Review

Biological Psychiatry: GOS

Single-Cell RNA Sequencing and Its Applications in the Study of Psychiatric Disorders

André S.L.M. Antunes and Daniel Martins-de-Souza

ABSTRACT

Neuroscience is currently one of the most challenging research fields owing to the enormous complexity of the mammalian nervous system. We are yet to understand precise transcriptional programs that govern cell fate during neurodevelopment, resolve the connectome of the mammalian brain, and determine the etiology of various neurodegenerative and psychiatric disorders. Technological advances in the past decade, notably single-cell RNA sequencing, have enabled huge progress in our understanding of such features. Our current knowledge of the transcriptome is largely derived from bulk RNA sequencing, which reveals only the average gene expression of millions of cells, potentially missing out on minor transcriptome differences between cells detectable only at single-cell resolution. Since 2009, several single-cell RNA sequencing techniques have emerged that enable the accurate classification of neuronal and glial cell subtypes beyond classical molecular markers and electrophysiological features and allow the identification of previously unknown cell types. Furthermore, it enables the interrogation of molecular and disease-relevant mechanisms and offers further possibilities for the discovery of new drug targets and disease biomarkers. This review intends to familiarize the reader with the main single-cell RNA sequencing techniques developed throughout the past decade and discusses their application in the fields of brain cell taxonomy, neurodevelopment, and psychiatric disorders.

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Our current knowledge of the transcriptome is largely based on bulk sequencing of RNA derived from homogenized tissue or heterogeneous cell populations. Yet, while bulk sequencing has led to a better understanding of gene expression, genomic rearrangements, and splice variants, it reveals only the average gene expression of millions of cells, potentially missing out on minor transcriptome differences between cells detectable only at single-cell resolution (1). Notably, even cells from supposedly homogeneous populations are known to display differential expression patterns among each other owing to intrinsic stochastic processes and influences of their surrounding microenvironment (2,3). The same type of biological sample (e.g., cultured cells and tissues, blood, and organs) is suitable for both bulk and single-cell transcriptomics, and the major difference between both techniques is that single-cell RNA sequencing (scRNA-seq) generally requires single-cell isolation prior to RNA extraction. This step can itself alter the transcriptomic state of a cell when handling morphologically complex or large cells, such as mature neurons and cardiomyocytes (4,5). In contrast, bulk transcriptomics allows tissue lysis directly in trizol, thereby freezing the cell transcriptome in the very first step of the protocol. In addition, most scRNA-seq protocols only detect polyadenylated RNA, which excludes all noncoding RNAs, and they generally detect fewer transcripts compared with bulk experiments. Although this imperfect coverage can lead to biased transcript quantifications, bioinformatics tools have been developed to correct this flaw (6).

Despite the existing challenges, scRNA-seq protocols and bioinformatics tools are rapidly expanding and have unlocked the potential to answer questions that were less accurate or even impossible with bulk transcriptomics. For instance, scRNA-seq has rendered the molecular characterization of every human cell a feasible endeavor in the Human Cell Atlas Project (7), and it has enabled the identification of rare cell types in complex tissues (8,9) and cell lineage relationships in early development (10,11), inferring cellular trajectory and cell fate (12,13). Further, it has revealed the occurrence of random allelic gene expression (14,15), the distinction between normal versus abnormal cells (16), and the identification of pathology-associated cell types (17,18). scRNA-seq is itself not a novel technique, yet the volume and complexity of the data it generates places it in the realm of big data as a massive source of untapped knowledge (19). In general, scRNA-seq methods require 1) isolation of single cells or single nuclei, 2) complementary DNA (cDNA) synthesis by reverse transcription, 3) cDNA amplification to generate high-throughput sequencing libraries, 4) sequencing, and 5) data analysis (Figure 1).

Psychiatric disorders such as autism spectrum disorder, major depressive disorder, bipolar disorder, and schizophrenia pose an enormous burden to patients and have limited treatment options. They are highly heritable and present a complex, polygenic architecture, with multiple discrete loci collectively involved in the molecular mechanisms underlying pathology (20). Thus, to elucidate disease mechanisms, it is imperative to perform comprehensive molecular analyses in the healthy and

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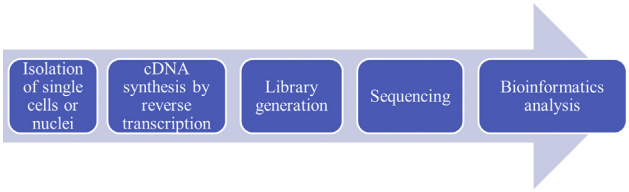

Figure 1. General workflow for single-cell RNA sequencing. cDNA, complementary DNA.

the disease-affected brain across different developmental stages, regions, and cell types. Until recently, no comprehensive dataset or systematic characterization of the genome and omic phenotypes linked to psychiatric disorders were available. Substantial progress in our understanding of psychiatric disorders was made possible by projects such as the Genotype Tissue Expression (21), BrainSpan (22), and psychENCODE (23) consortia. Together, they characterized thousands of postmortem brain samples from neurotypical and disease-affected individuals, thereby creating a molecular brain atlas, investigating genetic variants that associate with gene expression levels, and integrating multiple data modalities to identify global mechanisms relevant to disease. By integrating genomic and bulk transcriptomic data, these studies revealed differential gene splicing and expression in psychiatric disorders, with disease effect being caused largely by isoform-level (24) and shared molecular phenotypes across disorders (25). Notwithstanding the knowledge gained from bulk transcriptomic studies, they only reveal the average transcriptomic of the brain region investigated. Furthermore, bulk assays risk yielding false positives due to variation in cell-type composition between samples. Notably, scRNA-seq enables precise classification of cell subtypes based on their transcriptome with an accuracy that goes beyond molecular markers and electrophysiological features and allows the identification of previously unknown cell types (9,26). Consequently, precise association between shared molecular phenotypes and specific cell types across disorders is now possible, and better understanding of temporal dynamics underlying variation in gene expression has been gained (27). Finally, scRNA-seq enables the interrogation of cell-specific molecular and disease-relevant mechanisms, offering further possibilities for the discovery of new drug targets and disease biomarkers (28).

This review discusses the most widely used scRNA-seq methods and how they differ from each other. In the second part, it discusses the impact of scRNA-seq in our current understanding of psychiatric disorders. The bioinformatics aspect of scRNA-seq is out of the scope of this article, and the interested reader is referred to reviews published elsewhere (6,29,30).

SINGLE-CELL ISOLATION

Over the past years, different single-cell isolation techniques have been developed and refined, each one with upsides and downsides. The main cell isolation methods and their main aspects are summarized in Figure 2.

REVERSE TRANSCRIPTION AND cDNA AMPLIFICATION

During cDNA synthesis, some protocols introduce molecular tags known as unique molecular identifiers (UMIs). These consist of 4 to 10 random nucleotide sequences that function as a bar code for the identification and quantification of individual transcripts present in the original biological sample (31). During this step, some protocols introduce adapters required for the sequencing reaction in the different platforms commercially available. The newly synthesized cDNA must be amplified by polymerase chain reaction (PCR) to generate enough material for preparing sequencing libraries. The two main strategies used, template switching and in vitro transcription, depicted in Figures 3 and 4 and discussed in detail in (32).

scRNA TECHNOLOGIES: SEQUENCING METHODS

To date, more than 15 scRNA-seq methods have been described, which can be divided into two categories: full-length and tag-based methods. Full-length scRNA-seq methods deliver reads that cover the gene body uniformly and are thus suitable for quantitative analysis of gene expression

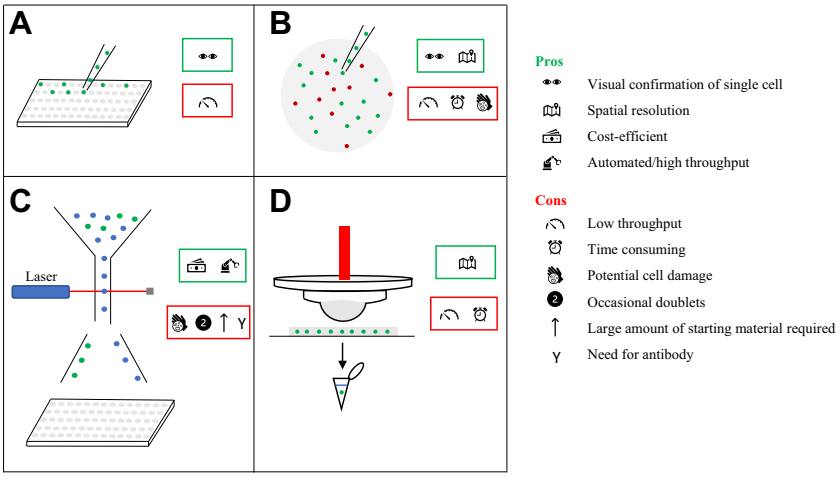

Figure 2. Commonly used techniques for single cell isolation. **(A)** Limiting dilution. **(B)** Micromanipulation. **(C)** Fluorescence-activated cell sorting. **(D)** Laser capture microdissection.

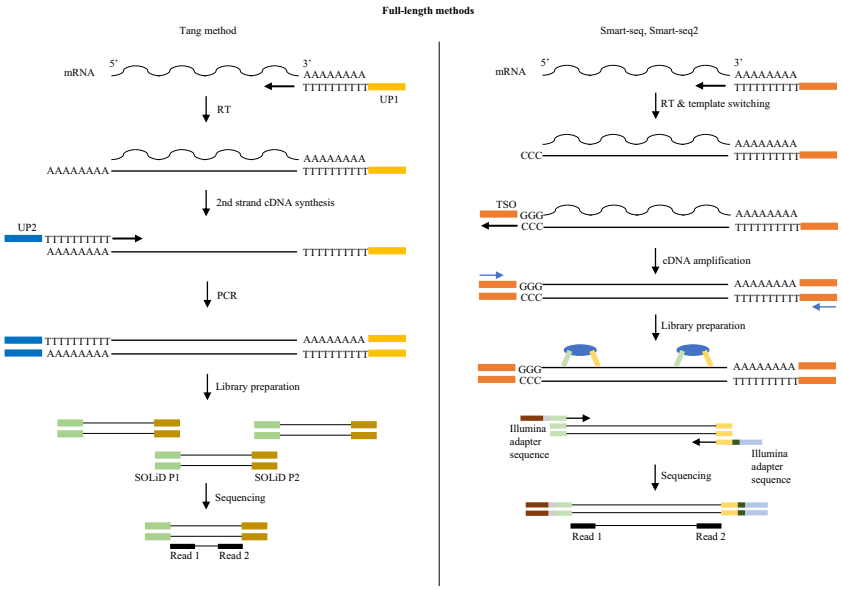

Figure 3. Full-length sequencing methods. Left: Tang method. Right: Smart-seq and Smart-seq2 methods. mRNA, messenger RNA; PCR, polymerase chain reaction; RT, reverse transcription; Smart-seq, switch mechanism at the 5′ end of the RNA transcript sequencing; SOLiD P1/P2, SOLiD platform adapters; TSO, template switching oligonucleotide; UP, universal primer.

and detection of single nucleotide polymorphisms, splice variants, and mutations (33,34). In contrast, tag-based methods allow the sequencing only of the 5′ or 3′ end of the transcripts. Sacrificing full-length coverage in tag-based methods is traded off by the capability of estimating transcript abundance for every RNA molecule in the original sample and multiplexing of cDNA synthesis (35,36).

Full-Length Methods

Tang Method. Tang *et al.* (37,38) manually isolated single cells and synthesized the first cDNA strand using an oligo-dT primer carrying an anchor sequence for the subsequent cDNA amplification by PCR. After first strand synthesis, a polyA tail was enzymatically added to the 3′ end of the molecule, which served as a template for a second oligo-dT primer carrying a second anchor sequence for PCR-mediated library amplification. The known anchor sequences at both ends of the newly synthesized cDNA allowed for efficient library amplification and downstream processing. (Figure 3, left panel).

Smart-seq, Smart-seq2, and Quartz-seq. Improved protocols termed Smart-seq (switch mechanism at the 5′ end of the RNA transcript sequencing) and Smart-seq2 (33,34) use the template-switching strategy for cDNA synthesis, which enhances transcript isoform analysis and identification of single nucleotide polymorphisms. Smart-seq relies on an expensive commercial kit for its

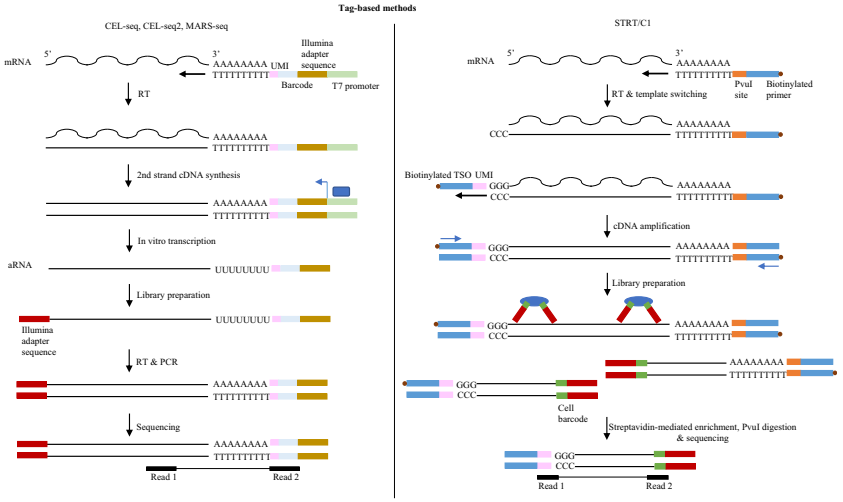

Figure 4. Tag-based sequencing methods. Left: CEL-seq, CEL-seq2, and MARS-seq. Right: STRT/C1. aRNA, antisense RNA; cDNA, complementary DNA; CEL-seq, cell expression by linear amplification and sequencing; MARS-seq, massively parallel single-cell RNA sequencing; mRNA, messenger RNA; PCR, polymerase chain reaction; RT, reverse transcription; Smart-seq, switch mechanism at the 5′ end of the RNA transcript sequencing; STRT, single-cell tagged reverse transcription; UMI, unique molecular identifier.

execution, while Smart-seq2 presents decreased costs by using off-the-shelf reagents.

The quartz-seq method is even simpler and is performed in a single PCR tube without any purification, thereby ensuring increased sensitivity, greater reproducibility, and less processing time (39) (Figure 3, right panel).

Tag-Based Methods

CEL-seq, CEL-seq2, and MARS-seq. CEL-seq (cell expression by linear amplification and sequencing) adopts linear in vitro transcription for RNA amplification instead of the exponential PCR-based approach used in the aforementioned methods. It uses an oligo-dT primer containing a bar code, the 5′-Illumina adapter sequence, and a T7 promoter sequence. The bar code allows for the identification of transcripts from a given cell, whereas the T7 promoter is responsible for initiating RNA amplification by in vitro transcription (35). CEL-seq2 presents reduced cost and hands-on time and was the first protocol to introduce UMIs (40).

CEL-seq was refined to what is called MARS-seq (massively parallel single-cell RNA sequencing) (41), which consists of a fully automated process that uses cell bar codes and UMIs, thus allowing for multiplexing. MARS-seq represented a leap in throughput and reproducibility in single-cell transcriptomics, dramatically reducing sequencing costs (Figure 4, left panel).

STRT-seq. The STRT-seq (single-cell tagged reverse transcription sequencing) protocol uses the template-switching approach for cDNA synthesis (42). However, unlike previously described methods, modifications on the oligo-dT primers used for cDNA synthesis and PCR amplification induce a 5′-end enrichment library preparation. STRT-seq was modified to include the commercially available C1 Single-Cell Auto Prep System microfluidic platform (Fluidigm) and became STRT/C1 (43), which includes UMI tags in the oligo-dT used for second strand cDNA synthesis (Figure 4, right panel).

Microfluidic-Based Methods

The abovementioned methods are known as plate based because of the need to sort cells into well plates. They can process thousands of cells but require a lot of pipetting and manual work. Despite the upscaling, automation, increased efficiency, and decreased costs achieved with different protocols, these methods remain cumbersome and labor intensive. Recently developed array- and emulsion-based methods allow the sequencing of thousands of cells per day and require nanoliters of reagents for sample preparation at a fraction of the cost of the previous methods (Figure 3).

CytoSeq, BD Rhapsody, Drop-seq, and InDrop. The CytoSeq and BD Rhapsody methods use a liquid handling robot to separate cells in arrays containing up to 100,000-picoliter wells followed by the loading of DNA-barcoded beads into the wells. The oligos attached to the beads contain a universal PCR priming sequence for library amplification, a cell bar code, a UMI, and an oligo-dT sequence. Once cells and beads are together in the wells, the robot dispenses the cell lysis solution to allow messenger RNA (mRNA) hybridization with the oligo-dT portion of the primers attached to the beads. The beads, now attached to the transcripts, are pooled into a single tube where the downstream steps are performed (44). The nanoliter scale and downstream processing in a single tube dramatically simplify the whole process and reduce costs.

Drop-seq and InDrop differ from the array-based methods in that they do not require physical support in an array format; instead, they use a microfluidic device to encapsulate single cells together with a barcoded bead in a nanoliter-scale emulsion droplet (Figure 5) (36,45).

10X Genomics Chromium. Two years after Drop-seq came out, 10X Genomics launched a very similar method that uses a microfluidic device to encapsulate single cells together with a barcoded bead in a droplet, inside which both capture and reverse transcription occur (46). After the reverse transcription reaction, the emulsion is broken, the cDNA is amplified, and the library is constructed in a single tube for subsequent sequencing.

Combinatorial Indexing Methods

Combinatorial indexing methods are the latest technology that allow reading the transcriptome of single cells without the need to isolate and work with individual cells at any given moment (13,47). Instead, fixed and permeabilized cells are plated in groups of a few dozen cells in a single well of a 96-well plate where reverse transcription with barcoded oligo-dTs takes place within the cells. Next, multiple rounds of pooling, splitting, and bar coding generate single-cell combinatorial tags from mixed-cell populations. This multistep bar coding allows a combination of about 2×10^7 possibilities. Thus, by sequencing hundreds of thousands of cells, there is only a very low chance of having two cells with the same set of bar codes.

A diagram depicting the time of publication, throughput, and technical details of the different methods described is shown in Figure 6 and Table 1.

LIBRARY PREPARATION

Nextera (48) is the most common protocol used for library preparation. It uses transposases to fragment the cDNA libraries into smaller bits and add transposons with partial Illumina adapter sequences to these fragments. Next, a simple PCR is used to amplify the material and complete the library preparation process. The final product is a library compatible with the Illumina system.

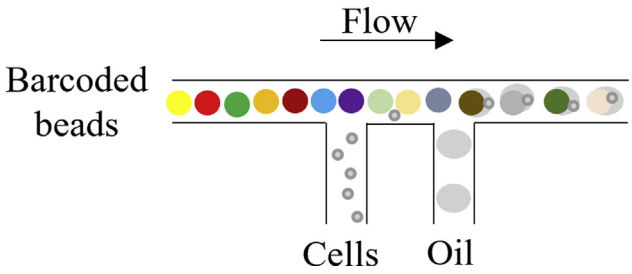

Figure 5. Microfluidics device for single-cell and barcoded bead encapsulation in oil emulsion.

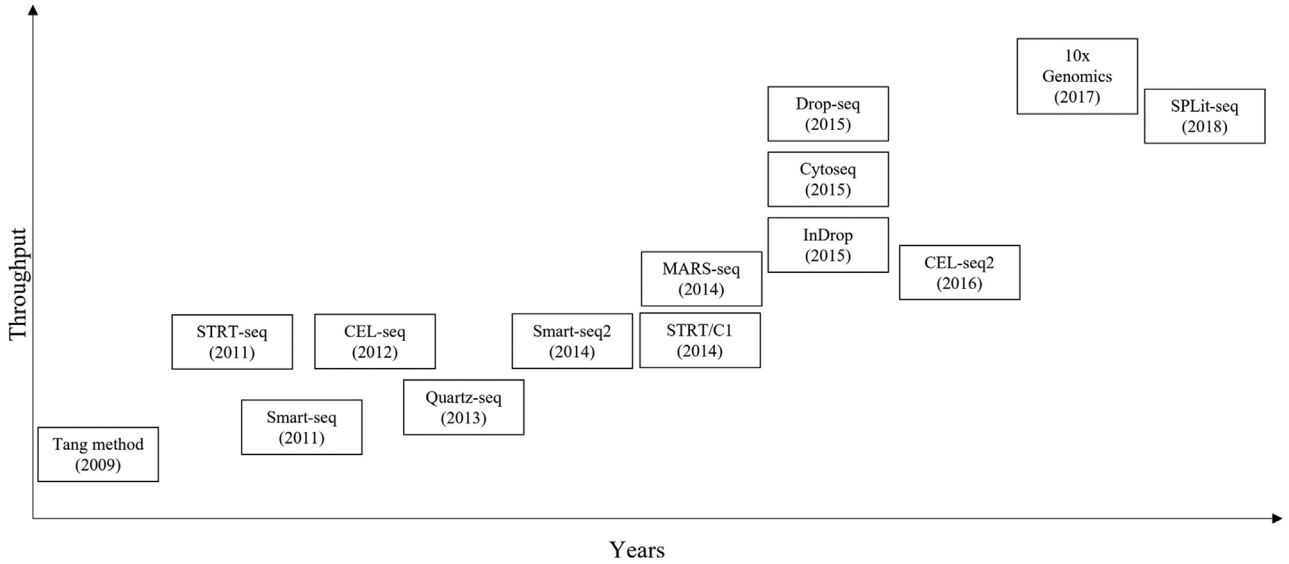

Figure 6. Time of publication and throughput of different single-cell RNA sequencing methods. CEL-seq, cell expression by linear amplification and sequencing; MARS, massively parallel single-cell RNA sequencing; Smart-seq, switch mechanism at the 5′ end of the RNA transcript sequencing; STRT, single-cell tagged reverse transcription sequencing.

scRNA SEQUENCING AND MULTIMODAL DATA

Single-parameter inspection of cells is unable to provide comprehensive biological information on their dynamic processes. Studies reveal poor correlation among (epi)genomes, transcriptomes, and proteomes (49,50) owing to complex regulatory networks within different omic strata (51–53). Thus, multidimensional analysis is required to understand single-cell states and processes. Lately, protocols have been developed that allow simultaneous measurement of (epi)genomic variation and transcript/protein expression. G&T-seq (genome and transcriptome sequencing) and scM&T-seq (54,55) enable integrative genome/methylome and transcriptome analysis through parallel extraction of genomic DNA and cytoplasmic mRNA; methods targeting single-cell epigenomics have been developed such as DNA-seq and chromatin immunoprecipitation sequencing (56,57); ATAC-seq (assay for transposase-accessible chromatin using sequencing) (58), an approach targeting chromatin accessibility through the use of a transposase,

Table 1. Main Technical Details of Different scRNA-Seq Methods

Method	Transcript Coverage	UMI	Early Multiplexing	Strand Specificity	Reference (DOI)
Homopolymer Tailing-Based PCR					
Tang method	Nearly full-length	No	No	No	10.1038/nmeth.1315
Quartz-seq	Full-length	No	No	No	10.1186/gb-2013-14-4-r31
Template Switching-Based PCR					
Smart-seq	Full-length	No	No	No	10.1038/nbt.2282
Smart-seq2	Full-length	No	No	No	10.1038/nmeth.2639
STRT-seq	5′-only	Yes	Yes	Yes	10.1101/gr.110882.110
STRT/C1	5′-only	Yes	Yes	Yes	10.1038/nmeth.2772
Drop-seq	3′-only	Yes	Yes	Yes	10.1016/j.cell.2015.05.002
In Vitro Transcription-Based Linear Amplification					
CEL-seq	3′-only	Yes	Yes	Yes	10.1007/978-1-4939-9240-9_4
CEL-seq2	3′-only	Yes	Yes	Yes	10.1186/s13059-016-0938-8
MARS-seq	3′-only	Yes	Yes	Yes	10.1126/science.1247651
InDrop	3′-only	Yes	Yes	Yes	10.1016/j.cell.2015.04.044
Designed Primers-Based PCR					
Cytoseq	3′-only	Yes	Yes	No	10.1126/science.1258367
In-Cell Barcode Tailing-Based PCR					
SPLiT-seq	Decreased 3′ bias	Yes	Yes	Yes	10.1126/science.aam8999

CEL-seq, cell expression by linear amplification and sequencing; MARS-seq, massively parallel single-cell RNA sequencing; PCR, polymerase chain reaction; scRNA-seq, single-cell RNA sequencing; Smart-seq, switch mechanism at the 5′ end of the RNA transcript sequencing; STRT-seq, single-cell tagged reverse transcription sequencing; UMI, unique molecular identifier.

has been integrated with combinatorial indexing to jointly profile chromatin accessibility and mRNA in a method called sci-CAR (59).

Recent advances toward multidimensional transcriptomic analysis have tackled the spatial component of scRNA-seq. Currently, most protocols process tissue and cells into a suspension prior to mRNA sequencing; however, the emerging field of spatially resolved transcriptomics aims to preserve the positional information of single cells in a tissue (60).

scRNA SEQUENCING IN THE STUDY OF THE BRAIN AND PSYCHIATRIC DISORDERS

Knowledge gained through scRNA-seq is being increasingly applied in neuroscience to achieve a more detailed understanding of the cellular diversity, regulatory programs, and biological contexts related to neurodevelopment and psychiatric disorders. Next, we will discuss some findings where scRNA-seq has led to advances of biological and clinical relevance in these fields.

scRNA Sequencing in the Taxonomy of Brain Cells

scRNA-seq has been extensively applied to classify and characterize cells from different brain regions in unprecedented molecular resolution. Understanding the molecular signature of cell types in the brain offers insights into their functionality and relationship to health and disease.

Mouse Studies. Transcriptomic characterization at single-cell resolution of the mouse striatum revealed important findings that would have been impossible to observe with bulk RNA-seq. For instance, striatal medium spiny neurons (MSNs) are typically classified as D_1 or D_2 according to the dopaminergic receptor they express. scRNA-seq identified previously unknown MSN subtypes, which overexpress genes associated with the risk of developing psychiatric disorders (61). In fact, the link between striatal dysfunction and psychiatric disorders such as schizophrenia and bipolar disorder is well established (62,63).

Direct anatomical and electrophysiological correlation with gene expression profiles can be achieved by Patch-seq, a combination of whole-cell patch clamp with scRNA-seq (64). Patch-seq performed on pyramidal neurons and fast-spiking interneurons of the mouse prefrontal cortex revealed novel cell-specific enriched transcripts in addition to already known classical markers (65). Some of the differentially expressed transcripts code for ion channels and receptor subunits linked to psychiatric disorders that were, for the first time, reported to be enriched in a specific neuronal subtype (e.g., *Kcnn2* and *Gria3* in pyramidal and *Kcnk2* in fast-spiking interneurons) (66–68). Unlike classical markers of pyramidal and fast-spiking cells (e.g., VGLUT1 and GAD65), many of the newly identified markers code for membrane proteins and are therefore suitable for cell-selective therapeutic targeting. However, how well transcript and protein levels correlate remains to be determined with the advancement of single-cell proteomics technologies and multimodal data integration.

Human Studies. Despite its utility, the mouse brain does not encompass the molecular complexity and diversity of the human brain. Single-nucleus RNA-seq (snRNA-seq) identified a subtype of cortical GABAergic (gamma-aminobutyric acidergic) neurons not seen in the mouse brain (69). Rosehip cells, as they were called, display distinct functional properties and express selective markers implicated as risk factors for Rett syndrome and intellectual disability. This is an important finding made possible by single-nucleus transcriptomics, because knowledge of the diversity and function of human-specific cell types is important for our comprehension of neuropsychiatric disorders. Of note, freshly isolated tissue is necessary for scRNA-seq. Difficulties in the obtention of fresh brain tissue has been overcome by snRNA-seq, which can be performed on frozen postmortem samples. Thus, most of the single-cell transcriptomic data on the human brain available to date stems from isolated nuclei. It is noteworthy that while snRNA-seq allows convenient access to frozen tissue, it trades off the information contained in transcripts from the cytoplasm and proximal dendrites (70,71).

Using snRNA-seq, Lake *et al.* (9) identified 16 different neuronal subtypes across 6 different cortical regions. Another study analyzed the human temporal lobe and identified 7 neuronal subtypes, of which 2 corresponded to excitatory neurons and 5 to inhibitory neurons (72). A study of the BRAIN Initiative Cell Census Network generated a multimodal cell census and atlas of the mammalian motor cortex by integrating conventional and spatially resolved sc/snRNA-seq, snATAC-seq, sc-methylome, and scPatch-seq of mouse, marmoset, and human (73,74). The BRAIN Initiative Cell Census Network was able to elaborate a consensus cross-species cell taxonomy and elucidate their conserved hierarchical organization. Furthermore, this multimodal approach revealed a unified molecular genetic architecture of cortical cell types that links transcriptomic and epigenomic information in mammalian species. These findings have implications for neuropsychiatric research using animals, which many times fail to model such complex disorders.

To date, most studies of neural taxonomy have focused on the brain cortex. In a recent study, Tran *et al.* (75) defined the molecular taxonomy of cells in limbic brain regions. They identified novel subpopulations of interneurons and MSNs in the nucleus accumbens and revealed that distinct populations of D_1- and D_2-expressing MSNs in this region may be differentially associated with schizophrenia and bipolar disorder. Furthermore, they identified classes of GABAergic inhibitory neurons in the amygdala that were preferentially associated with schizophrenia.

scRNA Sequencing in Neurodevelopment

scRNA-seq is also a powerful tool for the comprehension of development. In early brain development, several cells are present that often look morphologically and histologically similar to each other, yet they follow a variety of differentiation paths to form distinct cell types. Precise and detailed mapping of transcriptional programs that drive brain development is only possible if single-cell resolution is achieved and is indispensable for a better understanding of psychiatric diseases,

which have a genetic component and in many cases a neurodevelopmental origin (76).

scRNA-seq of the developing human prefrontal cortex indicates that excitatory neurons appear and mature at an earlier stage relative to inhibitory neurons and that this pattern is intrinsically regulated (77). This is an important finding given that disruption of the fine excitatory/inhibitory balance in the prefrontal cortex is linked with the manifestation of psychiatric disorders, as demonstrated by scRNA-seq analysis of cerebral organoids from schizophrenia-discordant twins (78,79). Another study demonstrated that neurons in the amygdala undergo unique changes in maturation and migration in a time window spanning from gestation into adulthood (80). Such processes could underlie decreased neuron numbers seen in the amygdala of individuals with autism (81). Without the level of resolution provided by scRNA-seq, such findings would not be possible, thus highlighting how novel insights into the pathophysiology of psychiatric disorders can be gained by in-depth analysis of neurodevelopmental processes of brain regions implicated in psychiatric disorders (82).

Dissection of interspecies neurodevelopmental differences has also leveraged the power of scRNA-seq and multimodal data integration to achieve unprecedented resolution and in-depth understanding of molecular and cellular processes, thus providing better interpretation of neuropsychiatric research using model animals. In a study from the psychENCODE Consortium, Li *et al.* (27) used tissue- and single cell–level transcriptomics and epigenomics in multiple brain regions ranging in age from embryonic development through adulthood. They accomplished a spatiotemporal and multimodal data integration illuminating a broad span of human neurodevelopmental dynamics, revealing genes and co-expression modules related to multiple brain traits and psychiatric disorders. Furthermore, scRNA-seq has provided a transcriptional landscape of the developing brain in humans and nonhuman primates (11) and of neurogenic trajectories in the hippocampal-entorhinal system of the adult pig, macaque, and human (83). Together, these studies reveal regional and temporal differences in the formation and maturation of brain circuits across species. In addition, using cerebral organoids, scRNA-seq, and scATAC-seq integration revealed delayed maturation of the human brain relative to nonhuman primates (84). Such an in-depth picture of the primate transcriptional architecture is only possible at single-cell resolution and is crucial for modeling neurodevelopmental and psychiatric disorders in primates. Furthermore, using multimodal data integration, human-specific changes in gene expression were seen in the developing cortex, which correlate with chromatin accessibility differences between humans and nonhuman primates. Finally, fetal tissue and brain organoids have also been used to provide a comprehensive molecular and spatial atlas of early stages of human brain and cortical development (85).

scRNA-seq has also been used for trajectory inference and RNA velocity analysis to determine transcriptional trajectories of cell lineage determination and to predict the future state of individual cells (86,87). Velocity analysis describes the rate of gene expression change for an individual gene at a given time point based on the ratio of its spliced and unspliced mRNA. This approach has been applied on scRNA-seq data of developing cortical cells, revealing that genes associated with neurodevelopmental or psychiatric disorders exert high influence on the velocity stream, suggesting that susceptibility to these disorders may result from early events in cortical development (85).

Cell Types Associated With Psychiatric Disorders

Determining the contribution of discrete cell types to disease is important to understand their etiology and pathophysiology. Bulk transcriptomics is unable to detect the relatively low contribution of rare cell types to the total RNA in a given tissue; thus, until a few years ago, RNA-seq studies performed failed in detecting genetic dysregulations in discrete cell subtypes (88). scRNA-seq integration with (epi)genomic data has helped mapping such risk factors to defined cell types (75,89,90); yet, relating genetic risk factors for complex and polygenic diseases to casual disease mechanisms remains a challenging task. To date, there are not many scRNA-seq studies describing cell-type associations with psychiatric disorders. Several preprints have been released, and thus more studies are expected in a short time (91–93).

Autism Spectrum Disorder. In autism spectrum disorder, snRNA-seq has implicated upper layer cortical excitatory neurons and microglial cells. This study went beyond cell-type association with pathology and related the observed gene expression signatures with the clinical severity of autism (94). Another study using cortical organoid models implicated risk genes in the asynchronous development of two specific neuronal populations in autism spectrum disorder, namely GABAergic and deep-layer excitatory projection neurons (95). In addition, it revealed that despite affecting distinctive molecular pathways, the risk genes analyzed converged onto the same phenotype and that phenotypic expressivity was modulated by the human genomic context, suggesting that higher-order processes of neuronal development and circuit wiring underlie the pathophysiology of autism spectrum disorder. This study highlights insights provided by scRNA-seq and multimodal data analysis in associating risk factors and disease mechanisms for complex and polygenic pathologies. Finally, the power of sc/snRNA-seq and multimodal data analysis has also been demonstrated through the reliable characterization of individual cells in the mosaic brain from females with Rett syndrome. It was demonstrated that in individuals with *MECP2* mutations, highly methylated genes are repressed in a cell type–specific manner in wild-type but not in mutant neurons (96).

Schizophrenia. Schizophrenia is one of the psychiatric disorders that is most difficult to treat owing to its complex and not yet fully understood genetic/environmental etiology, neurodevelopmental origin, molecular neuropathology, and variety of symptoms, including psychosis. Genomic data have pointed to interneurons and pyramidal neurons as cell types primarily affected by genetic susceptibility in schizophrenia. Integration with single-cell transcriptomic data identified that this primary cell type pathology extends to and has secondary effects on brain endothelial and glial cells (97). Recent studies used snRNA-seq to investigate the association between genetic risk

factors and cell types in the brain cortex and other regions (75,98). They identified a cell-type association with psychiatric disease and substance use, thus highlighting the clinical relevance of single-cell data. Furthermore, they found a significant association of excitatory and inhibitory neurons in the cortex and GABAergic neurons in the midbrain with schizophrenia. Different loci were associated between midbrain and cortical cells, suggesting a distinct role for midbrain neurons in schizophrenia. Curiously, as in the study of Skene and Grant (97), a convergence of psychiatric disorders onto shared loci within cells of oligodendroglial lineage was observed. Further, analysis of cellular processes linked to the gene expression patterns in the oligodendroglial cells associated with schizophrenia risk was enriched for lipid metabolism and neuron development. It is noteworthy that dopaminergic hyperfunction is considered one of the main pathophysiological features of schizophrenia (99); yet, focus on dopaminergic receptor targeting by antipsychotics have brought limited improvements in the quality of life of patients (100). Proteomic studies have already pointed to the involvement of oligodendrocytes in schizophrenia (101,102), and single-cell transcriptomics further strengthens this notion.

Major Depressive Disorder. Major depressive disorder is another complex and heterogeneous psychiatric disorder and a leading cause of disability. A study analyzed the transcriptome of ~80,000 nuclei from cortical samples and identified major dysregulations in excitatory neurons and oligodendrocyte progenitor cells (OPCs) (18). The nuclear transcriptome of the cortical cells clustered into 26 distinct cell subtypes, 2 of which were OPCs ($NG2^+$). Between both OPC clusters, 1 stood out as highly dysregulated with concomitant transcriptional overlap with genes previously implicated with major depressive disorder. Furthermore, the OPC cluster that appeared strongly dysregulated presented a distinct phenotype relative to OPCs committed with lineage differentiation, further strengthening the notion of functional heterogeneity among OPCs (103–105).

CONCLUSIONS AND FUTURE PERSPECTIVES

In the past decade, next-generation sequencing technology has developed significantly, notably the substantial decrease in the cost of each experiment and the increase in quality, throughput, and yield. This has rendered single-cell transcriptomics a reality despite costs remaining prohibitive for routine use in many labs.

Most scRNA-seq studies to date have focused on dissecting the cellular heterogeneity of brain cells and inferring cell types associated with the development and manifestation of brain disorders. Improved molecular taxonomy and novel insights into neurodevelopment gained through scRNA-seq have shed light onto processes in the brain that are not yet fully understood, having direct implications to our comprehension of psychiatric disorders. We can now trace with unprecedented resolution the origin and maturation of distinct cellular types and their distribution and balance in different brain regions and neural tracts in the context of health and disease. More specifically, we have gained a better understanding on how risk genes affect distinct cell types and processes in the brain and can elucidate how they modulate distinct molecular pathways that lead to distinctive phenotypes (95). Moreover, single-cell studies have drawn increased attention to the role of glial and other non-neuronal cells in psychiatric disorders, warranting a less neurocentric and more integrative approach for understanding the brain (18,97).

Future challenges lie in inferring causality, predicting disease mechanisms from scRNA-seq data, and relating those findings with symptomology and disease severity. For that matter, single-cell multiomics approaches will be of great benefit, and the development and consolidation of single-cell proteomic technologies is highly anticipated.

Better success rates in therapeutic intervention remain a major issue in psychiatric disorders, and technological advances are expected to allow more precise patient stratification and help selecting patients for clinical trials and therapeutic approaches based on their chances of success.

Finally, evolving techniques for spatial transcriptomics will allow the precise correlation of scRNA-seq data to the anatomical context of every cell in the brain, further advancing our understanding of brain disorders and benefiting medical research.

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ARTICLE INFORMATION

From the Laboratory of Neuroproteomics (ASLMA, DM-d-S), Department of Biochemistry and Tissue Biology, Institute of Biology, State University of Campinas; Experimental Medicine Research Cluster (DM-d-S), University of Campinas, Campinas; D'Or Institute for Research and Education (DM-d-S); and the Instituto Nacional de Biomarcadores em Neuropsiquiatria (DM-d-S), Conselho Nacional de Desenvolvimento Científico e Tecnológico, São Paulo, Brazil.

Address correspondence to André S.L.M. Antunes, Ph.D., at aslmantunes@gmail.com.

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