Single cell sequencing of brain cell transcriptomes and epigenomes

Ethan J. Armand[1,*], **Junhao Li**[1,*], **Fangming Xie**[2,*], **Chongyuan Luo**[3], **Eran A. Mukamel**[1,†]

[1]Department of Cognitive Science, University of California, San Diego, La Jolla, CA 92037, USA

[2]Department of Physics, University of California, San Diego, La Jolla, CA 92037, USA

[3]Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA 90095, USA

Summary

Single cell sequencing technologies, including transcriptomic and epigenomic assays, are transforming our understanding of the cellular building blocks of neural circuits. By directly measuring multiple molecular signatures in thousands to millions of individual cells, single cell sequencing methods can comprehensively characterize the diversity of brain cell types. These measurements uncover gene regulatory mechanisms that shape cellular identity and provide insight into developmental and evolutionary relationships between brain cell populations. Single cell sequencing data can aid the design of tools for targeted functional studies of brain circuit components, linking molecular signatures with anatomy, connectivity, morphology and physiology. Here, we discuss the fundamental principles of single cell transcriptome and epigenome sequencing, integrative computational analysis of the data, and key applications in neuroscience.
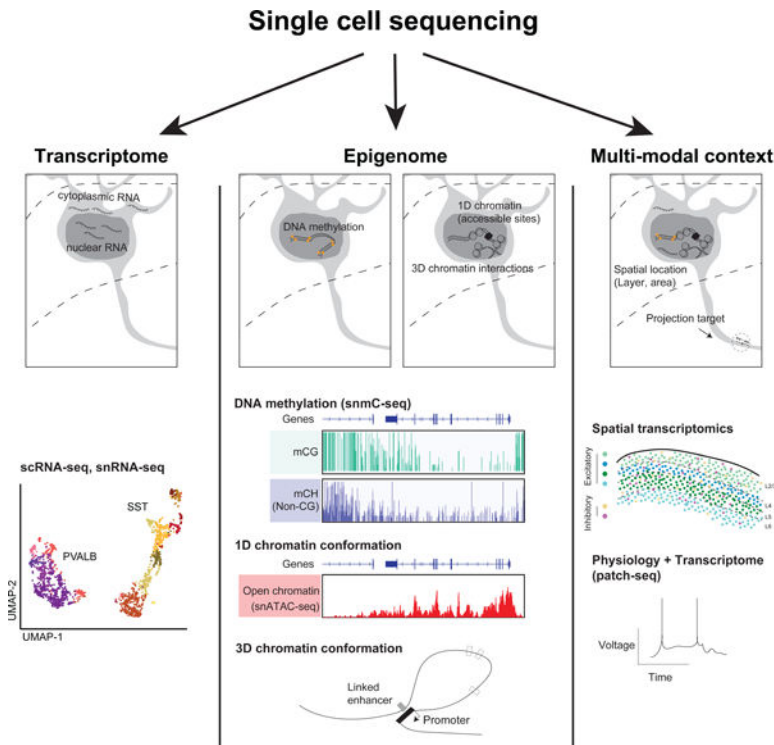
Graphical Abstract

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Single cell sequencing

eTOC/In Brief:

Sequencing the transcriptomes and epigenomes of single cells enables deep molecular characterization of brain cells with high throughput. Armand, Li, Xie *et al.* review the key experimental principles and computational methods and discuss recent landmark applications in neuroscience.

Keywords

Single cell sequencing; Transcriptome; Epigenome; DNA methylation; ATAC-seq; Open chromatin; Cell type; Spatial transcriptomics; Multi-omics; Cell state

Introduction

Brain cells are complex biological machines whose function is defined at the molecular level by conserved gene expression programs. Understanding the molecular identity of neural cell types requires measuring thousands of genes to uncover fine-grained distinctions between cells. At the same time, a large sample of cells is required to capture rare types and accurately assess heterogeneity. Fine-grained analysis using many genes and broad scope of coverage for many cells have often been viewed as mutually exclusive goals: experiments typically probe many molecular signatures in a limited number of samples or target a small number of genes in a large number of samples. In this Primer, we describe how high-throughput single-cell sequencing assays enable high-resolution, broad-scope analysis of brain cell types.

Sequencing DNA and/or RNA quantifies the information-bearing molecules that encode and enact each cell's biological identity. The abundance of RNA molecules defines the transcriptome, while chemical modifications of DNA and histone proteins along with the physical conformation of DNA within the nucleus define the epigenome (Fig. 1a). The interaction among thousands of genes and hundreds of thousands of gene regulatory DNA sequence elements gives rise in each cell to one of hundreds of robust cellular phenotypes. Neuroscience is fundamentally oriented toward understanding information processing in biological networks, and computational- and systems-neuroscientists seek to understand the emergence of complex adaptive behavior. It is thus natural that neuroscientists recognize the opportunity to use fine-grained, broad-scope single cell sequencing data to advance understanding of the transcriptomic and epigenomic identity of brain cell types.

We focus on the fundamental principles that define the resolution and scope of single cell transcriptome and epigenome sequencing; recent reviews provide more technical introductions to specific technologies (Luecken and Theis, 2019; Rostom et al., 2017; Stuart and Satija, 2019; Zhu et al., 2020) and applications to neurobiology (Fishell and Kepecs, 2019; Huang and Paul, 2019; Tosches and Laurent, 2019; Zeng and Sanes, 2017). We also describe key computational techniques for analyzing and interpreting single cell sequencing data. Although some bioinformatic methods may be unfamiliar to neuroscientists, many computational techniques used for electrophysiology and microscopy data also play a prominent role in single-cell sequencing. Indeed, computational neuroscience has a distinct perspective on understanding complex biological datasets compared with the field of bioinformatics (De Schutter, 2008), emphasizing synthetic modeling rather than hypothesis testing. We believe that this neuroscience perspective can inspire new ways of understanding the rich information provided by single cell genomics.

1. Single cell sequencing samples the cell's information-bearing molecules

Until recently, analysis of RNA and DNA in brain tissue was largely limited to bulk assays of the average composition of a tissue. By making a molecular smoothie, bulk assays destroy the association of transcripts or DNA modifications with individual cells and may fail to detect rare cell type signatures. Gene expression signatures of one cell type may be masked by complementary patterns in other cell types. Techniques for purifying whole cells or nuclei from specific cell populations can partially overcome these limitations. Yet, these require highly selective RNA or protein markers or genetic tools (e.g. mouse CRE lines), and can only be applied to previously characterized cell types.

Single-cell sequencing technologies measure RNA or DNA from individual cells without the need for selective cell purification. These techniques can be summarized by three characteristics: scope (number of cells), granularity (number of genes or epigenetic features), and spatial resolution (Fig. 1b). Moreover, single cell technologies measure different types of RNA transcripts or epigenetic modifications. Although single cell sequencing does not require cell purification, it does rely on high-quality primary or frozen tissue obtained from careful expert dissection of neuroanatomical regions.

The transcriptome of mammalian cells consists of 10^5–10^6 individual messenger RNA (mRNA) molecules (Shapiro et al., 2013). These messages represent some 4,000–12,000 different genes per cell (Yao et al., 2020a), including many distinct mRNA isoforms encoding multiple variants of each gene. Although post-transcriptional regulation is complex, the number of mRNA transcripts in a cell correlates with the abundance of the encoded protein and is thus a signature of cell identity and function (Liu et al., 2016).

Single cell epigenome assays measure DNA methylation, chromatin accessibility and/or chromatin conformation. These chemical and physical modifications of DNA are established during development and regulate gene expression through the lifespan. Whereas the transcriptome is a snapshot of gene expression, reflecting in part the cell's state and the impact of neural activity around the time of tissue collection (Hrvatin et al., 2018), epigenetic marks include both transient and stable modifications of long-lived chromatin components. Epigenomic data also reveal cis-regulatory elements such as enhancers that establish and maintain cell type-specific gene expression.

Together, single cell transcriptome and epigenome sequencing constitute a powerful toolkit for understanding the brain's cellular components, enabling functional studies of the roles of specific cell types in healthy and diseased brains.

2. Single cell transcriptomes

Single-cell mRNA sequencing (scRNA-seq) initiated a new era of molecular studies of brain cell types (Darmanis et al., 2015; Macosko et al., 2015; Tasic et al., 2016; Zeisel et al., 2015). Like bulk RNA-Seq, scRNA-Seq performs reverse transcription using mRNA molecules as the template to synthesize complementary DNA (cDNA). Next-generation sequencing (NGS) libraries are generated by amplifying cDNA, followed by fragmentation, additional amplification, and quantification by NGS.

2a. Plates and droplets

Individual cells or nuclei can be physically separated using plate-based cell sorting or droplets. Plate-based methods distribute individual cells or groups of cells into wells using fluorescence-activated cell sorting (FACS) (Tasic et al., 2016) or microscope-guided capillary pipettes (Paul et al., 2017). Droplet-based methods segregate cells into individual aqueous compartments in a lipid suspension (Macosko et al., 2015). Cells are lysed within droplets and mRNA molecules are reverse-transcribed and tagged with oligonucleotide barcodes that uniquely identify the cell. Droplet-based scRNA-seq is efficient due to the small reaction volume, and rapidly processes thousands of cells by continuous flow in microfluidic devices.

An alternative to physically isolating cells is multiplexed sequencing of cDNA indexed with unique combinations of cell-specific barcodes (Rosenberg et al., 2018). Groups of cells are distributed into wells and tagged with well-specific barcodes, then pooled, re-split, and tagged with another barcode. By repeatedly pooling, splitting and tagging, cells acquire random barcode combinations. The number of cells per pool can be adjusted to ensure most cells have a unique barcode combination with high probability. The cDNA from all cells is

then pooled and sequenced, and data computationally demultiplexed. Combinatorial indexing and droplet-based methods have higher throughput and lower cost per cell, whereas plate-based methods provide more sensitive gene detection and are more customizable (Ziegenhain et al., 2017).

Importantly, these strategies differ in how they quantify mRNA abundance (Fig. 2A). Droplet-based scRNA-Seq methods count the 5' or 3' ends of mRNA molecules, incorporating unique molecular identifiers (UMIs) to avoid double-counting PCR products of the same molecule (Kivioja et al., 2011). These data do not distinguish mRNA isoforms that share the same promoter (5' end) or transcription termination site (3' end). By contrast, plate-based strategies can sequence fragments of full-length transcripts, including internal fragments covering all expressed exons and splice junctions (Gupta et al., 2018; Tasic et al., 2016). These methods have shown that isoform usage differs between brain cell types. By combining full-transcript sequencing with UMIs, thousands of unique mRNA molecules can be reconstructed in single cells (Hagemann-Jensen et al., 2020).

2b. Single cells and single nuclei

The intricate network of dendrites, axons and glial processes in brain tissues present multiple challenges for scRNA-seq. Distal cellular compartments contain a substantial population of mRNAs, which enable dendritic protein synthesis (Cajigas et al., 2012; Tushev et al., 2018). Yet, these transcripts are largely lost following dissection and cell dissociation. Because all transcripts originate in the nucleus, scRNA-seq can still capture newly synthesized dendritic and axonal mRNAs in the nucleus or soma before transport to distal cell compartments.

Moreover, scRNA-seq cannot be applied to frozen human post-mortem brain tissue, since cell membranes are ruptured during freezing. An alternative is to isolate and sequence RNA from single nuclei (snRNA-seq) (Grindberg et al., 2013). Nuclear transcripts comprise ~20% −50% of all the RNA in the cell (Bakken et al., 2018), including immature and unspliced RNA molecules containing introns (Fig. 2a). Despite this, snRNA-seq provides robust markers of brain cell types (Ding et al., 2020; Lake et al., 2017) with resolution comparable to scRNA-seq (Bakken et al., 2018; Yao et al., 2020a). Dendritic transcripts may be separately detected in single cells by manual microdissection (Middleton et al., 2019).

snRNA-Seq data may be less affected by some technical artifacts than scRNA-Seq. Because nuclei are relatively uniform in size and morphology, snRNA-seq is more likely than scRNA-seq to capture an unbiased sample of all cell types. Some cells, such as cortical layer 5 pyramidal tract neurons, may be more likely to die during cell isolation for scRNA-seq (Tasic et al., 2018). Moreover, snRNA-seq is less susceptible to spurious activation of gene expression during tissue dissection and cell isolation (Lacar et al., 2016), although this can be mitigated with a transcription inhibitor (Wu et al., 2017).

The technical differences between sc and snRNA-seq call for care in data analysis and interpretation. Differential expression in sc vs. snRNA data may reflect the localization of transcripts, such as the highly abundant nuclear-localized non-coding RNA *Malat1* that regulates RNA splicing among other functions (Bakken et al., 2018; Yao et al., 2020a).

While scRNA-seq data are analyzed by mapping sequenced fragments to exons, snRNA-seq data analysis should also consider the abundance of intronic reads from pre-mRNA, which may account for >75% of fragments (Bakken et al., 2018; Gaidatzis et al., 2015). Indeed, the distinct information in exonic vs. intronic reads can be used to estimate changes in mRNA expression over time (Bergen et al., 2020; La Manno et al., 2018).

2c. Neuroscience applications of single cell transcriptomics

Cell type discovery and characterization—Single cell technologies have transformed our understanding of the diversity of brain cell types. Dozens of morphologically and functionally distinct brain cell types have been recognized, but traditional methods of microscopy and physiology have limited scope for comprehensively characterizing cell types in mammalian brain circuits with millions of cells. Recent scRNA-seq studies sampled >20,000–750,000 cells in individual mouse brain regions (Kozareva et al., 2020; Shekhar et al., 2016; Tasic et al., 2018; Yao et al., 2020a). Broader surveys have covered multiple regions in both the central and peripheral nervous systems of mouse (Marques et al., 2016; Saunders et al., 2018; Zeisel et al., 2018) with as many as 1.2 million cells comprising 379 cell types (Yao et al., 2020b). These are not yet comprehensive, and additional coverage is needed especially in subcortical regions. The largest individual sc/snRNA-seq datasets represent ~1% of the ~100 million neuronal and glial cells in the adult mouse brain.

The human brain, with ~1000 times more neuronal cells than the mouse brain (Azevedo et al., 2009), is more daunting to sample comprehensively. Single-nucleus transcriptomes from multiple regions of the human brain have established cell type taxonomies (Fig. 2B–D) (Darmanis et al., 2015; Habib et al., 2017; Lake et al., 2016). Comparing human with mouse and non-human primates reveals both conserved and distinct cell type signatures, including species-specific differences in the proportions of cell populations and in gene expression within homologous cell classes and types (Bakken et al., 2020; Hodge et al., 2019; Krienen et al., 2020). sc/snRNA-seq also provides insight into the diversity of glial cells including the oligodendrocyte lineage (Marques et al., 2016) and functional states of microglia (Masuda et al., 2019).

A recurring theme from single-cell studies of the brain is the greater diversity of glutamatergic vs. GABAergic neurons across brain regions (Tasic et al., 2018; Yao et al., 2020b; Zeisel et al., 2015) or species (Bakken et al., 2020; Lake et al., 2016). This is also supported by single cell DNA methylation data (see below) (Luo et al., 2017). Cortical excitatory neuron transcriptomes may be more heterogeneous due to their developmental origin spread across the dorsal telencephalon, compared with GABAergic populations that originate in the ganglionic eminences and migrate to cortex (Mayer et al., 2018).

scRNA-Seq has also been applied in non-mammalian brains, illuminating basic questions about development, cell regulation and aging. The transcriptomes of *Drosophila* olfactory projection neurons targeting different glomeruli are distinct during development but very similar in the adult (Li et al., 2017). Aging dramatically reduces the amount of RNA in *Drosophila* brain cells (Davie et al., 2018). Correlations in gene expression across 52 transcriptomic cell types in the *Drosophila* optic lobe support a model of phenotypic convergence, in which multiple transcription factors regulate the same set of effector genes

(Konstantinides et al., 2018). In the zebrafish brain, distinct neural stem cell populations were differentially affected by amyloid toxicity in an Alzheimer's disease model (Cosacak et al., 2019).

Development and plasticity—scRNA-seq reveals how neuronal and glial cell type identities emerge in developing mouse (Mayer et al., 2018; Telley et al., 2019; Tiklová et al., 2019) and human brain (Li et al., 2018; Nowakowski et al., 2017; Pollen et al., 2015), and even across whole organisms (Farrell et al., 2018). sc/snRNA-seq is particularly suited to studying brain development, because critical stem cell and neural progenitor populations may be transient and difficult to isolate without prior knowledge of specific molecular markers. scRNA-seq at multiple timepoints can reconstruct developmental trajectories, revealing the dynamics of transcription factors and downstream effectors regulating specialized neural populations (Di Bella et al., 2020; La Manno et al., 2020; Zhong et al., 2018, 2020). Differentiation trajectories of newborn neurons and oligodendrocytes in adults can also be reconstructed (Habib et al., 2016; Marques et al., 2016). An exciting frontier is the combination of sc/snRNA-seq with lineage tracing by artificial labeling (Raj et al., 2018) or using natural somatic mutations shared by cells within a lineage (Huang et al., 2020).

In addition to developmental dynamics, changes in gene expression due to neural activity and plasticity can be assessed by sn/scRNA-seq, as shown for visual cortical neurons following light exposure (Hrvatin et al., 2018). Moreover, analyzing activity-regulated genes can identify active cells (Hu et al., 2017). Given the key role of activity- and calcium signaling-dependent gene regulation in synaptic plasticity, single cell techniques will be essential for understanding the cell type-specific context of transcriptome dynamics.

The role of cell types in disease—Transcriptomic signatures of disease can be studied by snRNA-seq applied to post-mortem brain tissue from patients. By performing snRNA-seq in >80,000 single nuclei from 48 individuals with Alzheimer's disease (AD) pathology (Mathys et al., 2019), many more differentially expressed (DE) genes were detected in individual cell types than bulk tissue. In particular, gene dysregulation in glial cells was detected in snRNA-seq, but not bulk RNA-seq, due to the relatively low contribution of glia to the total RNA in the tissue. snRNA-seq has been applied to brain tissue from patients with disorders including AD (Grubman et al., 2019; Zhou et al., 2020), major depression (Nagy et al., 2020), autism (Velmeshev et al., 2019), Rett syndrome (Renthal et al., 2018) and multiple sclerosis (Schirmer et al., 2019).

Notably, disease studies have focused on a relatively modest number of transcriptomic or epigenomic clusters corresponding to cell classes, not individual types. Dissecting the contributions of fine-grained cell types to disease is important but challenging, as biases in the computational assignment of single cells to clusters could significantly impact the findings.

3. Single cell epigenomes

Stable patterns of gene expression are maintained partly through epigenetic modifications of DNA, such as genomic cytosine methylation, and of histone proteins. These marks impact

gene expression by directing the binding of transcription factor proteins (TF) to specific genomic regions, which can enhancer or reduce transcription of nearby or distal genes. Whereas individual mRNA molecules are relatively transient messengers with a half-life on the order of minutes to hours (Chen et al., 2008), covalent modifications of DNA and some histone proteins in post-mitotic neurons can persist for months to years (Maze et al., 2015). Yet, parts of the chromatin landscape can also be flexibly reconfigured following neuronal activity (Su et al., 2017). The dynamic regulation of the epigenome across brain cell types and through the lifespan establishes and maintains cell identity, and may support plasticity and behavior. Single cell epigenomes complement transcriptomes, providing insight into cell type-specific gene expression regulation.

Like snRNA-seq, epigenomic assays can be applied to dissociated nuclei obtained from frozen and archived post-mortem tissue. These methods assess the whole genome, including non-protein coding regions that comprise over 95% of the genome. Non-coding regions contain millions of candidate *cis*-regulatory elements (cCREs) that influence gene expression, including promoters and enhancers (Nord and West, 2019; Roadmap Epigenomics Consortium et al., 2015). cCREs are defined by open chromatin, low levels of DNA methylation, and physical interactions with gene promoters via the 3-dimensional conformation of DNA in the nucleus.

Identifying cell type-specific enhancers can guide development of viral vectors and transgenic lines targeting cell types for functional studies (Graybuck et al., 2019; Hrvatin et al., 2019). In the future, it will be interesting to investigate dynamic regulation of the epigenome, including developmental trajectories (Trevino et al., 2020) and neuronal activity dependent modifications of the genome during learning and memory (Day et al., 2013), at the single cell level.

3a. DNA methylation

In mammals, genomic cytosine can be covalently modified by a methyl group. Methylcytosine (mC) is frequently found at CG dinucleotides, where it often correlates with transcriptional repression. DNA methylation in brain cells has two unique features compared with other cells (Kinde et al., 2015). First, neurons have high levels of hydroxymethylcytosine (hmC), a derivative of mC that may have distinct functional roles (Kriaucionis and Heintz, 2009; Mellén et al., 2017). Second, neurons accumulate substantial mC at non-CG sites, primarily CA and CT dinucleotides, during postnatal development (Lister et al., 2013; Xie et al., 2012). The pattern of DNA mC and hmC at nearly 1 billion CG and non-CG sites throughout the genome is a rich signature of cell type-specific gene regulation in neurons and glia (Kozlenkov et al., 2018; Mo et al., 2015).

DNA methylation can be measured in single cells by plate-based sorting of individual nuclei, followed by bisulfite conversion of DNA and multiplexed sequencing (Luo et al., 2017; Mulqueen et al., 2018; Smallwood et al., 2014), capturing up to ~30% of the genome per cell (Luo et al., 2018a). Sodium bisulfite treatment converts cytosine to uracil, while methylcytosine is protected from conversion; sequencing the bisulfite-converted DNA fragments reveals the methylation status at each cytosine position. This technique produced over 110,000 single cell DNA methylomes from 45 mouse brain regions (Liu et al., 2020),

identifying ~161 fine-grained neuronal cell types across the cortex, striatum, and olfactory areas. Each neuron population has unique DNA methylation signatures at gene bodies, which largely correlate with gene expression, as well as thousands of small (100–500 base-pair) differentially methylated regions (DMRs) which define cCREs (Fig. 3b). Droplet-based bisulfite chemistry could potentially increase the efficiency of single cell DNA methylome profiling, but the high cost of whole-genome compared with transcriptome sequencing would remain a factor limiting the application of this technique to substantially larger numbers of cells. In the future, single cell analysis of hydroxymethylation will elucidate the relative amount of mC and hmC in neuronal cell types (Mooijman et al., 2016).

3b. Mapping chromatin accessibility in 1D

Just as genes are basic units of transcriptome analysis, cCREs are a foundation for analysis of the epigenome. cCREs can be defined by the displacement of nucleosomes due to the binding of transcription factor proteins, forming regions of accessible chromatin. The single nucleus assay of transposase-accessible chromatin (snATAC-Seq) tags, captures and maps such regions using the Tn5 transposase enzyme (Buenrostro et al., 2015; Cusanovich et al., 2015).

Like sc/snRNA-seq, snATAC-seq can use droplets or combinatorial indexing (Cusanovich et al., 2015; Preissl et al., 2018). Combinatorial indexing snATAC-seq can be applied to frozen brain tissue (Preissl et al., 2018), and scales to $>10^4$ cells per experiment (Li et al., 2020). Notably, of all the distal cCREs that can be detected in a particular cell type, snATAC-seq captures around ~2–3% in each cell (Li et al., 2020; Preissl et al., 2018). This sparse coverage could reflect the sensitivity of the assay, and/or heterogeneous accessibility across individual cells of the same type.

Despite its sparseness, snATAC-seq data reliably distinguish fine-grained neuron and glia types. For example, 160 cell types were identified using snATAC with a median of ~5,000 unique sequenced fragments per cell in >800,000 mouse brain cells (Li et al., 2020). A total of ~500,000 accessible regions (cCREs) were found across all cell types. Alternatively, droplets can be used to physically separate nuclei for barcoding after tagging open chromatin with Tn5 transposase in bulk, yielding up to a median of ~34,000 unique sequenced fragments per cell (Lareau et al., 2019).

Whereas sc/snRNA-seq data are quantified using known genes and transcripts, regions of accessible chromatin are distributed throughout the genome and generally not known *a priori*. Many types of features have been used to quantify snATAC data, including gene promoters, putative enhancers, or combinations of regions sharing common DNA sequence motifs (Bravo González-Blas et al., 2019; Cusanovich et al., 2018; Fang et al., 2019; Schep et al., 2017). Each method is sensitive to different biological signals and is affected differently by the noise introduced by sampling a sparse set of fragments. Systematic benchmarking and comparison of computational analysis methods is important to achieve the maximum possible resolution (Chen et al., 2019a).

3c. Mapping chromatin conformation in 3D

CREs and gene promoters interact via the 3-dimensional organization of chromatin loops and domains (Fig. 3D). Single-cell assays of 3D chromatin conformation, closely related to Hi-C used in bulk tissue (Lieberman-Aiden et al., 2009), identify pairs of interacting regions by cross-linking, fragmenting, and re-ligating DNA fragments that are in physical proximity. Sequencing the resulting libraries detects chimeric DNA fragments that contain sequences separated by long distances in the linear (1D) genome, but which are closely associated in 3D, forming a contact matrix (Fig. 3C). A single-cell Hi-C study in neurons obtained ~1 million contacts per cell, illuminating the development of inverted chromatin in rod photoreceptor cells and the segregation of olfactory receptor genes in olfactory sensory neurons (Tan et al., 2019).

A challenge for 3D chromatin assays is the large space of possible pairwise contacts among chromosomal locations. Even if a substantial portion of the genome could be captured in a single cell Hi-C assay, the resulting pairwise contact matrix would contain only a small fraction of the 3D contacts between genomic locations because each DNA fragment can only participate in one ligation event. Cluster analysis of single cell Hi-C data can identify groups of cells with similar chromatin organization (Zhou et al., 2019), but on its own it does not reliably distinguish cortical neuron types (Lee et al., 2019; Li et al., 2019a).

An alternative strategy uses thin (~0.2 μm) tissue slices, followed by laser-capture microdissection of single nuclei and sequencing fragments contained in the same section (Beagrie et al., 2017). This confirms differences in chromatin organization between broad neuron classes without proximity ligation, but it does not provide single cell resolution contact estimates (Winick-Ng et al., 2020).

3d. Applications of single cell epigenomics

Dissecting neuronal gene regulatory networks—Single cell epigenomes can be used to characterize the networks of transcription factors (TFs) and cCREs that establish and maintain brain cell identity. DNA-binding TF proteins recognize specific sequences, or motifs, typically 6–10 base pairs long. The TFs that regulate specific brain cell types can be identified by detecting enriched sequences within cCREs, and comparing them with known TF binding motifs (Lake et al., 2018; Li et al., 2020; Liu et al., 2020; Luo et al., 2017; Preissl et al., 2018). This approach was applied to integrated multimodal (transcriptomic and epigenomic) data from mouse motor cortex, revealing a role for the TF *Rfx3* in regulating Layer 2/3 excitatory neurons (Yao et al., 2020a). High precision analysis of the exact location of TF binding can be performed by detecting so-called footprints, i.e. characteristic chromatin accessibility patterns at the binding site (Li et al., 2019b; Mo et al., 2015). However, footprint analysis has so far been limited to bulk ATAC-seq.

Linking genetic elements with disease risk—Although genome-wide association studies (GWAS) have identified risk loci for some neurological and psychiatric disorders, the selective vulnerability of specific brain cell types in each disease remains largely unknown (Wang et al., 2018a). Partitioned heritability analysis using cell type-specific transcriptomes or epigenomes can identify cell types whose active genes are enriched near GWAS risk loci,

suggesting a potential direct or indirect role in the disease (Fig. 3F) (Calderon et al., 2017). This does not require molecular data from patient tissue samples, instead using sc/snRNA-seq from healthy subjects together with GWAS information. Partitioned heritability analysis can also use epigenomic data to link genetic risk variants with cell type-specific cCREs (Lake et al., 2018; Luo et al., 2019).

4. Single-cell spatial transcriptomics

sc/snRNA-seq disrupts tissues to isolate cells, sacrificing the spatial context of neural circuits. Brain cells migrate to unique laminar and areal positions before birth, which constrain their connectivity. For neurons, spatial context and connectivity are essential to cell identity and function.

Two approaches have emerged to provide spatially resolved single-cell transcriptomes (Fig. 1B). Spatially resolved sequencing uses slides pre-patterned with beads carrying unique DNA barcodes to tag mRNA molecules based on their location in a brain slice, enabling whole-transcriptome sequencing with ~10 μm spatial resolution (Rodriques et al., 2019; Ståhl et al., 2016). Assigning transcripts to individual cells requires finer spatial resolution, below ~1 μm, which can be achieved by optical imaging-based methods. Single molecule fluorescence *in situ* hybridization (smFISH) detects individual mRNA transcripts with high sensitivity using fluorescent oligonucleotide probes (Raj et al., 2008). Probes can be localized with diffraction-limited resolution of ~250 nm or larger, sufficient to assign transcripts within cell somata (Moffitt et al., 2018; Zhang et al., 2020a). Finer spatial resolution can be achieved by super-resolution imaging (Lubeck and Cai, 2012) or isotropic physical expansion of tissue prior to imaging (Chen et al., 2015a; Xia et al., 2019).

smFISH uses pre-defined probes to detect specific transcripts rather than directly sequencing mRNA molecules. To achieve broad transcriptome coverage of hundreds to thousands of transcripts, smFISH may be combined with barcoding strategies and multiple rounds of optical decoding (Chen et al., 2015b; Wang et al., 2018b; Zhang et al., 2020a). Transcripts are identified by unique combinations of probes. Error-correction can be achieved with redundant probes. The number of detectable transcripts scales exponentially with the number of probes. While most studies in brain have used panels of several hundred genes (Kim et al., 2019a; Moffitt et al., 2018; Zhang et al., 2020a), up to 10,000 transcripts have been imaged in cell lines (Xia et al., 2019) and in mouse brain (Eng et al., 2019). These methods detect up to 3,300 genes per cell in mouse brain (Eng et al., 2019), ~3-fold less than the highest-resolution sc/snRNA-seq (Yao et al., 2020a).

Spatial transcriptomics can dissect neural circuits with complex anatomical organization, such as specific subdivisions of mouse hypothalamus that are critical for social and reproductive behaviors (Kim et al., 2019b; Moffitt et al., 2018). Spatial maps of ~1 million cells in the hypothalamic preoptic region revealed over 70 molecularly distinct cell types (Moffitt et al., 2018). Notably, fine-grained cell type resolution was achieved despite probing only 155 genes, suggesting that neuron types can often be defined by a modest set of judiciously chosen mRNA targets.

While these methods have clear advantages for interrogating brain cells in their spatial context, they currently have key limitations compared with dissociated single cell transcriptome sequencing. First, smFISH requires designing probes to query a predefined list of genes. Marker gene selection and probe design are critical, and the data cannot provide information about genes which are not pre-selected for study. Second, it remains challenging to unambiguously assign transcripts to single cells. For Slide-seq (Rodriques et al., 2019), this is limited by spatial resolution (~10 μm), while smFISH based methods use computational image processing to define cell boundaries in dense brain tissue. Because many neuronal mRNAs localize to distal dendrites and axons, they cannot be assigned to a cell. Third, smFISH methods may require complex experimental set up and time-consuming imaging, a challenge for large tissue volumes such as whole mammalian brains.

5. Multi-omics: Single cells, multiple data modalities

Fully characterizing cell identity requires jointly measuring gene expression and epigenetic regulation in the same cell. Although single cell epigenetic data can identify cCREs and link them with genes via chromatin looping in 3D, they cannot establish whether an active cCRE drives gene expression. For this, RNA measurements must be obtained from the same cell. Single cell transcriptome and epigenome datasets can be computationally integrated (see below) (Butler et al., 2018; Haghverdi et al., 2018; Hie et al., 2019; Stuart et al., 2019; Welch et al., 2019), but empirical confirmation requires multiomic sequencing in single cells. Moreover, functional validation of cell types identified by single cell sequencing requires techniques that combine sequencing with physiology, connectivity, and/or morphology.

5a. Multimodal sequencing in single cells

Multiomic sequencing combines DNA and RNA measurements in single cells. As many as 24 distinct multiomic techniques have been published since 2015, covering 11 combinations of 7 modalities (Zhu et al., 2020). Here we focus on the main techniques that have been applied in brain tissue to date.

An important class of multiomic methods combines transcriptome (sc/snRNA-seq) and chromatin accessibility (snATAC-seq) measurements in the same cell (Cao et al., 2018; Chen et al., 2019b; Zhu et al., 2019). Tagging open chromatin with transposase and reverse transcription of mRNA can be performed sequentially, in either order, without damaging nuclear structure. Combinatorial indexing or droplet-based techniques can be used to achieve high throughput. Paired snRNA-seq and snATAC-seq of over 15,000 cells in fetal and adult mouse cortex revealed cell type-specific dynamic regulation of promoter accessibility during brain development (Chen et al., 2019b; Zhu et al., 2019). These assays currently provide ~1000-fold less RNA coverage than state-of-the-art sc/snRNA-seq from fresh brain tissue, with 400–4,000 RNA-seq reads per cell and 1,000–6,000 ATAC-seq reads (Fig. 1b). As a result, high-throughput multi-omics studies in mouse cerebral cortex identified 9–22 cell types (Chen et al., 2019b; Zhu et al., 2019), compared with over 100 cell types found using scRNA-seq (Tasic et al., 2018; Yao et al., 2020a, 2020b).

Multiomic sequencing can also assay the transcriptome and DNA methylome from the same cell (Angermueller et al., 2016; Hou et al., 2016; Hu et al., 2016; Luo et al., 2018b). These methods have been applied to modest numbers of cells (up to several thousand) due to the large amount of sequencing required to sample DNA methylation genome-wide. Yet, the depth of RNA and DNA coverage are comparable to scRNA-seq or single cell DNA methylome sequencing, respectively. The methods can be extended to measure open chromatin using single cell nucleosome occupancy and methylation sequencing (NOME-seq) (Pott, 2017), as recently demonstrated in human brain samples (Luo et al., 2019).

Finally, DNA methylomes can be combined with 3D chromatin conformation by bisulfite conversion of chimeric DNA fragments from proximity ligation (Lee et al., 2019; Li et al., 2019a). Each sequenced read does double duty, providing contact information via the location of mapped read ends together with DNA methylation information about cytosines within the fragments. This was applied to over 4,000 neurons in human prefrontal cortex, generating deep coverage with 1.2 million reads per cell (Lee et al., 2019). The chromatin conformation data from single cells distinguish major cell classes such as neurons vs. astrocytes, but not neuron subtypes. Multiomic sequencing solves this problem by clustering cells using high resolution DNA methylation information, followed by separate analysis of the pooled (pseudo-bulk) chromatin conformation profiles from each cell type. The authors found chromatin loops connecting the excitatory-neuron specific transcription factor gene *SATB2* with a nearby non-coding RNA in excitatory, but not inhibitory, neurons (Lee et al., 2019).

5b. Combining single cell transcriptomes with physiology and morphology

Single cell sequencing and neuronal physiology can be directly linked by performing whole-cell patch recordings in brain slices, followed by cytosol extraction through the patch pipette for transcriptome sequencing (patch-seq) (Cadwell et al., 2016; Fuzik et al., 2016). Cells can be filled with biocytin following patch recording, for subsequent imaging and morphological reconstruction. Compared with sc/snRNA-seq, patch-seq is laborious and requires individually characterizing cells by patch-clamp recording followed by labeling, imaging and scRNA sequencing. Despite this, it was applied to 1,300 cells in the mouse primary motor cortex, identifying >70 cell types with their transcriptomic and morpho-electrical properties (Scala et al., 2020). Another study profiled 3,700 GABAergic neurons in mouse visual cortex (Gouwens et al., 2020), showing that inhibitory interneurons in specific transcriptomic classes have distinct laminar distributions and electrophysiological properties. Conversely this study also highlights the difficulty in identifying a one-to-one correspondence between morpho-electric and transcriptomic cell types.

5c. Combining single cell sequencing with connectivity

By infecting axon terminals in a defined brain region with a retrograde virus, the cell bodies of source neurons projecting to that region can be labeled, sorted, and their transcriptomes measured by sc/snRNA-seq (Retro-seq) (Tasic et al., 2018). Retro-seq data can be jointly analyzed with other sc/snRNA-seq datasets to add axon projection information onto transcriptomic signatures. This approach helped identify sub-populations of layer 5 pyramidal-tract neurons in the mouse motor cortex with distinct projections in the thalamus

and medulla, and different roles in motor control (Economo et al., 2018). Retro-seq can also be used with single cell DNA methylation to link projections with gene regulatory signatures (Zhang et al., 2020b). Retrograde labeling in combination with single-cell spatial transcriptomics provides the laminar and regional location of neurons with specific projections (Zhang et al., 2020a). This showed that mouse motor cortical intratalencephalic-projecting (IT) neurons form a many-to-many projection pattern: cells of the same transcriptomic type project to multiple cortical regions, while those regions receive input from multiple cell types (Zhang et al., 2020a). Although transcriptomic or epigenomic cell types often align with cell populations defined by axonal projections, this is not always the case. For example, Retro-seq analysis of mouse primary visual cortical pyramidal cells projecting to different higher-order visual areas form a continuous transcriptomic population, although some differentially expressed genes were detected (Kim et al., 2020). Likewise, uniform transcriptomic cell types in the ventromedial nucleus of the hypothalamus comprise mixed populations with distinct projection targets (Kim et al., 2019b).

Individual neurons can make multiple collateral projections that may not be captured by labeling individual targets, as in Retro-seq. An alternative strategy uses short oligonucleotide barcodes to detect projections from individual neurons to multiple regions (MAPseq) (Kebschull et al., 2016). Neurons in a starting region are labeled with a library of barcoded Sindbis viruses, followed by microdissection of small volumes from putative target regions and sequencing of the barcodes. MAPseq can be combined with *in situ* barcode sequencing in the source region to obtain spatial and laminar information about the projection cells, and with FISH to assess expression of marker genes (Chen et al., 2019c). Further development of these methods, e.g. by combining single cell transcriptome sequencing with multiplex projection labeling, will help distill the relationship between transcriptomic and anatomic signatures of neuron types.

6. Computational analysis of single cell sequencing data

Single cell sequencing datasets have thousands of features (e.g. genes, or genomic bins) measured across thousands to millions of single cells. Analysing these data requires sophisticated computational procedures, and analysis pipelines can significantly impact the findings. Open source software packages allow neurobiologists to apply sophisticated analytic strategies (Butler et al., 2018; Kharchenko et al., 2014; Stuart et al., 2019; Wolf et al., 2018). Here we outline analysis steps that are common to many analysis workflows, emphasizing the conceptual foundations. More technical details can be found in recent reviews (Efremova and Teichmann, 2020; Luecken and Theis, 2019).

1. **Quality control, mapping and quantification.** The first step in analyzing sc/snRNA-seq, snATAC-seq or single cell DNA methylation data is computational demultiplexing and alignment of sequenced reads from each cell with a reference genome or transcriptome. At this stage, it is also important to remove low-quality cells or reads. Each single cell sequencing technology has its own potential pitfalls, but common themes include low library complexity due to inefficient RNA/DNA capture; contamination by high levels of mitochondrial RNA; or

incomplete bisulfite conversion for DNA methylation assays. In most cases, low-quality cells can be removed by appropriate choice of thresholds.

Following quality control and quantification, transcriptome data can be represented as a table containing a row for each cell, and a column for each gene or transcript. The entries of the table describe the estimated abundance of each gene. Single cell DNA methylation and ATAC-seq data have more diverse representations, which may include gene-oriented signatures (*e.g.* promoter chromatin accessibility or gene body DNA methylation) or non-gene signatures (*e.g.* accessibility or methylation in genomic bins).

2. **Feature selection and dimensionality reduction.** Gene expression and epigenomic measurements define high-dimensional feature spaces, where the data dimension is the number of quantified genes or features. However, correlations among the features reduce the intrinsic data dimensionality, *i.e.* the number of independent parameters needed to capture the meaningful biological variability. Dimensionality reduction removes noise and simplifies subsequent analysis (Sun et al., 2019). First, features with little detectable expression or low variance across cells are removed. Next, linear projection, *e.g.* using principal component analysis (PCA), reduces the dimension from several thousand to ~50–100 features. The exact number of PCs to be retained is an unconstrained parameter, but in practice results may not be highly sensitive to this choice.

3. **Reducing data sparseness.** Single cell data are often sparse: genes which are expressed in a cell may be missed due to random sampling in sc/snRNA-seq, while snATAC-seq captures only a fraction of all regions of accessible chromatin in each cell. Pre-processing by data diffusion can help to combat sparseness and may improve visualization and cluster analysis (van Dijk et al., 2018).

4. **Doublets.** A common pitfall for single cell transcriptome and epigenome assays is the potential for incorrectly combining information from two or more cells, called doublets. This can occur due to physical association of cells that are captured in the same droplet or well. Doublets can also occur due to barcode collisions in combinatorial indexing experiments, in which multiple cells acquire the same combination of index barcodes by chance. Some doublet events can be removed by computational methods that detect signatures of apparent hybrid gene expression (Wolock et al., 2019). Although computational doublet removal may reduce the extent of contamination, it should be treated with caution when the true number of cell types is not known. Ultimately, putative cell types described by single cell sequencing must be confirmed by alternative techniques, such as spatial transcriptomics, which may be less susceptible to doublets.

5. **Visualization.** Exploratory data visualization builds intuition about the structure of abstract, high-dimensional single cell sequencing data, and helps formulate hypotheses for subsequent formal statistical analysis. Useful visualizations embed cells in a 2- or 3-dimensional space, optimizing the layout of cells so that their mutual distances approximate the distances of the high-dimensional data. Nonlinear manifold learning and data embedding techniques, including tSNE,

UMAP and force-directed layout embeddings (McInnes et al., 2018), make different compromises to force the complex relationships between single cell feature vectors into a 2- or 3-dimensional layout. More sophisticated approaches using variational autoencoders have also been applied to single cell data (Eraslan et al., 2019; Lopez et al., 2018). Low-dimensional embeddings are always imperfect; they are distorted and non-unique representations of the high-dimensional data. They should be used for exploration, but not hypothesis testing or drawing conclusions.

6. **Discrete clusters and continuous latent factors.** The simplest description of a single cell sequencing dataset is in terms of discrete clusters, or groups of cells with distinct molecular signatures. Clusters can often be arranged in a hierarchy, with groups of cells forming closely related sub-classes and classes (Zeng and Sanes, 2017). Clustering large-scale datasets with millions of cells can be accomplished with efficient community detection algorithms that optimize a modularity index (Traag et al., 2019). Yet, cluster analysis on its own does not provide evidence of the statistical reliability or biological reality of resulting cell types.

In addition to discrete clusters, cells may also differ along one or more continuous gradients. Continuous trajectories occur during brain development (Nowakowski et al., 2017) and in differentiating cell populations in the adult, such as oligodendrocyte lineage cells (Marques et al., 2016) or newborn granule cells in the dentate gyrus of the hippocampus (Habib et al., 2016). Trajectory or pseudotime estimation procedures seek to estimate the continuous relationships between single cells (Cao et al., 2019; Trapnell et al., 2014). Continuous gradients in post-mitotic neurons can reflect the spatial distribution of neurons across lamina or cortical regions, or graded functional properties (Liu et al., 2020). Methods to distinguish continuous and discrete variation in neural populations are a focus of current research (Harris et al., 2018; Stanley et al., 2020).

7. **Computational integration of multiple datasets.** Correlated transcriptomic and epigenomic signatures can allow computational methods to integrate independent datasets despite batch effects and systematic differences (Efremova and Teichmann, 2020). Parametric methods, such as approximate canonical correlation analysis (CCA) implemented by Seurat (Stuart et al., 2019) or non-negative matrix factorization (NMF) (Welch et al., 2019), project cells from multiple datasets into a common, low-dimensional space where they can be directly compared, clustered, and analyzed. Non-parametric methods such as mutual nearest neighbors (MNN) can also link cells across datasets, without learning a linear or nonlinear embedding in a common space (Haghverdi et al., 2018; Hie et al., 2019; Luo et al., 2019). These techniques link cells in one dataset with closely matching cells in another dataset, e.g. by selecting the cells with the most correlated gene-oriented signatures. A recent study integrated 9 multimodal datasets from the mouse primary motor cortex, including 7 sc/

snRNA-seq datasets as well as single cell DNA methylation and ATAC-seq data (Yao et al., 2020a).

8. **Cluster validation.** Analysis of the statistical significance and reproducibility of clusters can help to define an appropriate cluster resolution, providing objective criteria to resolve the classic tension between lumpers (who tend to combine things in groups) and splitters (who emphasize fine distinctions and granular categories). A stringent test of cell type replicability uses transcriptomic signatures of a cell type learned from one or more datasets to try to predict the identity of cells in an independent dataset, providing a statistical score (area under the ROC curve) that quantifies the match between clusters (Crow et al., 2018). Using this approach, signatures of cardinal GABAergic neuron types defined by transgenic mouse lines were validated in independent scRNA-seq datasets (Paul et al., 2017). This approach also verified the replicability of over 70 transcriptomic cell types in mouse primary motor cortex across 7 scRNA and snRNA-seq datasets (Yao et al., 2020a).

    Epigenomic signatures can further validate cell type distinctions and help to dissect their molecular regulation. By cross-validation using independent genes for clustering and data integration followed by testing cluster validity on held-out gene sets, a systematic comparison of scRNA, snRNA, DNA methylation and open chromatin data validated the cross-modal identity of mouse cortical cell types (Yao et al., 2020a).

9. **Downstream analysis.** After clustering, a wide variety of analyses can be used to further explore and characterize single cell sequencing data. Differentially expressed genes between cell types, or across samples or conditions, can be identified with statistical procedures (Finak et al., 2015; Kharchenko et al., 2014). Unfortunately, different computational methods diverge significantly in estimating differential expression (Soneson and Robinson, 2018), and techniques specifically designed for sc/snRNA-seq may not perform better than standard methods used for bulk RNA-seq (Wang et al., 2019).

    Single cell epigenomic data can be used to identify cCREs by detecting snATAC-seq peaks (Preissl et al., 2018), and/or differentially methylated regions (Luo et al., 2017). Moreover, snATAC-seq data can link cCREs and the genes they directly regulate (Pliner et al., 2018) (Fig. 3e). These data can be used as a starting point for analysis of gene regulatory networks, and for predicting cell type specific enhancers for use in viral targeting tools (Hrvatin et al., 2019; Mich et al., 2020).

7. Limitations, opportunities and outlook

Single cell sequencing is transforming many areas of biology, and the impact may be especially profound in neuroscience. The diversity of brain cell types and the complex regulation of cell identity and experience-dependent plasticity throughout the life of a neuron make single cell analysis essential for understanding brain circuits.

Like any emerging technology, single cell sequencing has important limitations. Doublets, in which two or more cells are captured in a single well or droplet or tagged with the same barcode, can distort single cell datasets and create spurious hybrid cell types. Contamination of scRNA-seq libraries, e.g. by cytoplasmic RNA from other cells in the same tissue, can lead to false positive gene detection. False negatives, or dropouts, are an even greater concern, since the sampling strategy typically recovers only a fraction of the total RNA or DNA. Single cell techniques can also be affected by non-physiological transcription caused by tissue dissection and cell dissociation (Lacar et al., 2016; Wu et al., 2017).

A more fundamental limitation of single cell techniques is the need to destroy the cell in order to extract its molecular signatures for sequencing. Although multiomic and multimodal techniques can increase the information obtained per cell, these methods are inherently snapshots of the life of a cell and do not record the dynamic changes in neural regulation that are critical to brain function. Some dynamic information can be inferred by analyzing mature vs. immature RNA transcripts (La Manno et al., 2018), but *bona fide* measurements of transcriptome dynamics in single cells (Cao et al., 2020) would be valuable in the brain.

The complexity of single cell datasets is both an opportunity and a challenge. Sophisticated computational methods for clustering, visualization and analysis of single cell data provide rich insight into the function of cellular machines. However, different computational methods or parameter choices can lead to divergent results for the same dataset. Clustering procedures are notorious for their inconsistency and lack of robustness, leading to subjectivity in the description of single cell data in terms of cell types. Single cell studies must address this by transparently investigating and reporting how biological conclusions depend on analytic choices.

The greatest challenge is to unify information from single cell sequencing with functional and physiological measures of neuronal cell type identity. Although there is remarkable correspondence between cell types defined by transcriptomic, epigenomic, anatomical and morphological criteria, efforts to harmonize these data at the level of hundreds of fine-grained brain cell types are still at an early stage.

As innovations in single cell technologies overcome these challenges, they are opening new frontiers for neuroscience research. Single cell sequencing is providing comprehensive, whole-brain analyses of the cellular components of brains in multiple species and across the stages of development. Techniques that link single cell sequencing with electrophysiology, morphology, and connectivity will profoundly change our understanding of the dimensions of neuronal diversity. Cross-species and developmental comparisons of brain cell types will transform our understanding of brain ontogeny and phylogeny, potentially leading to a detailed mechanistic understanding of the evolutionary relationships among neural populations (Arendt et al., 2016). Evolutionary conservation and divergence provide insight into the gene regulatory programs that were selected for their impact on cognitive function (Bakken et al., 2020; Hodge et al., 2019; Krienen et al., 2020). By combining single cell sequencing with techniques for activity-dependent labeling, neuroscientists will be able to link transcriptomes and epigenomes with the roles of specific cell types in behavior (Kim et

al., 2019b). Alongside the technical progress described here, we expect that new conceptual and theoretical frameworks will emerge to turn the enormous information from single cell sequencing into organized and systematic knowledge about brain cell ontogeny and function.

Acknowledgments

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Highlights:

- Single cell sequencing provides deep molecular information about neuronal and glial transcriptomes and epigenomes

- Multiple experimental strategies balance efficient high-throughput data collection with deep coverage of mRNA transcripts, DNA methylation, or open chromatin regions

- Multi-omic methods combine different data modalities in the same cell

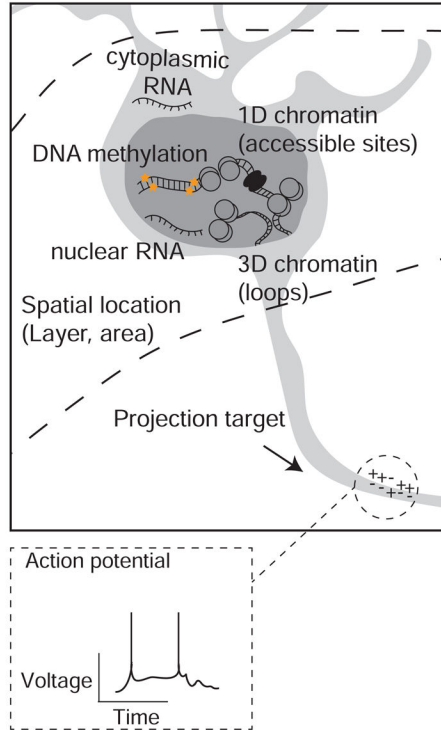- Techniques such as spatial transcriptomics and patch-seq provide functional and anatomical information in combination with molecular signatures
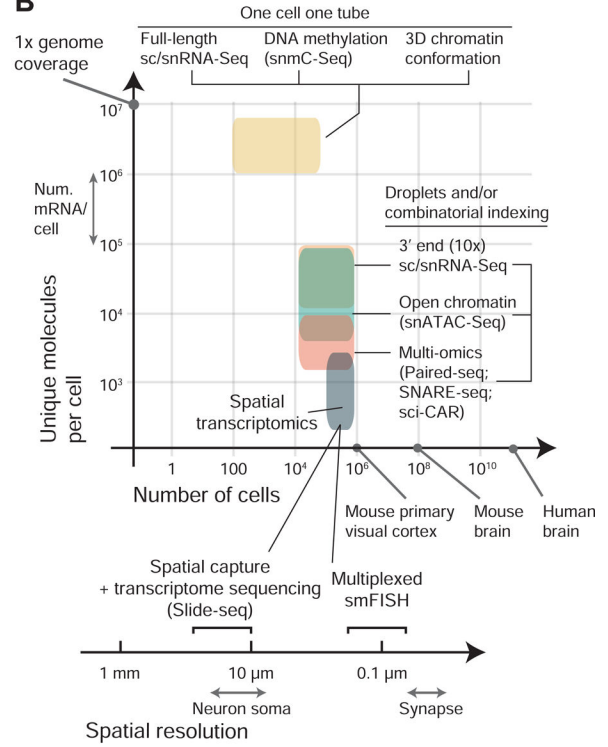
Figure 1.
Single cell sequencing modalities for neuroscience. (A) Transcriptome and epigenome signatures that can be assayed by single cell sequencing, alongside spatial context, connectivity, and physiology. (B) Comparison of single-cell molecular assays in terms of the number of cells (scope, x axis) and number of unique molecules characterized per cell (depth, y axis). Below: spatial resolution for imaging and spatial transcriptomics. Shaded boxes show the range of values achieved in recent single-cell studies of brain cells. The estimated number of cells in an adult human brain (Azevedo et al., 2009) or mouse brain (Erö et al., 2018), the typical size of a neuronal cell body (Zhang et al., 2020a), and the estimated number of mRNA molecules in a mammalian cell (Shapiro et al., 2013) are indicated for comparison.
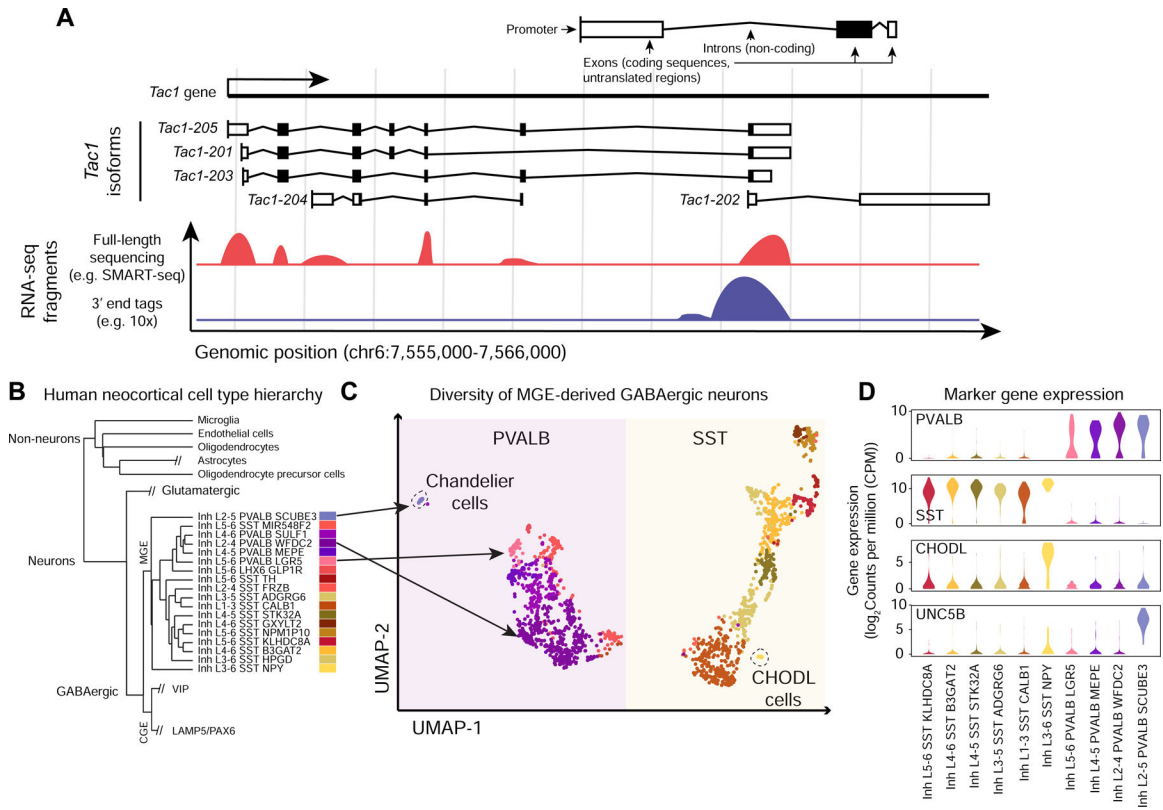
Figure 2.
Single cell transcriptomics applications in neuroscience. (A) The mouse gene *Tac1*, encoding the neuropeptide precursor Tachykinin-1 which is a specific marker of a subset of MGE-derived GABAergic neurons in cortex (Yao et al., 2020a), has five isoforms with different promoters, exon usage, and end sites (black gene models). Schematic tracks illustrate full-length RNA-sequencing (red) or 3'-end tagging (blue). (B) Hierarchical clustering of cell types in human medial temporal gyrus adapted from (Hodge et al., 2019), focusing on medial ganglionic eminence (MGE)-derived GABAergic interneurons. Branches from other cell classes truncated to (double-slash). CGE: caudal ganglionic eminence. (C) A two-dimensional embedding of 2,260 single nucleus transcriptomes computed using Uniform Manifold Approximation and Projection (UMAP) (McInnes et al., 2018). Each point represents one cell, and the arrangement of cells is an approximate representation of their degree of similarity (nearby points) or difference (distant points). Points are colored according to the cell type clusters from (B) (arrows). Rare subpopulations of *SST CHODL* and Chandelier cells are circled. (D) Violin plots show expression of marker genes in MGE-derived interneurons.
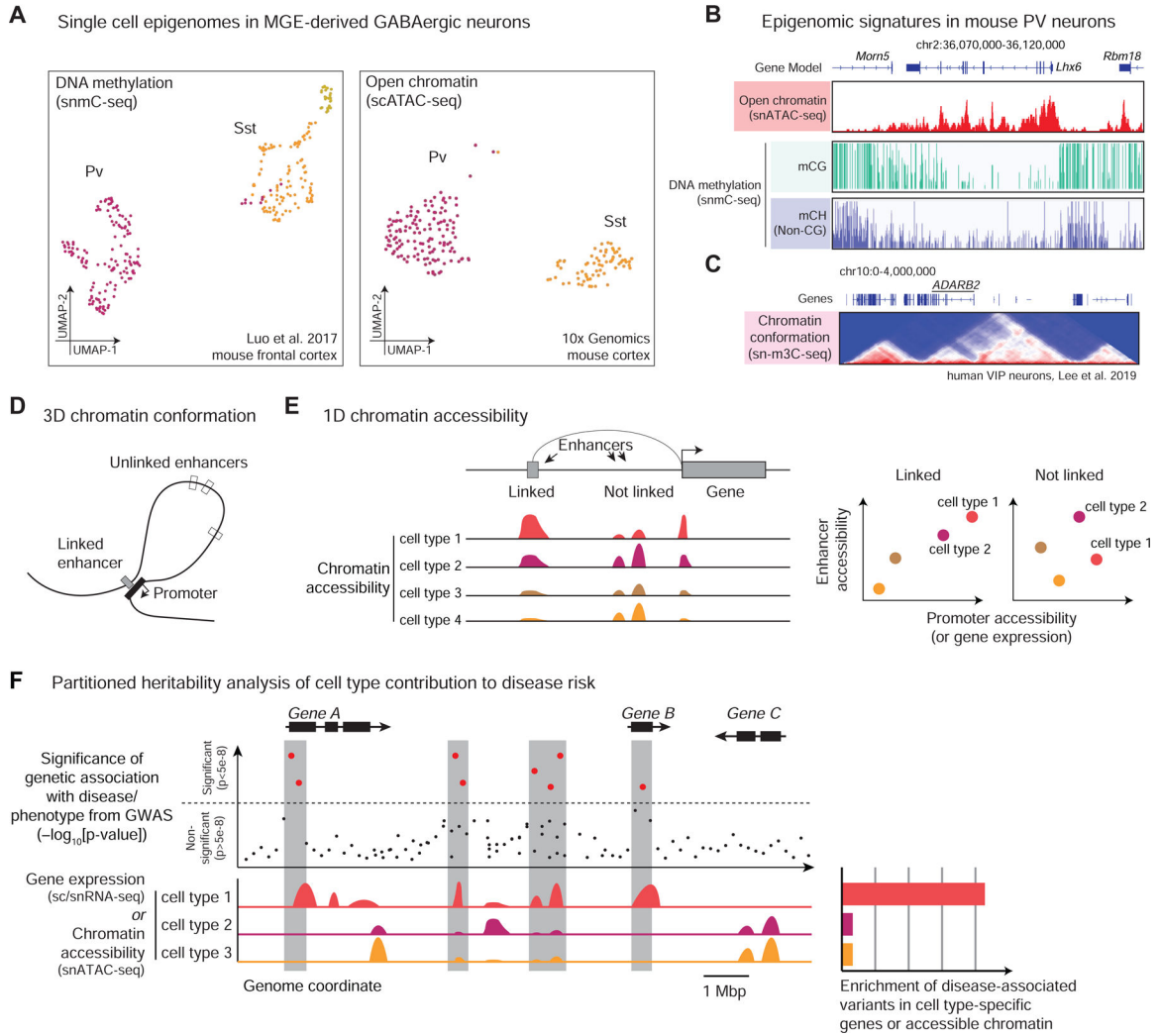
Figure 3.

Single cell epigenomics. (A) 2-dimensional embedding of MGE-derived inhibitory neurons profiled by DNA methylation (snmC-seq, (Luo et al., 2017)) and open chromatin (snATAC-seq, 10x Genomics). (B) Genome browser tracks showing pseudo-bulk signals pooled from many single cells of the same cell type (PV-expressing inhibitory interneurons from mouse cortex) around the marker gene *Lhx6*, showing open chromatin (10x Genomics), CG and non-CG DNA methylation (Luo et al., 2017). (C) 3D chromatin conformation from sn-m3C-seq of human frontal cortex VIP cells (Lee et al., 2019). Heatmap shows the number of contacts detected between pairs of genomic bins; red triangular blocks correspond to domains of frequent chromatin interactions. (D) Illustration of enhancer-gene interaction, in which a 3D chromatin loop brings an enhancer in physical proximity with a gene promoter. (E) Enhancer-gene interaction inferred from the correlation of chromatin accessibility across cell types. (F) Schematic illustration of partitioned heritability analysis. Top: Genome-wide association studies (GWAS) provide a statistical measure of genetic association with a disease (y-axis; higher points are more significant) for individual genetic variants (x-axis: genomic coordinate). Red dots/shaded regions are loci passing a stringent threshold for

genome-wide significance (p<5e-8). Bottom: Gene expression or accessible chromatin profiles in three cell types. Right: Enrichment of active gene expression or accessible chromatin near significant disease-associated variants indicates a potential vulnerability specifically in cell type 1.