through marker genes and nearby chromatin modification sites (Armand et al., 2021; Yao et al., 2021a) or assigning molecular identities to neurons in EM and LM datasets using morphologies obtained from Patch-seq data. Integration between transcriptomics and epigenomics is now further empowered by various single-cell multi-omic techniques (Armand et al., 2021; Zhu et al., 2020).
- Third, create cell type-targeting genetic tools (e.g., transgenic lines or recombinant viral vectors) using marker genes, promoters, and enhancer elements identified from transcriptomic and epigenomic cell atlases (Chan et al., 2017; Daigle et al., 2018; Dimidschstein et al., 2016; Graybuck et al., 2021; Hrvatin et al., 2019; Matho et al., 2021; Mich et al., 2021; Vormstein-Schneider et al., 2020) and use these tools for structural and functional studies. Currently available genetic tools are mostly targeting more coarse-level cell classes or subclasses or a mixture of cell types. These tools have nonetheless proven to be tremendously powerful, as the vast majority of our current knowledge of cell types in the brain and body and their functions has been derived from studies utilizing these tools. The emergence of comprehensive transcriptomic and epigenomic cell atlases now makes it possible to create highly specific tools targeting nearly all identified cell types, and even extended to non-genetic-model organisms and species (Ngai, 2022). This will have a paradigm-shifting effect to the study of function and dysfunction of broad biological systems.

Overall, application of these approaches to characterize cell types in different brain regions and tissue organs as well as across species has begun to reveal generalizable organizing principles of cell types. Below, I will discuss the large body of studies supporting these principles and then conclude with a proposed roadmap based on these principles for taking a multi-level, iterative approach to define cell types and build an overarching knowledge base of cell types across the brain and body, across lifespan and across species.

CELL TYPES ARE THE PRODUCT OF EVOLUTION

The concept of cell types needs to be established based on where cell types originated and how they have diversified. Cell type classification has been compared with species classification (Stadler et al., 2021; Tanay and Sebé-Pedrós, 2021; Zeng and Sanes, 2017). Indeed, species specialization is an overall culmination of the function of all the cell types within that species; thus, they may follow similar evolutionary principles. There have been several ways proposed to classify species. One is based on the notion of reproductive isolation. However, this approach is not universally implementable, and many exceptions have been found. A more fruitful approach is phylogenetic analysis, that is, comparing the relatedness between species using a wide range of structural and functional phenotypic features. Such analysis has led to the foundational "tree of life" as we understand it today. Nonetheless, many issues remain unresolvable in the phylogenetic classification of species, often due to the highly specialized phenotypic features acquired by some species as they adapt to their ecological niches, as well as convergent phenotypic evolution in other cases, both of which could skew comparative analysis. Over the past decade, evolutionary approach based on comparative genomics (i.e., phylogenomics) has brought an entirely new paradigm to species classification, providing a systematic, rational, unbiased, universally applicable, and extensible classification scheme (Murphy et al., 2021; Preuss and Wise, 2022; Stephan et al., 2022).

Similarly, cell types are inherited through the genome. Relatedness between cell types reflects their evolutionary distance as they were created through cell type duplication and segregation events. It has been proposed that the formation of new cell type identity requires the evolution of a unique cell type regulatory signature that includes a cell type-specific core regulatory complex (CoRC) of transcription factors, which defines the identity and coordinated gene expression pattern of the new cell type (Arendt et al., 2016). This set of master regulatory transcription factors, sometimes called terminal selectors, have been identified in a number of neuron types in various species including *C. elegans*, *Drosophila*, and mouse (Hobert and Kratsios, 2019; Reilly et al., 2020). The master transcription factors should be identifiable when the transcriptomes of evolutionarily related cell types are compared. A large body of studies (see above) have now shown that clustering of single-cell transcriptomes can systematically categorize cells into putative types, many of which are consistent with existing knowledge and thus can be considered bona fide cell types. Evolutionarily conserved cell types can be systematically identified by cross-species comparison of single-cell transcriptomic types in the brain (Bakken et al., 2021; Colquitt et al., 2021; Hodge et al., 2019; Kebschull et al., 2020; Krienen et al., 2020; Tosches et al., 2018; Tosches and Laurent, 2019; Yamagata et al., 2021). Thus, this approach appears to *make sense*; it is not coincidental but strongly supports the notion that transcriptomes harbor the molecular genetic code for cell type identity.

However, there are several challenges that must be surmounted to arrive at a complete and accurate evolutionary definition of cell types through cross-species comparisons. Accurate cross-species comparison of cell types at transcriptomic level requires well-annotated genomes, comparative gene ontologies, and consistently high-quality transcriptomic data generation from

many species (Tanay and Sebé-Pedrós, 2021). Furthermore, species mostly diverged millions of years ago, as did cellular identities. Cell type homologies between related species are often discernible only at a relatively coarse level which do not fully capture the biological complexity. Many gaps also exist due to the extinction of intermediate species. These challenges could limit a deeper understanding of cell types (see below) and how they contribute to the body or brain function. On the other hand, one does not need to characterize cells from a large number of evolutionarily related species to define cell types. It is possible to gain a deep understanding of cell types from even a single species, since each species has evolved from its simpler ancestors through many rounds of cellular and regional duplications in which the newly created cell types and regions adopt new functions, and thus, comparing between cell types and between regions within the species (in the same way as comparing between species) can reveal the evolutionary relationships between cell types as well. Then, we can expand the investigation into as many other species as possible, which will further clarify the description of cell types, their origins, and how their functions manifest.

Therefore, the first and foremost principle is that cell types are the product of evolution and cell type identities are encoded in the genome. Like phylogenomics for species classification, transcriptomic (and epigenomic) classification is a good proxy of cell type classification as the gene regulatory mechanisms that encode and maintain cell type identities are embedded in the transcriptomes and epigenomes. This core concept has led to a systematic delineation of the relationship between cell types both within a species and, increasingly, across species. At the same time, cell type conservation may be imposed more by function than natural selection directly as in organismal evolution. As such, evolution of individual cell types may be more complicated than organismal evolution as a whole, and it will be interesting to see if different cell types evolve in similar or different ways as the whole organism. Finally, a transcriptome also contains gene expression profiles that underlie arguably all phenotypic features of the cell at the time or state when the cell is characterized. What else are transcriptomes and transcriptomic clusters telling us?

HIERARCHICAL ORGANIZATION OF TRANSCRIPTOMICALLY DEFINED CELL TYPES

Transcriptomically derived cell type taxonomies in the adult mammalian brain, with majority of the studies conducted in mouse, have consistently revealed a hierarchical organization of cell types (Figure 2; Brain Initiative Cell Census Network, 2021; Macosko et al., 2015; Romanov et al., 2017; Russ et al., 2021; Saunders et al., 2018; Shekhar et al., 2016; Tasic et al., 2016, 2018; Yao et al., 2021b; Zeisel et al., 2015, 2018; Zeng and Sanes, 2017). The first (highest) level of branches is the separation of neuronal and various non-neuronal cell classes (Figure 2A). For neurons, the second level of branches is driven by major brain structures/regions, and the third level comprises various cell subclasses and types within each major brain structure, although there may be cell types crossing or shared between brain structures due to cell migration during development.

The basic architecture of the mammalian brain (Swanson, 2000, 2012) is composed of telencephalon, diencephalon, mesencephalon (midbrain), and rhombencephalon (hindbrain). Telencephalon (consisting of five major brain structures—isocortex, hippocampal formation [HPF], olfactory areas, cortical subplate, and cerebral nuclei) and diencephalon (including thalamus and hypothalamus) are collectively called forebrain. Midbrain is divided into tectum and tegmentum, and hindbrain is divided into pons, medulla, and cerebellum. Within each of these major brain structures, there are multiple regions and subregions, each with many cell types. A cell type can be specific to a subregion, a region, or a major brain structure.

Here, I use isocortex (or simply called cortex) as an example to illustrate the organization of cell types within a major brain structure. Isocortex is composed of multiple cortical areas, each mediating sensory, motor, or associational functions. Transcriptomic cell type taxonomies from visual cortex and motor cortex display a similar organization (Figure 2B; Brain Initiative Cell Census Network, 2021; Tasic et al., 2018; Yao et al., 2021a). In each of these areas, there are two neuronal classes based on the dominant neurotransmitters they release, glutamatergic and GABAergic, as well as several non-neuronal classes. The glutamatergic excitatory neurons mostly have long-range axon projections to other cortical and/or subcortical regions. They are divided into nine subclasses based on their layer specificity and long-range projection patterns: L2/3 intratelencephalic projecting [IT], L4/5 IT, L5 IT, L6 IT, Car3 IT, L5 extratelencephalic projecting [ET], L5/6 near-projecting [NP], L6 corticothalamic projecting [CT], and L6b. The GABAergic inhibitory neurons mostly have their axon projections confined within the local area. They are divided into six subclasses named after canonical marker genes: Lamp5, Sncg, Vip, Sst, Sst-Chodl, and Pvalb. Within each of the glutamatergic or GABAergic subclasses, as well as each non-neuronal class, there are several transcriptomic clusters or types, resulting in a total of ~110 transcriptomic cell types in each cortical area (Brain Initiative Cell Census Network, 2021; Tasic et al., 2018). This organization is highly consistent with the existing knowledge about cortical cell types that have been extensively studied in a variety of phenotypic modalities over the past 50 years (Harris and Shepherd, 2015; Tremblay et al., 2016; Yuste et al., 2020; Zeng and Sanes, 2017), suggesting that single-cell transcriptomics alone can faithfully capture the overall cell type organization at class and subclass levels, although the validity of transcriptomic clusters at the lowest branch level has yet to be fully tested.

Comparison of transcriptomic cell types across different cortical areas reveals new insights. Glutamatergic neuron types are distinct between visual and motor cortical areas, whereas GABAergic neuron types are shared between the two areas (Tasic et al., 2018). This dichotomy may be rooted in the developmental origins of these two cell classes. During development, glutamatergic neurons are generated within the cortex in which different areas are laid out by gradient expression of morphogens (Cadwell et al., 2019; O'Leary et al., 2007), whereas GABAergic neurons are generated in the subcortical ganglionic eminence and migrate into cortex (Hu et al., 2017; Lim et al., 2018). A larger transcriptomic study covering all areas from both isocortex and HPF further identifies hundreds of transcriptomic types and a high degree of diversity in the glutamatergic neuron class across both brain structures (Figure 2C; Yao et al., 2021b). Within isocortex, cell types that are specific to a

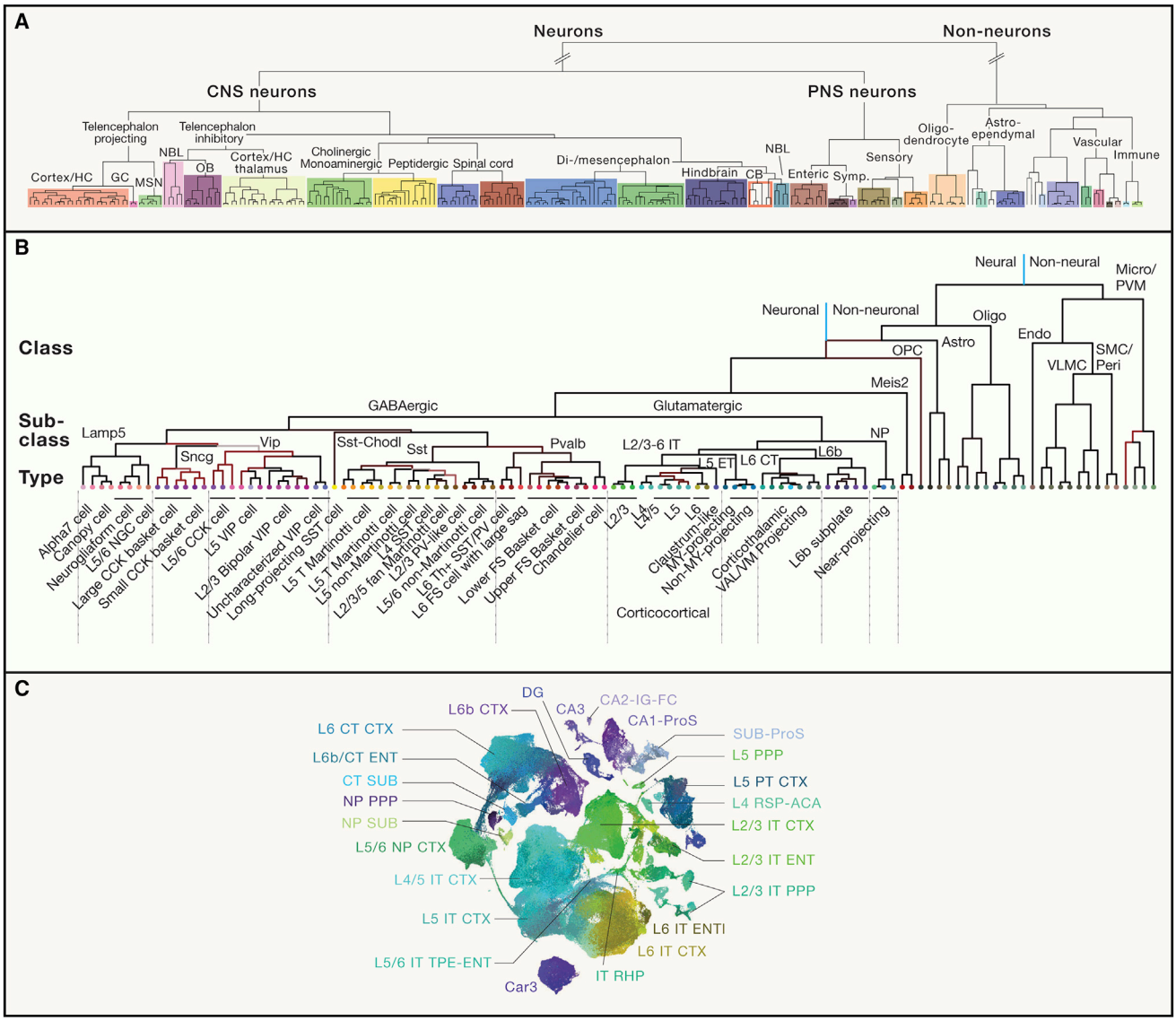

Figure 2. Hierarchical organization of cell types

(A) A transcriptomic cell atlas for the mouse nervous system (image adapted from Zeisel et al., 2018).

(B) A transcriptomic cell type taxonomy for the mouse primary motor cortex, with annotation (image adapted from Brain Initiative Cell Census Network, 2021).

(C) UMAP representation of a transcriptomic cell type taxonomy for the glutamatergic neuron types in mouse isocortex and hippocampal formation, revealing discrete and continuous variations (image adapted from Yao et al., 2021b).

cortical area or shared among areas are both identified, and the shared cell types often exhibit gradient distribution or gradient gene expression across areas. This highly complex transcriptomic cell type landscape along the cortical sheet likely results from the series of cortical developmental events from "Protomap" to "Protocortex" (Cadwell et al., 2019; O'Leary et al., 2007). Compared between the two brain structures, the glutamatergic cell types in isocortex and HPF are highly distinct from each other; however, they also display one-to-one homology at subclass level suggesting a similar evolutionary origin (Yao et al., 2021b). This homologous relationship suggests that parallel neural networks can be formed by homologous sets of cell types.

Overall, based on these findings, we may hypothesize that the adult-stage transcriptomic landscape can reveal the organization of cell types within and between brain regions that reflect their evolutionary and developmental histories. The hierarchical organization of transcriptomic cell types likely represents evolutionary origins of and distinctions between cell types; major branches represent earlier division of cell classes, and minor branches represent more recent segregation events. This hierarchical organization is laid out via an elaborate developmental program involving a series of highly coordinated processes and events. This hypothesis can be tested by studying the evolution and development of cell types (see below).

Another prominent feature of the relationship between cell types revealed by transcriptomic studies is the coexistence of discrete and continuous variations between types. Continuous variations have been observed in a variety of forms in cortical excitatory and inhibitory neurons, medium spiny neurons in the striatum, and excitatory projection neurons across different nuclei of the thalamus (Phillips et al., 2019; Stanley et al., 2020; Tasic et al., 2018; Yao et al., 2021b). Discrete variations exist among cell subclasses and major types that are usually at the higher branches of the hierarchy. Continuous variations are usually found among closely related transcriptomic clusters or subtypes at lower branches, such as the many IT neuron types across the cortical depth from L2/3 to L6 (Figure 2C). Cells at opposite ends of the continuum have clearly distinct transcriptomic profiles, but the transition from one end to the other is gradual among the cells composing the continuum. This makes it difficult to subdivide the cells into types using statistical criteria and name an exact number of cell types. However, the continuous variation itself is still biologically meaningful and needs to be properly represented in cell type descriptions. One way to better understand the significance of the continuous variation is to examine how it correlates with other modalities of cell type properties (see below).

Regarding non-neuronal cells in the mammalian brain, there are multiple classes that can be divided into neural and non-neural groups (Figures 2A and 2B; Brain Initiative Cell Census Network, 2021; Zeisel et al., 2018). The non-neural group contains cell classes of the immune origin, i.e., microglia and border-associated macrophages (BAMs) (Butovsky and Weiner, 2018; Masuda et al., 2019; Munro et al., 2022; Prinz et al., 2019; Thion and Garel, 2020; Van Hove et al., 2019), and of the vascular origin, i.e., endothelial cells, smooth muscle cells (SMCs), pericytes, and vascular leptomeningeal cells (VLMCs) (Ross et al., 2020; Schaeffer and Iadecola, 2021; Sweeney et al., 2019; Vanlandewijck et al., 2018). The neural group contains cell classes of the neuroectoderm origin (same as the origin of neurons), including oligodendrocytes, oligodendrocyte progenitor cells (OPCs), astrocytes, and ependymal cells (Ben Haim and Rowitch, 2017; Dimou and Simons, 2017; Escartin et al., 2021; Khakh and Deneen, 2019; Kuhn et al., 2019; Ortiz-Álvarez et al., 2019; Redmond et al., 2019). In brain transcriptomic cell type taxonomies, non-neuronal cells generally display less diversity than neurons, with little regional specificity except for astrocytes. There are two major subclasses of astrocytes, one specific to the telencephalon and the other to non-telencephalon regions, in addition to several other highly specialized astrocyte-like cell types such as Müller glia of the retina and Bergmann glia of the cerebellum (Zeisel et al., 2018). Immature and mature oligodendrocytes form a long continuous trajectory originating from OPCs, indicating coexistence of multiple states of gradually maturing oligodendrocytes (Marques et al., 2016; Zeisel et al., 2018).

Like comparative genomics for species classification, single-cell transcriptomics is highly effective for cross-species comparison of cell types to reveal their evolutionary relationships. Comparative studies of cortical cell types among mouse, human, and non-human primates (Bakken et al., 2021; Brain Initiative Cell Census Network, 2021; Hodge et al., 2019; Krienen et al., 2020) show that the hierarchical organization described above along with all the neuronal and non-neuronal cell classes and subclasses (major branches of the hierarchy) is well conserved across these mammalian species. Main differences between species lie in the heterogeneity of individual gene expression within each subclass and type, as well as the ambiguity of cross-species correspondence of the leaf-node transcriptomic types which likely will require multimodal characterization to clarify. Similarly, comparative transcriptomic studies reveal homologous cortical glutamatergic and GABAergic cell classes between mammal and reptile (Tosches et al., 2018; Tosches and Laurent, 2019), as well as homologies and variations of neuron subclasses and types in the cerebellar nuclei or the retina of mouse, chicken, and primates (Kebschull et al., 2020; Yamagata et al., 2021). These studies further suggest that the hierarchical organization of brain cell types is a framework extensible to describing cell type evolution.

CORRESPONDENCE BETWEEN TRANSCRIPTOMIC CELL TYPES AND OTHER CELLULAR PROPERTIES

Cell types are also considered to be the basic functional units of an organism. For the categorization of cell types based on their transcriptomes to be meaningful and to understand their relevance to the structure and function of the tissue organ where the cells reside, it is necessary to characterize other modalities of cellular properties. Multimodal correspondence of cell types in the mouse retina is a classic example where independent anatomical, functional, and transcriptomic studies uncover similar numbers of neuron types (~130) and find that molecular profiles and anatomical distribution patterns (laminar specificity and mosaicism) are well correlated with visual response properties (Baden et al., 2020; Masland, 2012; Sanes and Masland, 2015; Seung and Sümbül, 2014; Shekhar and Sanes, 2021). Recent work from the BRAIN Initiative Cell Census Network (BICCN) in creating a multimodal cell census and atlas of the mammalian primary motor cortex represents the most comprehensive multimodal study to date, integrating transcriptomics with epigenomics, spatially resolved transcriptomics, morpho-electrical (ME) properties, and connectivity (Brain Initiative Cell Census Network, 2021).

Integration of transcriptomic and epigenomic datasets using computational approaches allows consolidation of robust molecular cell types and identification of hundreds of thousands of *cis*-regulatory elements (CREs) associated with specific cell types (Yao et al., 2021a). Some of these CREs are associated with specific marker genes, whereas others may represent past events.

Integration of transcriptomics and MERFISH, a spatially resolved transcriptomic method, reveals the spatial organization of mouse motor cortex cell types (Zhang et al., 2021a). A major finding of the study is that in addition to the laminar distribution of glutamatergic neuron subclasses as expected, even GABAergic types within each subclass exhibit layer-selective localization, and the continuous variations among individual glutamatergic types or GABAergic types correlate well with their continuous distribution along cortical layers/depth (with a prominent example being that all the excitatory L2/3-L6 IT types line up along the cortical depth from L2/3 to L6) (Figure 3A). Thus, a strong correspondence is demonstrated here between the continuous variations among cortical neuron types in the transcriptomic domain

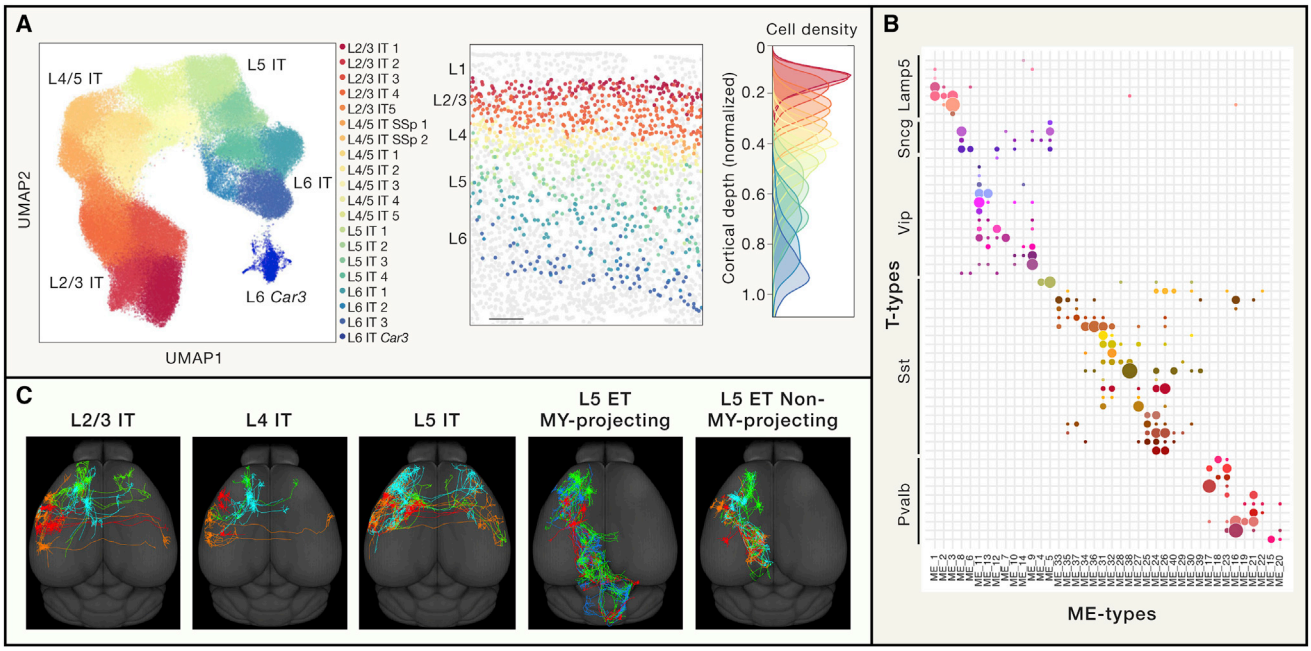

Figure 3. Multimodal correspondence of cell type phenotypic properties

(A) MERFISH data from mouse motor cortex shows that continuous variation of glutamatergic IT transcriptomic types is correlated with their continuous spatial distribution along the cortical depth from L2/3 to L6 (image adapted from Zhang et al., 2021a).

(B) Patch-seq data on GABAergic interneurons from mouse visual cortex shows correspondence between transcriptomic (T) types and morpho-electrical (ME) types (image adapted from Gouwens et al., 2020).

(C) Brain-wide complete morphology reconstruction of cortical glutamatergic neurons shows distinct axon projection patterns between major transcriptomic types and further heterogeneity within each type (image adapted from Peng et al., 2021). Each color outlines the projection of one neuron within the type in each panel.

and their continuous and directed spatial distribution patterns. Other MERFISH studies of neuron types in hypothalamus or nucleus accumbens also reveal strong correlation between transcriptomic specificity and anatomical/subregional specificity (Chen et al., 2021; Moffitt et al., 2018).

Integration of transcriptomic, electrophysiological, and morphological properties by Patch-seq reveals multimodal corresponding distinctions of mouse motor cortex cell types at subclass level (Scala et al., 2021). Within each subclass, the ME properties vary continuously along with the transcriptomic types. There is also additional heterogeneity of ME properties within some transcriptomic types, indicating a more complex picture. Another Patch-seq study of mouse visual cortical GABAergic neurons also reveals a relatively high degree of corresponding continuous variations of transcriptomic (T) types with their anatomical distribution along the cortical depth and the variations of their ME properties (Figure 3B; Gouwens et al., 2020). To overcome heterogeneity at individual type level, a set of triple-modality MET types are defined at an intermediate level of granularity (between transcriptomic subclasses and types). These visual cortex GABAergic MET types show robust cross-modality concordance and mutual predictability.

The vast majority of cortical and subcortical neuron types have long-range axon projections to form circuit networks throughout the brain. To examine the long-range axon projection specificity of transcriptomic cell types, Retro-seq and related methods (e.g., Epi-retro-seq, Retro-MERFISH) have been used (Kim et al., 2020; Tasic et al., 2018; Zhang et al., 2021a, 2021b). Since a neuron type usually has multiple projection targets and a neuron within that type can choose a subset of those targets either specifically or randomly, a single-target-site Retro-seq assay is often insufficient to resolve the target specificity of a transcriptomic type except in special cases. Brain-wide complete reconstruction of single-neuron morphology is currently the only approach to capture the full extent of a neuron's axon projection pattern and define projection neuron types (Gao et al., 2022; Peng et al., 2021; Winnubst et al., 2019). A study using this approach in cortical excitatory neuron subclass-specific Cre driver lines (Peng et al., 2021) reveals distinct projection patterns between subclasses, e.g., not only between IT and ET but also between L2/3 IT and L5 IT neurons, confirming subclass level projection specificity (Figure 3C). Within each subclass, the study also finds extensive heterogeneity among individual neurons; this heterogeneity reflects three axes of variations: regional specificity, topographic specificity, and individual (potentially stochastic) variation, which do not readily correlate with transcriptomic types within the subclass. Thus, it remains an open question how axon projection patterns correlate with transcriptomic types, which needs to be addressed in future studies using approaches such as coupling complete morphology reconstruction with multiplexed FISH or performing MAPseq/BARseq with sequencing of both starter cells and axon targets. It may also be necessary to extend such studies into developmental periods to identify potentially clearer molecular

correlates when the projection specificity is established (Klingler et al., 2021).

Systematic investigation of connectivity among transcriptomic types at synaptic level and relating them to conventional studies where morphology and individual molecular markers were used to identify cell types is much needed to better understand the connectional specificity between transcriptomic types. It has been suggested that neuron types may be defined by their unique communication properties implemented as synaptic input-output patterns (Huang and Paul, 2019; Paul et al., 2017). The emerging large-scale EM datasets (e.g., from the MICrONS project, https://www.iarpa.gov/research-programs/microns) hold great promise to tackle synaptic-level connectivity between cell types and individual cells in the mammalian brain (Abbott et al., 2020). Perhaps, a greater opportunity lies in the *Drosophila* field where comprehensive catalogs of both transcriptomic cell types and connectional cell types have been obtained independently (Bates et al., 2019; Hulse et al., 2021; Li et al., 2022; Scheffer et al., 2020), and a systematic comparison and cross-correlation between them may be realized soon.

To compare functional properties among transcriptomic cell types, two general approaches have been taken—coupling *in vivo* calcium imaging with post hoc multiplexed FISH to decode the molecular identities of the imaged cells (Bugeon et al., 2022; Condylis et al., 2022; Lovett-Barron et al., 2020; von Buchholtz et al., 2021; Xu et al., 2020) or mapping immediate early gene (IEG) activation during sensory response or behavior using scRNA-seq or MERFISH (Hrvatin et al., 2018; Kim et al., 2019; Moffitt et al., 2018; Sathyamurthy et al., 2018; Wu et al., 2017). Using the former approach, it has been shown that GABAergic transcriptomic types in mouse visual cortex differ in their response to behavioral states (e.g., running versus resting), whereas visual response properties (e.g., orientation or direction selectivity) only differ at subclass level (Bugeon et al., 2022); in somatosensory cortex, higher sensory response is seen in a specific L2/3 IT excitatory transcriptomic type (Condylis et al., 2022). In hypothalamus, several studies using either of the two approaches in mice demonstrate that activated neurons during a specific behavioral state are often distributed across a range of transcriptomic cell types (Kim et al., 2019; Moffitt et al., 2018; Xu et al., 2020). Understanding the functional roles of different transcriptomic cell types is a huge undertaking. Studies mentioned here are just the beginning; many more will come in the future and will allow us to gain a much deeper understanding.

In summary, for a definition of cell types to be meaningful, it must be associated with what cell types do. A transcriptomic cell type taxonomy must be linked to anatomical and functional information to evaluate the validity of the transcriptomic taxonomy and determine the appropriate level of granularity (since in theory, transcriptomic clusters can be infinitely subdivided, and the more cells profiled, the more clusters can be obtained). So far, it has been shown that transcriptomic types have excellent correspondence with their spatial distribution patterns. Since the spatial distribution pattern is defined during development, this suggests that transcriptomes may retain the developmental plan. At the same time, whether specific transcriptomic types (beyond the subclass level) have specific connectional or functional attributes is still unclear in many cases. Since transcriptomes are rich in containing the molecular correlates of all sorts of cellular properties, specific molecular signatures responsible for certain essential anatomical or functional features may be hidden below noise and will need to be brought out through supervised approaches and used to refine the classification of cell types toward more functional relevance. It is also necessary to trace back into development to identify potentially clearer molecular correlates as different connectional or functional properties may be established at different developmental time points. On the other hand, it is also reasonable to propose that some connectional or functional properties should not be used to define cell types because they may be emerging properties arising from the interaction of a network of cell types or from experience and/or activity-dependent processes that represent a cell *state* rather than a defining feature for a cell *type*.

CELL TYPES VERSUS CELL STATES

A key question arising when evaluating a transcriptomic taxonomy is whether some clusters actually represent a particular cell state—a transient or dynamically responsive property of a cell to a context—rather than a cell type, as a cell type can exist in different states. This is a difficult question to address since most of the phenotypic measurements including the single-cell transcriptome are only a one-time snapshot of the cell. However, one can compare transcriptomes collected from different time points or different behavioral, physiological, or pathological states and see which clusters appear, disappear, or shift under different conditions. Cell type-specific gene expression changes associated with different cell states may be seen during circadian cycles, variable metabolic states, development, aging, or under behavioral, pharmacological, or diseased conditions (Figure 4; Mayr et al., 2019; Morris, 2019). Furthermore, individual variations within a species (e.g., within the human population) that are driven by genetic or environmental factors may be manifested as a variety of cell type or cell state variations. Studying the various states of cell types will enhance our ability to distinguish core gene sets (e.g., master transcription factors) maintaining cell type identities versus genes associated with specific functional states, and further our understanding of the diverse function of cell types as well as the biological basis of individual variability.

The distinction between cell types and cell states is particularly challenging during development, as cells continually change their states, and at certain key time points, they may switch their cell type identities. Can single-cell phenotypic properties such as transcriptomes distinguish types versus states? Although not absolute, it is reasonable to assume that transcriptomic changes tend to be more continuous during cell state transitions and more abrupt or discrete when cells switch their types. More often, emergence of a new cell type during development is the consequence of cell division from which a daughter cell takes up a new cell type identity (Figure 4). Trajectory analysis or lineage tracing coupled with single-cell transcriptomics across developmental time points has now often been used to identify the time course of emergence and maturation of each cell type, as well as the ancestor-descendant relationship across

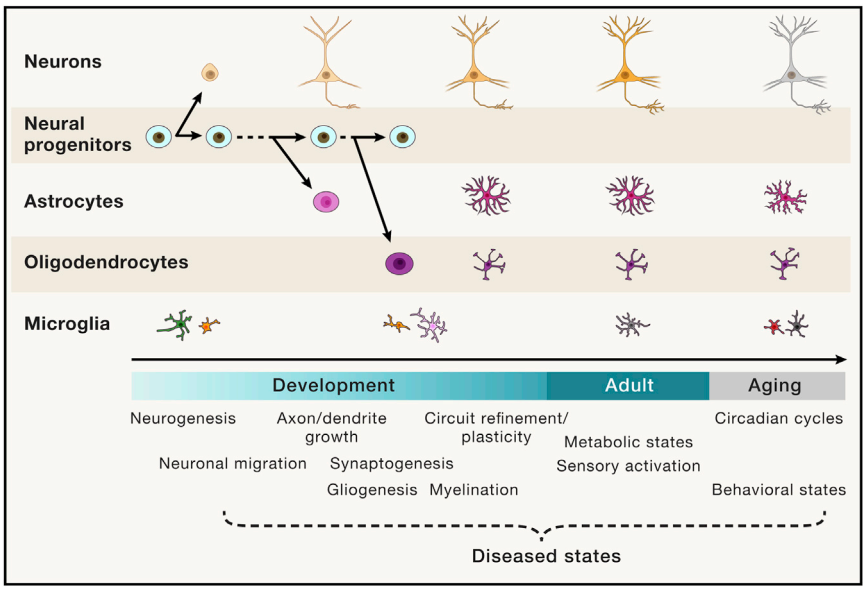

Figure 4. Dynamic changes of cell types and states during development, aging, and various physiological or pathological contexts

Major neuronal and non-neuronal classes are shown along the life stages of development, adulthood, and aging. Neural progenitors generate different neuronal types, astrocytes, and oligodendrocytes at different developmental time points, whereas microglia have a separate developmental origin. Major developmental events, various physiological states in adulthood, and different diseased states throughout lifespan are shown below the timeline.

cell types that are present at different developmental stages (e.g., progenitors versus differentiated cells) (McKenna and Gagnon, 2019; Saelens et al., 2019; Tritschler et al., 2019; Wagner and Klein, 2020).

Coordinated neuronal activities in brain circuits generate sensory perception and behavior. Specific neuronal populations activated during a particular perceptual or behavioral episode can be identified by screening for the activation of IEGs in them, via immunostaining, transgenic reporter lines, or single-cell or spatial transcriptomics in more recent years (DeNardo and Luo, 2017; Hrvatin et al., 2018; Moffitt et al., 2018; Wu et al., 2017). IEG activation leads to expression of downstream effectors, such as ion channels or synaptic proteins, that shape the cell states and remodel neuronal connections. Cell state changes in the brain are closely related to neural plasticity. Similarly, various diseased conditions can induce pathological changes in cell states in different brain regions or tissue organs. Numerous studies have revealed selective vulnerability of specific cell types for specific diseases. Pharmacological or genetic (e.g., CRISPR-based) perturbations in normal or diseased conditions, in combination with single-cell profiling (e.g., Perturb-seq), are powerful approaches to gain a mechanistic understanding of how disruptive or restorative cell state changes can affect cell type function or dysfunction (Adamson et al., 2016; Dixit et al., 2016; Jaitin et al., 2016; Replogle et al., 2022).

Here, I highlight a prominent feature of the non-neuronal cell types in the brain, which is that despite having lower diversity than neurons in baseline adult state, many non-neuronal cell types undergo pronounced changes, i.e., they exhibit many different cell states, under different physiological or diseased situations. Astrocytes exhibit diverse morphological and physiological properties in different brain regions and contribute to essential functions in blood-brain barrier, synaptogenesis, neurotransmitter buffering, ion homeostasis, and secretion of neuroactive agents (Ben Haim and Rowitch, 2017; Khakh and Deneen, 2019). Astrocytes become reactive under pathological conditions. Reactive astrocytes undergo morphological, molecular, and functional changes in response to injury or central nervous system (CNS) diseases; they may adopt multiple, heterogeneous states depending on context (Escartin et al., 2021). Oligodendrocytes are the myelinating cells of the CNS that are generated from OPCs throughout life. Myelination process also exhibits activity-dependent plasticity (Monje, 2018). Oligodendrocyte pathology is evident in a range of disorders including multiple sclerosis, schizophrenia, and Alzheimer's disease (Kuhn et al., 2019). Regarding cerebrovascular cell types, recent single-cell transcriptomic studies in the human brain reveal gene expression changes in them that can impact blood-brain barrier integrity in stroke and Huntington's and Alzheimer's diseases (Garcia et al., 2022; Winkler et al., 2022; Yang et al., 2022). Finally, microglia are the primary innate immune cells in the CNS and have a distinct developmental origin from peripheral immune cells. They are generated from mesodermal progenitors that arise from the yolk sac and are among the earliest residential cell types in the brain. Microglia display diverse and dynamic phenotypic states and play a plethora of roles in development, adulthood (homeostasis), aging, and diseases (Butovsky and Weiner, 2018; Prinz et al., 2019; Thion and Garel, 2020). Single-cell transcriptomic studies reveal a relatively homogeneous adult microglia population and greater heterogeneity of microglia states at different developmental stages, during aging and in pathological conditions (Hammond et al., 2019; Li et al., 2019; Masuda et al., 2019). In particular, microglia can be both responders to and inducers of various neurodegenerative, neuroinflammatory, and neurodevelopmental diseases. Taken together, these studies paint a collective picture on how the variety of non-neuronal cell types actively respond to and contribute to different physiological and pathological changes in the brain.

CELL TYPE DEVELOPMENT

A deep understanding of a subject often comes from understanding how it is built. The entire repertoire of cell types in the brain and the body is built through a sequential and parallel series of spatially and temporally coordinated developmental events starting from a single fertilized egg, the zygote. This developmental program carries out a remarkable implementation plan that unravels the identities of all cell types which are encoded in the genome through

evolution. Transcriptional and epigenetic regulatory programs are unfolded from the genome sequences and drive a cascading series of cell proliferation and differentiation processes, leading to the manifestation of diverse cellular phenotypes. In the developmental ontogeny of cell types, earlier-stage ancestral cell types are fewer and are more multipotent, and they give rise to a larger number of descendant cell types with increasingly restricted fates. The developmental program rolls out not only a temporal but also an elaborate spatial plan, specifying the location of each tissue organ and the spatial organization of all the cell types within each. This is a highly dynamic spatiotemporal process involving specific cell-cell interactions, cell migration streams, and formation of niches and microenvironments that allow functional specialization.

For the brain, the main series of events of brain development leading to the mature adult-stage cell types and circuits include: patterning and regionalization (laying out the master plan of brain architecture), neurogenesis and neuronal migration, gliogenesis and glia cell differentiation, neuronal differentiation and circuit formation (axonogenesis, dendritic arborization, synaptogenesis, and myelination), and circuit refinement and plasticity (Figure 4). Systematic single-cell transcriptomic, epigenomic and spatially resolved transcriptomic profiling with high temporal resolution, coupled with lineage tracing and other phenotypic characterization, holds tremendous potential to capture key sets of genes and genomic regulatory networks involved in these series of events and begin to resolve the extremely complex spatial and temporal transitions of cell types and states leading to the adult-stage repertoire of cell types (Allaway et al., 2021; Bandler et al., 2022; Bhaduri et al., 2021; Cao et al., 2019b; Chen et al., 2022; Delgado et al., 2022; Di Bella et al., 2021; Klingler et al., 2021; La Manno et al., 2021; Romanov et al., 2020; Schmitz et al., 2022; Sharma et al., 2020; Shekhar et al., 2022; Tiklová et al., 2019; Zhu et al., 2018). Studying brain cell type development using these approaches will allow us to establish the developmental trajectory for each cell type from progenitors to transitional cell types and states to adult mature cells, discover the set of master transcription factors that define and maintain the identity of each cell type, and identify key events and molecular correlates that lead to the acquisition of a cell type's specific connectional or functional properties.

The generation and patterning of mouse cortex and spinal cord cell types are two example systems where extensive historical studies have uncovered several common principles (Cadwell et al., 2019; Catela et al., 2015; Jessell, 2000; O'Leary et al., 2007; Osseward and Pfaff, 2019; Sagner and Briscoe, 2019). First, opposing morphogen gradients establish the basic plan for cortex (anterior-posterior) or spinal cord (dorsal-ventral) patterning and provide instructive signals for the expression of complementary sets of transcription factor activators and repressors, which in turn define distinct neural progenitor domains within cortex or spinal cord. Second, driven by the transcription factor network, each type of neural progenitors generates a series of neuronal cell types. The set of cell type-defining transcription factors in a progenitor or a daughter cell can change with time, such that different neuronal types emanate from the same progenitor in a precisely timed birth order. Later in development, the same neural progenitors also generate non-neuronal cell types such as astrocytes and oligodendrocytes. Third, specific sets of cell adhesion molecules provide guidance cues for axon path finding and synapse formation, leading to the assembly of region- and cell type-specific local and global circuit networks. Fourth, patterned neural activities spontaneously emerged from the circuits and/or influenced by external inputs further sculpt synaptic connections and circuit organization to refine functional specificity of the circuit networks.

In addition to these general principles, it is worth noting the many kinds of complexity already encountered. The development of a cell type may not follow a simple trajectory but involves multiple steps of divergent or convergent differentiation (Shekhar et al., 2022), the former due to the multipotency of progenitors or transitional cell types and the latter due to convergence of different transitional types. Developmental trajectory also is not the same as developmental lineage, as a lineage is defined as all the cells descended from a single precursor/progenitor, and it has been shown in multiple systems that a progenitor can produce cells belonging to several neuronal and non-neuronal types, ordered by developmental timing (Agathocleous and Harris, 2009; Sagner and Briscoe, 2019; Sulston et al., 1983; Zeng and Sanes, 2017). Finally, there are transient cell types and circuits that mainly exist during development and have developmental stage-specific functions (Cossart and Garel, 2022; Molnár et al., 2020). All these observations, and more to be discovered, contribute to a nuanced understanding that cell type development is not a simple linear process but a highly multifaceted process, leading to the complex cell type landscape described in the above sections, which underlies the richness of cell type function.

A comprehensive atlas of mammalian cell type development, likely first generated in mouse and then extended to other species including human (Haniffa et al., 2021), will provide the foundation for matching developmental events and their timelines across species, better understanding the evolutionary relationships between cell types, evaluating and guiding human iPSC and organoid *in vitro* development, and, ultimately, transforming our investigation and treatment of developmental disorders.

HOW TO DEFINE CELL TYPES?

In conclusion, cell types are the product of evolution, and they are the basic functional units of an organism. To unify these two concepts and define cell types properly, we need to take a multilevel approach, progressing from simple and singular to complex and multifaceted. In such a roadmap, with each iteration, the definition of cell types will become more mature and unified, and the repertoire of cell types defined will better align with the functional architecture of the organism.

The logical first step is to use single-cell transcriptomics-based cell type taxonomy as the initial framework and anchor for defining cell types. The transcriptomic taxonomy contains evolutionarily rooted molecular signatures and allows effective label transfer and linking with all other modalities. Conversely, relating other cellular properties will help to refine transcriptomic types. The transcriptomic taxonomy organizes cell types in a hierarchical manner, laying out different levels of descriptions from major divisions at class and subclass levels to more granular and fuzzier divisions at type and subtype levels (due to the more prevailing continuous variations at the latter levels). To account for

the biological reality, a hierarchical presentation of cell types is more meaningful than ascertaining an exact number of types. The transcriptomic taxonomy should be supported by comprehensive epigenomic and spatially resolved transcriptomic characterizations (Figure 1A) to associate chromatin modification and gene regulatory elements to transcriptomic profiles and assign precise spatial distribution patterns to transcriptomic cell types.

Second, we need to conduct comprehensive anatomical, physiological, and functional studies of transcriptomic types using approaches that allow molecular identification of the cells under study (Figures 1A and 1B). Such studies will help to resolve differing opinions in lumping or splitting cell types and provide rationales for determining the appropriate level of granularity in defining cell types. They will also provide a context for understanding cell type function and associated cell state changes. In particular, generating complete neuronal morphology reconstructions and comprehensive brain-wide connectomics datasets and relating them to transcriptomic types (Figure 1A) will be extremely informative in understanding the ultimate synaptic-level brain architecture and its underlying organizing principles, which will lay the foundation for understanding circuit-based brain function.

Third, we need to systematically study the entire developmental process of cell types, at least in mouse. Extending the above-mentioned approaches into development will reveal causal relationships and molecular mechanisms underlying the unique identities, connectivity or other forms of cell-cell interactions, and functions of the vast array of cell types. We should also extend such cell type studies into other species as much as possible to further uncover evolutionary principles of cell type diversity and how it supports the common or species-specific biological functions including those of the human itself. The studies of cell types and evolution/development (Evo-Devo) are truly interdependent; to achieve meaningful progress, one cannot just do one without the other.

Finally, to put all these together, we need a conceptual framework and knowledge base to organize all the knowledge gained from these studies. A tantalizing idea of a ''periodic table of cell types'' has been proposed (Xia and Yanai, 2019). Considering the Evo-Devo root and the consequential hierarchical organization of cell types, here I suggest that a ''tree of cell types'' might be more appropriate for an overarching classification of cell types and delineation of their origins and relationships (Stadler et al., 2021; Tanay and Sebé-Pedrós, 2021). One can define a tree of cell types for each species, covering its entire life span, and compare such trees across species. Obviously, the ''tree'' will be a very complex, multi-dimensional graph, and there will likely be multiple branches connecting each node to account for convergence, divergence, and other multipronged interrelatedness. To make the tree of cell types widely applicable, it will be critical to adopt explicitly definable and standardized criteria, develop a common cell type ontology and nomenclature, and create computational tools to allow mapping and comparison across datasets as well as genetic tools to enable consistent access of cell types (Osumi-Sutherland, 2017; Yuste et al., 2020). To extract knowledge and insights from the vast amount of data, a list of associated rules, logics, and principles will need to be established and articulated, and this will be greatly facilitated by computational modeling. Ultimately, this knowledge base of cell types, interweaving cell type function, development, and evolution, will provide the blueprint of life to enable a deeper understanding of the dynamic changes of cellular function under a wide range of healthy and diseased conditions, and lead to innovations that improve human health in many ways.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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