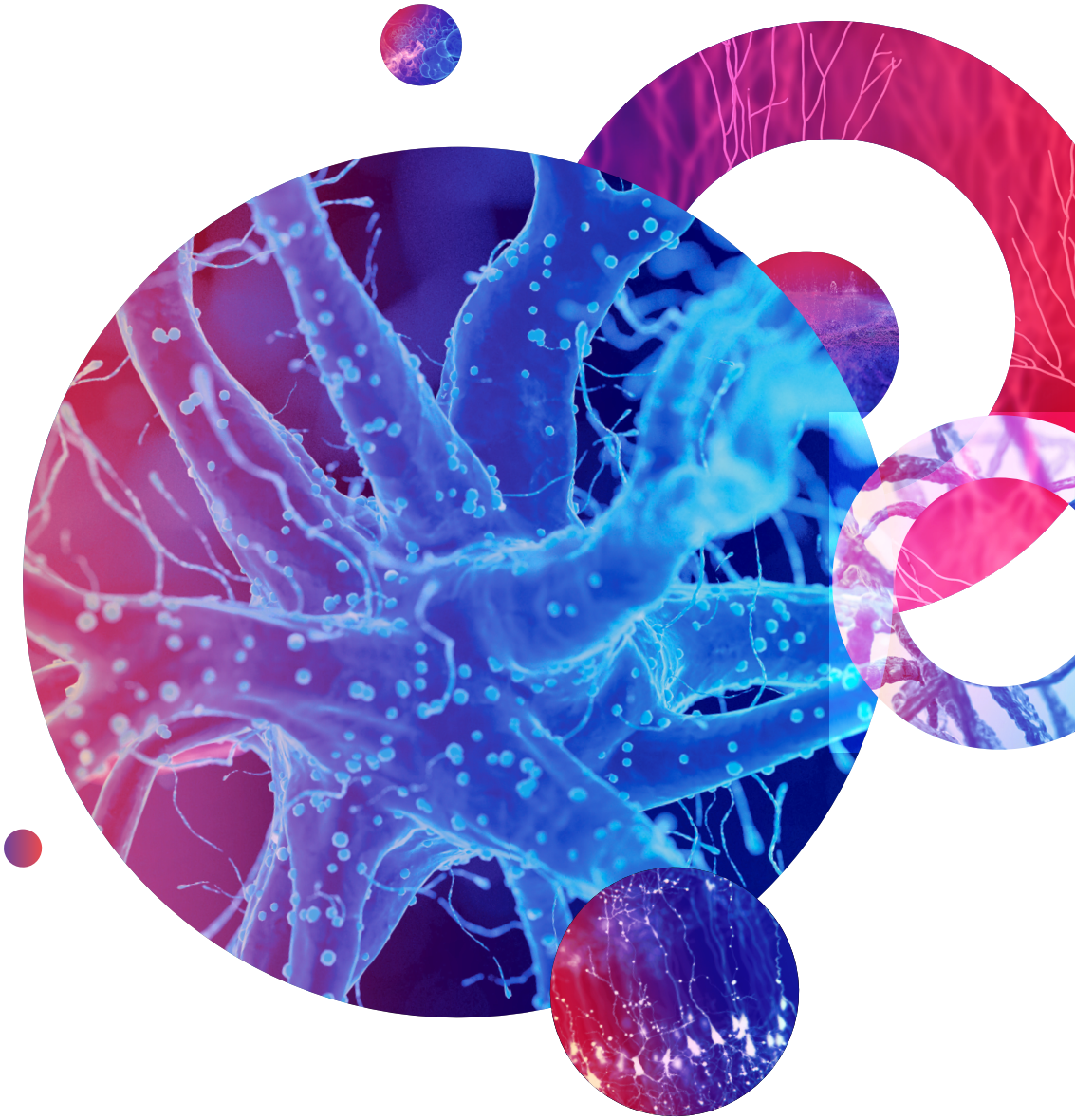
10x GENOMICS

Explore the complexity of the nervous system

Gain a new perspective and push the boundaries of neural discovery



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Chapter 1

In delicate balance: the central nervous system

The nervous system is a complex network of diverse cell types with a myriad of functions, communicating via dynamic signaling pathways and synaptic interactions. This diverse population of neural cell types develops from a relatively homogenous population of progenitor cells during development in a tightly regulated manner. Neural cells, comprising excitatory and inhibitory neurons, and glial cells, including astrocytes, oligodendrocytes, ependymal, and microglial cells, interact with each other and with cells outside of the central nervous system (CNS) in a delicate balancing act. These multitudes of cell types and subtypes are involved in many specialized functions. When any part of these intricate functions are disrupted—through trauma, disease, or genetic variation—neurological disease and disorders can result.

The search for insights into neurological disease

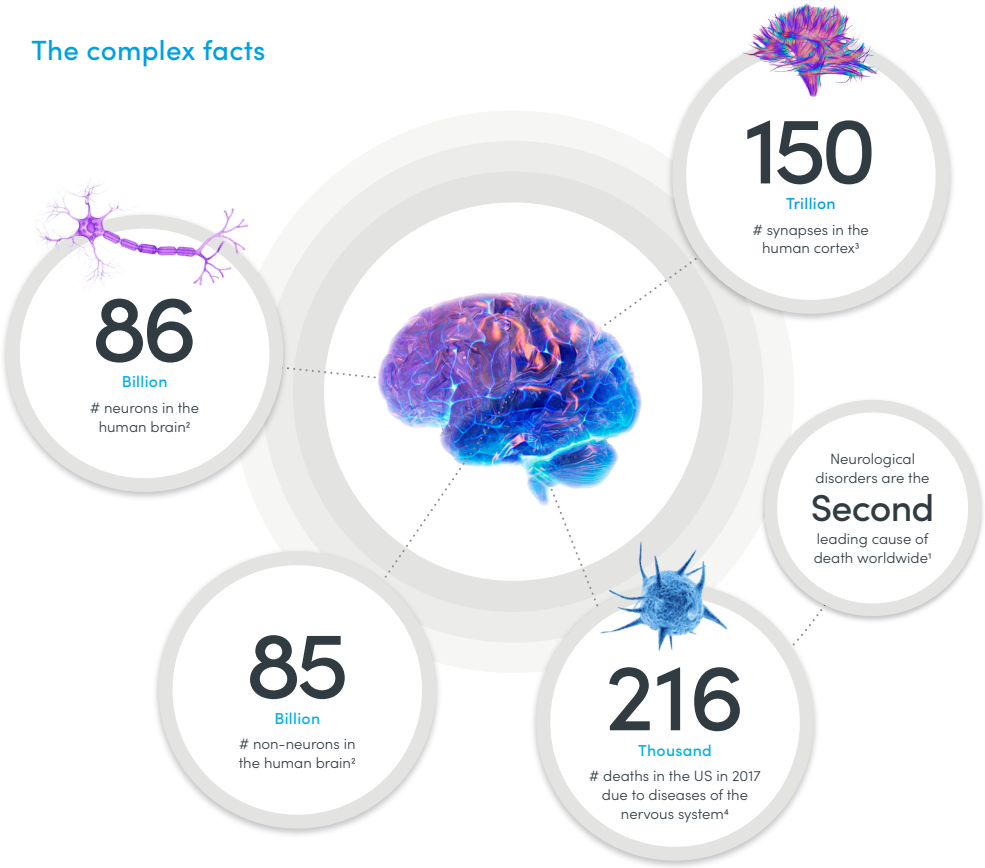
Neurological disorders, including epilepsy, dementia, Alzheimer's disease, and amyotrophic lateral sclerosis, represent the second-leading cause of death as evaluated in the Global Burden of Disease Study 2015 (1). Ongoing research is aimed at understanding the causative mechanisms of these disorders, with the ultimate goal of developing improved treatment options. Advancing our knowledge of a wide range of neural disorders relies on the ability to carefully identify cell types within the nervous system and understand molecular changes during disease progression and treatment.

To dissect the diversity of cell types and subtypes within the nervous system, neuroscientists rely on cellular characterization by molecular phenotype, electrophysiology, connectivity, morphology, neurotransmitter signaling, and physical location. However, the depth of information needed to fully characterize and understand neural cells, their subtypes, and ultimately their function, is often hampered by experimental limitations. Traditional methods for exploring cell types and subtypes in neural tissue include immunohistochemistry (IHC), in situ hybridization (ISH), and microarrays. These techniques rely on probing a small number of known targets, such as cell-type markers, myelinated axons, or amyloid plaques, and require relatively large amounts of sample, as well as an involved, extensive analysis. While these techniques continue to have an important place in neuroscience research, they fail to fully capture the diversity of the CNS with its magnitude of cell types and molecular interactions.

See neuroscience in a whole new way

To obtain deeper, more detailed information about the CNS and to advance neuroscience research, improved experimental methods are needed when studying cellular phenotypes and functions, cell location and morphology, and signaling pathways. Gene expression analysis with transcriptomics, achieved through various forms of RNA sequencing, allows researchers to build upon prior knowledge of the brain obtained through traditional techniques, helping them delve further into the intricacies of cellular phenotypes, signaling pathways, and cellular interactions within the CNS.

The complex facts



References

1. VL Feigin et al., Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Neurol.* 16, 877–897 (2017).
2. S Herculano-Houzel, The Human Brain in Numbers: A Linearly Scaled-up Primate Brain. *Front Hum Neurosci.* 3, 31 (2009).
3. B Pakkenberg et al., Aging and the Human Neocortex. *Exp Gerontol.* 38, 95–99 (2003).
4. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics. CDC WONDER online database: About underlying cause of death, 1999-2017. Available at: http://wonder.cdc.gov/ucd-icd10.html. Accessed May 8, 2019.

Chapter 2

Gene expression profiling: exploring the transcriptome

Function of the CNS is governed by the precise regulation of gene expression across diverse and distinct cell subtypes. For many years, neuroscientists have looked at gene expression patterns to determine when and where specific genes are switched on in the CNS, and to explore the molecular machinery that mediates health and disease. Understanding expression patterns across the many structures of the CNS is a difficult endeavor. Traditional techniques, such as ISH, probe gene expression of relatively small numbers of known targets, often requiring large numbers of samples for processing and extensive analysis. However, analysis of the entire transcriptome provides a comprehensive view of RNA expression, enabling an unbiased approach to study cellular and molecular changes that may have been missed with other techniques.

Approaches to transcriptome analysis

Several approaches are now available to examine the transcriptome, including bulk RNA sequencing (RNA-seq), single cell RNA-seq, and spatial gene expression profiling (Figure 1). These techniques are all enabled by next-generation sequencing (NGS) platforms. One way researchers can analyze the neural transcriptome is to first isolate mRNA. The mRNA sample is then reverse transcribed to cDNA, which is amplified and completed as a final library before being sequenced using an NGS platform (1).

While bulk RNA-seq provides insight into the transcriptome, this approach results in an averaged result of gene expression profiles from across a population of cells, which can mask the diversity and complexity of cell types and molecular characteristics under study. In addition, rare cells with unique transcriptomes are often not detected by traditional bulk approaches. Single cell RNA-seq analyzes transcriptomes of individual cells to profile entire populations in order to identify cell types, cell states, and molecular characteristics. Spatial gene expression analysis enables profiling of the full transcriptome in anatomically defined regions of the brain by combining histology and mRNA analysis, providing an understanding of cells in their morphological context.

Transcriptome information obtained using these capabilities has revolutionized not only our understanding of complex biological systems, but also how genetic-level alterations or signaling changes in one cell type can impact other cells in the environment and lead to pathological conditions. These types of revelations will help researchers understand and treat disease phenotypes, even for complex, multifactorial diseases that have evaded understanding with traditional technologies (2).

Transcriptome analysis

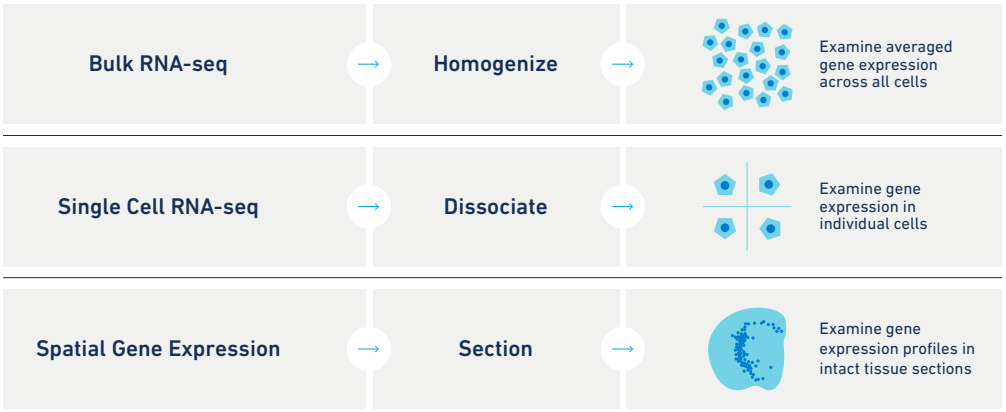


Figure 1: Methods of analyzing the transcriptome.

The cellular diversity of neural tissues relative to non-neural tissues has traditionally complicated data interpretation in neuroscience. However, in recent years, the enhanced precision and accessibility of RNA-seq has led to an increased focus on the transcriptome, with the aim of characterizing spatial and temporal gene expression patterns within neurological systems and determining relationships between the cellular transcriptome and phenotypic and behavioral manifestations (3).

Neuroscience at high resolution

Single cell RNA-seq can reveal complex and rare cell populations, uncover regulatory relationships between genes, and track distinct cell line trajectories (4). This data is now being incorporated into numerous databases and atlases including the Allen Brain Atlas, the Brain RNA-Seq database, the Human Brain Transcriptome project, scREAD, and the Single Cell Portal (4). Single cell RNA-seq has also proven instrumental to the characterization and cataloging of complex neural tissues as well as the identification of novel and rare cell types (5). Transcriptome analysis has been applied to numerous different experimental model species aside from humans and mice, including invertebrates, zebrafish, Drosophila, and non-human primates (6-10).

In recent years, transcriptome analysis has done much to unveil diversity within neural cell types and identify new cellular subtypes. Alexandra Grubman and colleagues used single nuclei RNA-seq of 13,213 nuclei to understand cell type–specific differences that arose in human Alzheimer's disease brains (11). Subclustering identified 31 distinct cell subtypes, resolved from the primary clusters of microglia, astrocytes, neurons, oligodendrocytes, oligodendrocyte progenitor cells, and endothelial cells. Cell clustering analysis highlighted the divergent transcriptional programs in Alzheimer's disease. For all non-neuronal cell types, distinct subclusters were formed by cells from control or disease patients, even when annotated cell identity was the same (11). In another study, single nuclei RNA-seq was able to resolve the heterogeneity of astrocyte subtypes present in individuals with Huntington's disease that was obscured by bulk RNA-seq, which could not distinguish between gene expression changes caused by alterations in cell population versus cell phenotype (12).

Beyond characterizing individual cells, comprehensive transcriptome analysis using bulk and single cell RNA-seq has greatly increased scientists' capacity to investigate the molecular mechanisms underlying neurological health, development, and disease, as well as how they translate to cellular proteomic and phenotypic alterations. In a comprehensive analysis of the murine microglial population, researchers from Boston Children's Hospital; the Broad Institute of MIT and Harvard; and the Agency for Science, Technology and

Experiment planning guide: Getting started with Single Cell Gene Expression

Whether you have questions about how to design your experiments, optimize sample preparation, or identify the computational tools necessary to best analyze your single cell gene expression data, we have you covered.

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Research (A*STAR) in Singapore used single cell RNA-seq to analyze the RNA expression patterns of more than 76,000 individual microglia in mice during development, old age, and after brain injury (13). Intriguingly, they found that while microglial diversity was greatest during youth, several microglial states either persisted throughout the mouse lifespan or actually increased in number with age. They also identified distinct microglial signatures in response to demyelinating injury which correlated with microglial subtypes found in human multiple sclerosis lesions (13). To delve deeper into molecular and cellular changes in Alzheimer's disease (AD), researchers from the Massachusetts Institute of Technology, the Broad Institute of MIT and Harvard, and Harvard Medical School used single cell RNA-seq to profile transcriptomes of 80,660 cortical cells from 48 individuals with varying disease pathology (14). Their research identified common and cell type–specific alterations of gene expression that correlated to the pathology, "as well as 40 transcriptionally distinct subpopulations of cells, some of which are preferentially overrepresented in AD pathology and differentially between sexes" (14).

Single cell transcriptomic analysis has also been used to explore the temporal properties underlying responses to stimuli. In a study on murine auditory system development, researchers from Johns Hopkins not only identified spiral ganglion neuron subclasses using single cell RNA-seq, but also found that specification for these subtypes requires hair cell–mediated, mechanotransduction-regulated spontaneous firing during auditory circuit maturation prior to the onset of hearing (15). In another study, Hannah Van Hove and colleagues used single cell transcriptomics for an unbiased characterization of the diversity of immune cell types in the brain, identifying regulators of the maturation and diversity of brain macrophages and providing "a framework for understanding host–macrophage interactions in both the healthy and diseased brain" (16).

Disease can also impact gene expression in regionally distinct ways. David Schafflick and researchers from the University Hospital Münster in Germany and University of California, Berkeley used single cell transcriptome profiling of almost 43,000 blood cells and over 22,000 cerebrospinal fluid (CSF) cells from control or multiple sclerosis patients to understand how disease states could differentially impact different cellular compartments (17). They found that changes in cell composition, including an expansion of $CD4^+$ cytotoxic T cells, occurred only in CSF, while transcriptional changes were observed in blood cells. In neuroscience, location matters. Having methods that can provide insight from limited numbers of cells can be critical for diagnostic and research goals, such as when sampling CSF, which has a cell concentration ~1,000-fold lower than blood (17).

Uncover new dimensions with spatial gene expression profiling

More recently, spatial gene expression profiling, which combines histology and mRNA analysis to measure the whole transcriptome in intact tissue sections, has emerged as an attractive alternative transcriptomic approach. In this method, tissue samples are placed on glass slides arrayed with spatially barcoded reverse transcriptase primers that confer two-dimensional positional information during sequencing (18). Measuring transcriptomes in this way allows scientists to examine the heterogeneous nature of gene expression patterns and regional gene expression alterations, and how they may contribute to normal brain function or neurodegenerative diseases.

A team of researchers from the Karolinska Institute, Sweden, affixed brain tissues directly to barcoded reverse transcriptase primers and performed reverse transcription followed by sequencing and computational reconstruction. The researchers demonstrated how high-quality RNA-seq data maintains two-dimensional positional information and how spatial gene expression profiling provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections (18). To investigate molecular mechanisms underlying amyotrophic lateral sclerosis (ALS), Silas Maniatis and colleagues used spatial gene expression profiling to examine gene expression in postmortem tissue of ALS patients and a murine model of the disease (19). They were able to follow the spatiotemporal dynamics of the disease progression in their model, including microglial activation, autophagy, and reactive gliosis, as well as gene regulation changes. The authors conclude that this approach enabled them to "provide a comprehensive spatiotemporal, transcriptome-wide gene expression dataset combining resolution, replication, and biological perturbation" (19).

To understand the role of amyloid plaques in neurodegeneration, Wei-Ting Chen, Ashley Lu, and colleagues led by Bart de Strooper and Mark Fiers from the Leuven Brain Institute in Belgium and University College London used Spatial Transcriptomics of mouse brain sections to identify molecular changes that take place in cells adjacent to amyloid deposits (20). They identified a network of 57 plaque-induced genes expressed by multiple cell types whose connectivity strengthened as plaque formation increased. The authors found that this cellular response led to inappropriate control of the classic complement cascade, leading to inflammation (20).

Spatial Gene Expression illuminates autism spectrum disorder

In a webinar from *The Scientist*, Kristen Maynard and Leonardo Collado-Torres at the Lieber Institute for Brain Development explain how they used Visium Spatial Gene Expression to identify autism spectrum disorder risk genes with layer-specific expression in the human dorsolateral prefrontal cortex. Read about this study and find a link to the recorded webinar on the 10x Genomics Blog.

Learn more ⟶

Resolve biological complexities

Ultimately, while the molecular biology revolution allowed scientists to use biomarkers to define cell types and subtypes within morphological classifications, progress has been limited by the amount of time required to individually investigate biomarker candidates and the difficulties involved in the identification of novel biomarkers. Additionally, the classification of cell types by a limited set of markers may bias analysis by obscuring cell subtypes under study, resulting in an inability to characterize the true extent of cellular diversity.

The advent of bulk RNA-seq, followed by single cell RNA-seq and now spatial gene expression profiling, has allowed these difficulties to be overcome; the depth and breadth already demonstrated by transcriptomics is redefining our understanding of neurological systems in previously unattainable ways (21). High-throughput transcriptome analysis gives researchers a way to identify novel differences between experimental groups and probe known marker candidates at the same time. Transcriptome analysis is helping scientists bridge the gap between the genome and proteome, which, in turn, supports the creation of comprehensive relational databases and enables multi-dimensional systems biology models of neurological systems (22).

Explore Spatial Gene Expression Profiling in the mouse brain

Want to explore the rich information that spatial gene expression profiling provides? Dive into our interactive technology highlight to learn more.

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References

1. AE Handel, G Disanto, SV Ramagopalan, Next-generation sequencing in understanding complex neurological disease. *Expert Rev Neurother.* 13, 215–227 (2013).
2. MJ Keogh, PF Chinnery, Next generation sequencing for neurological diseases: New hope or new hype? *Clin Neurol Neurosurg.* 115, 948–953 (2013).
3. R Hitzemann et al., Introduction to sequencing the brain transcriptome. *Int Rev Neurobiol.* 116, 1–19 (2014).
4. B Hwang, JH Lee, D Bang, Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp Mol Med.* 50, 96 (2018).
5. JM Keil, A Qalieh, KY Kwan, Brain transcriptome databases: A user's guide. *J Neurosci.* 38, 2399–2412 (2018).
6. R Horie et al., Shared evolutionary origin of vertebrate neural crest and cranial placodes. *Nature.* 560, 228–232 (2018).
7. T Horie, R Horie, K Chen, C Cao, M Nakagawa et al., Regulatory cocktail for dopaminergic neurons in a protovertebrate identified by whole-embryo single-cell transcriptomics. *Genes Dev.* 32, 1297–1302 (2018).

References

8. S Pandey et al., Comprehensive identification and spatial mapping of habenular neuronal types using single-cell RNA-seq. *Curr Biol.* 28, 1052–1065.e7 (2018).
9. K Davie et al., A single-cell transcriptome atlas of the aging drosophila brain. *Cell.* 174, 982–998.e20 (2018).
10. MC Marchetto et al., Species-specific maturation profiles of human, chimpanzee and bonobo neural cells. *Elife.* 8, e37527 (2019).
11. A Grubman et al., A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nat Neurosci.* 22, 2087–2097 (2019).
12. O Al-Dalahmah et al., Single-nucleus RNA-seq identifies Huntington disease astrocyte states. *Acta Neuropathol Commun.* 8, 19 (2020).
13. TR Hammond et al., Single-Cell RNA Sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. *Immunity.* 50, 253–271.e6 (2019).
14. H Mathys et al., Single-cell transcriptomic analysis of Alzheimer's disease. *Nature.* 570, 332–337 (2019).
15. S Sun et al., Hair cell mechanotransduction regulates spontaneous activity and spiral ganglion subtype specification in the auditory system. *Cell.* 174, 1247–1263.e15 (2018).
16. H Van Hove et al., A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. *Nat Neurosi.* 22, 1021-1035 (2019).
17. D Schafflick et al., Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis. *Nat Commun.* 11, 247–247 (2020).
18. PL Ståhl et al., Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science.* 353, 78–82 (2016).
19. S Maniatis et al., Spatiotemporal dynamics of molecular pathology in amyotrophic lateral sclerosis. *Science.* 364, 89–93 (2019).
20. W Chen et al., Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease. *Cell.* 182, 976-991. e19 (2020).
21. A Casamassimi et al., Transcriptome profiling in human diseases: New advances and perspectives. *Int J Mol Sci.* 18, (2017).
22. DH Geschwind, G Konopka, Neuroscience in the era of functional genomics and systems biology. *Nature.* 461, 908–915 (2009).

Chapter 3

Building your understanding of the nervous system, cell by cell

The brain comprises a complex system with distinct cell types that are spatially and temporally regulated. When bombarded by physiological or pathological events, the brain has an extraordinary ability to forge new or stronger connections, altering neural mechanisms based on current needs. This dynamism of the CNS translates to extensive cell plasticity and huge variation in cell function, type, and subtype. Without this constant cell fluctuation, memories could not be formed, and recovery from brain damage would likely be impossible. Disruption of a component within the system due to physiological elements or pathological events can alter the structure and function of the brain and its associated structures.

The immense diversity of the nervous system

The CNS exhibits vast interconnectivity, large cellular networks that travel both within and beyond the brain, and myriad cell types with varied and irregular cell shapes. Neurons show great diversity in shape and size, and, as such, limited genetic and molecular tools have been available to neuroscientists historically (1). Furthermore, due to the nature of CNS tissues, access may be limited and large sample sizes difficult to obtain. Traditional methods, such as histological staining, for studying cell-to-cell interactions are incredibly useful and widely used tools in neuroscience, but they cannot completely convey the immense number of parameters that need to be studied within the CNS for full comprehension. They also aren't suited when sample availability is limited.

To overcome challenges in single cell and cell-to-cell analysis in neuroscience, several methods have been developed for classifying cells in the CNS. Techniques that make use of morphological and electrophysical properties, as well as differentially expressed genes, have been used for classification with varying degrees of success, but these technologies barely touch upon describing the complete cellular phenotype (2). More recently, single cell approaches have emerged through advances in NGS of DNA and RNA, enabling analysis of small amounts of material, large numbers of samples, and a myriad of other molecular parameters (3).

Comprehensive profiles of single cells

Single cell RNA-seq analysis provides comprehensive profiles of individual cells and can be used to compare the profiles of one cell versus another (Figure 1). Through the examination of cell-to-cell differences in RNA transcripts and comparison of control and disease states, key neurobiological questions can be answered. Naomi Habib and researchers from The Hebrew University of Jerusalem, the Broad Institute of MIT and Harvard, The Weizmann Institute, and the Massachusetts Institute of Technology identified

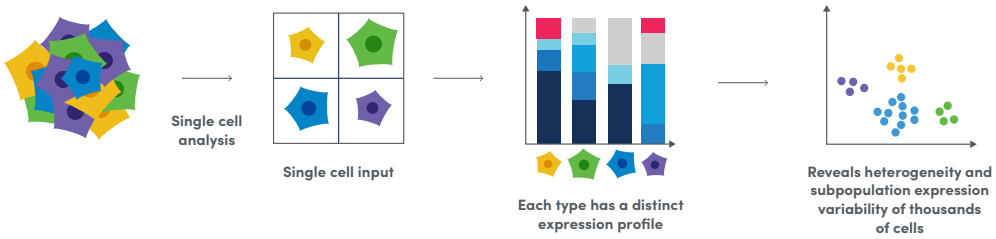

Figure 1: Single cell gene expression profiling reveals cellular heterogeneity.

a cellular map of Alzheimer's disease by examining 54,769 single-nucleus RNA profiles isolated from the hippocampus of transgenic mice modeling Alzheimer's disease or wild-type controls. In general, cell subtype proportions were consistent between control and diseased hippocampi (4). However, the researchers identified a unique population of astrocytes specific to Alzheimer's disease–model mice. These disease-associated astrocytes (DAAs) could be found in the hippocampus and cortex of Alzheimer's disease mice and appeared before the onset of cognitive decline, gradually increasing in number as the disease progressed. DAA-like cells could also be found in Alzheimer's disease human brains, suggesting physiological relevance of the mouse model to human disease (4).

Gaining insights into cell types and subtypes

Single cell analyses have been instrumental in the identification of cell subtype, phenotype, and function for a variety of cell types and states, diseases, and model organisms. In particular, Alzheimer's research has been transformed by both RNA-seq and single cell RNA-seq. In a review by Jan Verheijen and Kristel Sleegers, the authors discuss how single cell transcriptomics analysis has been pivotal in providing additional support for previously unidentified risk genes while also identifying novel associated genes, helping to elucidate mechanisms of Alzheimer's disease (5). The dynamic nature of the transcriptome and heterogeneity between tissues and cell types adds complexity to the process of elucidating molecular mechanisms contributing to Alzheimer's disease (5). As discussed in the previous chapter, researchers have used single cell RNA-seq to profile transcriptomes from 80,660 cortical cells from 48 individuals with varying stages of AD pathology, shedding insight into the cell type–specific alterations of gene expression that correlated to the disease pathology (6). For Alzheimer's disease risk genes like TREM2, transcriptome profiling at single cell resolution can resolve molecular mechanisms and identify potential therapeutic interventions (7). Using an agonistic antibody, researchers found that activation of control or an impaired variant of TREM2 in microglia could alleviate symptoms of Alzheimer's disease in a mouse model. Moving into human phase 1 clinical trials, the antibody was well tolerated and showed a reduction in TREM2 cleavage (7). Finally, by profiling both the blood and CSF of adults with Alzheimer's disease, mild cognitive impairment, or no evidence of neurodegeneration, researchers at Stanford University in California discovered that individuals with Alzheimer's disease or mild cognitive impairment had increased numbers of peripheral $CD8^+$ T effector memory cells and that the proportion of these cells negatively correlated with cognition (8). This study demonstrated a previously unrecognized adaptive immune response in the blood and CSF of Alzheimer's patients, shedding light on the pathogenesis of disease and paving the way for the development of novel diagnostics.

Due to the complex etiology of psychiatric disorders, the molecular mechanisms remain elusive and researchers are turning to new techniques, including single cell RNA sequencing, to unravel the genetics of mental disorders. To gain insight into cell type and molecular changes in autism spectrum disorder (ASD), Dmitry Velmeshev and colleagues

from Arnold Kriegstein's lab used single cell RNA-seq to examine a total of 104,559 cells from the cortex of 15 ASD patients and 16 controls (9). They identified gene expression changes in microglia and upper layer excitatory neurons, including genes involved in synaptic function and neural development, providing insight into the molecular changes involved in this disorder. Summer Thyme and colleagues used single cell RNA-seq to profile the forebrains of zebrafish models of schizophrenia and controls, identifying neuronal subpopulations that may be involved in the disease etiology, as well as potential therapeutic targets (10). In the severe X-linked neurodevelopmental disorder Rett syndrome, loss of function of methyl-CpG binding protein 2 (MeCP2) results in genome-wide transcriptional defects. In a study led by Yangfei Xiang and Yoshiaki Tanaka, a cell culture–based model of human Rett syndrome was developed using a hESC line (11). The researchers then identified a putative therapeutic treatment for Rett syndrome, the small molecule JQ1, and used single cell sequencing of human organoids to evaluate its transcriptional impact genome-wide in a cell type–specific manner. Not only did treatment with JQ1 ameliorate gene regulation defects in culture, it also prolonged lifespan in a mouse model of Rett syndrome (11).

Aside from pathogenesis, single cell analysis can also be applied to studies of basic neuroscience, including neurodevelopment and evolution. Suijuan Zhong and a team of researchers from Beijing, China, catalogued gene expression changes at single cell resolution across human brain development using 30,416 cells from the hippocampus at gestational weeks 16–27 (12). In combination with bulk epigenetic assays, they identified 47 cell subtypes, their developmental trajectories, and a key gene regulatory network (12). Researchers at the University of Connecticut School of Medicine analyzed more than 6,000 retinal ganglion cells (RGC) using single cell RNA-seq, classifying them into 40 subtypes (13). Previously, only 30 RGC subtypes had been identified; in addition to demonstrating the extent of gene expression variability needed for subtype segregation, it also revealed a hierarchy in diversification from cell-type population to subtypes (13).

In a study led by researchers from The Salk Institute for Biological Studies and UC San Diego, species-specific maturation profiles of human, chimpanzee, and bonobo neural cells were used to investigate the development of cortical pyramidal neurons, which are a common class of neuron found in virtually every mammal (14); they comprise about two-thirds of all neurons in the mammalian cerebral cortex, and their axons span long distances, sometimes outside of the brain, making them an attractive target for studies in evolution. As part of the study, the researchers performed single cell RNA-seq on neural progenitor cells (NPCs) to determine whether there was a difference in cortical layer identity between species at the population level. The results revealed that each species had similar proportions of cortical markers, indicating that within the cortical-fated cells there was no enrichment for any given layer between the species. Overall, results showed differential migration patterns in human NPCs compared to the non-human primates, suggesting heterochronic changes in human neurons (14).

Neural insights from epigenomics

Other notable single cell methods that were recently developed include single cell assays for accessible chromatin, which provide single cell information at the epigenetic level. Single cell ATAC-seq measures the degree to which specific regions of chromatin are accessible to regulatory factors. The technique relies on nuclei being transposed in bulk, followed by partitioning on a microfluidic chip. Single cell ATAC-seq can analyze thousands of cells, providing insights into the regulatory landscape of the genome and cell-to-cell variation.

Surveying the regulatory landscape with Single Cell Epigenomics

The control of gene expression via epigenetic mechanisms integrates both intrinsically programmed and environmental signals in neurons. Analyzing the epigenome at the single cell level is critical in understanding how neuronal populations develop and regulate their expression profiles. The new ability to combine readout of the epigenome and transcriptome from the same cell can provide even greater resolution of cellular heterogeneity and gene regulatory mechanisms. With single cell epigenomics, you can:

- Profile the chromatin landscape cell by cell and identify transcription factor binding sites
- Uncover cellular epigenetic variability
- Dissect the role of epigenetics in neural plasticity

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The combination of single cell transcriptomics and epigenomics can reveal intricate gene regulatory mechanisms. By integrating chromatin conformation capture (Hi-C) with single cell ATAC-seq and single cell RNA-seq, scientists at St. Jude Children's Research Hospital in Memphis, Tennessee uncovered a transcriptional super enhancer specific to bipolar neurons of the retina (15). Single cell analysis was necessary to find cell-specific enhancers of low-abundance cell types including bipolar cells and Müller glia. Disruption of the bipolar-specific enhancer led to a complete loss of bipolar cells in adult mice, confirming the enhancer's importance (15).

Identifying gene regulatory networks in neurodevelopment

Are you curious about what type of information can be obtained by combining single cell transcriptome and open chromatin profiling? In a webinar hosted by Biocompare, Tomasz Nowakowski from the University of California, San Francisco, describes how his lab found gene regulatory networks that may control cerebral cortex development by combining single cell RNA-seq and single cell ATAC-seq data.

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Single cell technologies are beginning to unlock the causes of neurodegenerative diseases and psychiatric disorders, among other pathologies, while also providing clues into neuronal changes occurring during evolution. Single cell RNA-seq and single cell ATAC-seq are helping to paint a more detailed picture of neurological mechanisms, allowing researchers to find new biomarkers for disease and injury, characterize new neuronal subtypes, and examine nervous system evolution.

References

1. H Zeng, JR Sanes, Neuronal cell-type classification: Challenges, opportunities and the path forward. *Nat Rev Neurosci.* 18, 530–546 (2017).
2. B Tasic, Single cell transcriptomics in neuroscience: cell classification and beyond. *Curr Opin Neurobiol.* 50, 242–249 (2018).
3. J Shendure, H Ji, Next-generation DNA sequencing. *Nat Biotechnol.* 26, 1135–1145 (2008).
4. N Habib et al., Disease-associated astrocytes in Alzheimer's disease and aging. *Nat Neurosci.* 23, 701–706 (2020).
5. J Verheijen, K Sleegers, Understanding Alzheimer disease at the interface between genetics and transcriptomics. *Trends Genet.* 34, 434–447 (2018).
6. H Mathys et al., Single-cell transcriptomic analysis of Alzheimer's disease. *Nature.* 570, 332–337 (2019).
7. S Wang et al., Anti-human TREM2 induces microglia proliferation and reduces pathology in an Alzheimer's disease model. *J Exp Med.* 217, e20200785 (2020).
8. D Gate et al., Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. *Nature.* 577, 399–404 (2020).
9. D Velmeshev et al., Single-cell genomics identifies cell type–specific molecular changes in autism. *Science.* 364, 685–689 (2019).
10. SB Thyme et al., Phenotypic landscape of schizophrenia-associated genes defines candidates and their shared functions. *Cell.* 177, 478–491 (2019).
11. Y Xiang et al., Dysregulation of BRD4 Function Underlies the Functional Abnormalities of MeCP2 Mutant Neurons. *Mol Cell.* 79, 84–98.e9 (2020).
12. S Zhong et al., Decoding the development of the human hippocampus. *Nature.* 577, 531–536 (2020).
13. BA Rheaume et al., Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. *Nat Commun.* 9, 2759 (2018).
14. MC Marchetto et al., Species-specific maturation profiles of human, chimpanzee and bonobo neural cells. *Elife.* 8, e37527 (2019).
15. JL Norrie et al., Nucleome Dynamics during Retinal Development. *Neuron.* 104, 512–528.e11 (2019).

Chapter 4

Unlock the potential of your research

To better understand neural function and disease, researchers need to find ways to unlock the secrets of the complex networks regulating the CNS. Understanding these cellular and molecular networks is pivotal for developing new treatments and for preventing or decelerating neurological disease.

A major challenge to fully understanding how the brain works, and to understanding and identifying the genes and transcripts that control neural health and viability, is the complexity of the CNS. Techniques that average signal across a whole population of cells, or those that rely on just a few molecular markers, do not provide a complete picture. For example, in a study by researchers at the University of Edinburgh, analysis of transcriptomic datasets from the mammalian nervous system available in the public domain was used to define the genes critical for influencing neural health and disease in different neural cell types and brain regions (1). Genes with minimal functional annotation were validated as being key nervous system genes, and some genes were identified as novel candidates for a role in neurological health and disease (1). Results from broader techniques such as this can be further investigated using single cell technologies to confirm their function and regulation during aging and neurodegeneration.

Similar analysis of public data was performed by researchers at the CHA University, Seoul, who used co-expression networks, built on post-mortem and Alzheimer's brain transcriptome data, to investigate key surrogate biomarkers of the aging brain. Results revealed that the complement system is likely to be a master regulator in initiating and regulating the immune system in the aging brain and could serve as reliable and surrogate biomarkers for the diagnosis of cognitive dysfunction (2).

Massively parallel RNA-seq platforms have transformed our understanding of complex neurological diseases, such as schizophrenia. Bulk RNA-seq studies have shown that there are extraordinary transcriptome dynamics, with significant levels of alternative splicing, associated with the disease (3). Single cell studies are now extending this research, exploring the transcriptional diversity in neuropsychiatric diseases and providing the basis for new biomarkers and improved treatments (4).

Push the boundaries of neuroscience research

The human brain—with its intricate network of synaptic interactions, distinct cell types, and dynamic responses to intrinsic and extrinsic stimuli, including disease—remains one of the greatest mysteries of life. However, with recent advances in transcriptomics, particularly in single cell transcriptomics, we are now beginning to appreciate the complexity of the brain and correlating pathological systems with physiological states. Neurological diseases and disorders are historically difficult to treat due to their convoluted etiological mechanisms. Both multicellular and single cell transcriptomics are helping to transform our understanding of neuropathologies, from the severity of traumatic brain injury to the diagnosis and progression of Alzheimer's disease. With transcriptomics, biomarkers for neurological disease can be discovered and further researched using single cell and spatial technologies to identify potential treatment targets. Transcriptomics of CNS and even of peripheral fluids and tissues, whether used to profile cell populations within an environment or to investigate single cells, are providing neuroscientists with the answers that they seek.

How can 10x Genomics accelerate your neuroscience research?

The nervous system is a complex network of diverse cell types with a myriad of functions, precisely regulated by distinct molecular processes. Neuroscience research relies on cellular characterization by molecular phenotype, electrophysiology, connectivity, morphology, neurochemistry, and physical location, but the depth of information needed to fully characterize and understand neural cells, their subtypes, and ultimately their function is often hampered by experimental limitations. 10x Genomics empowers neuroscientists to understand the cellular and molecular mechanisms underlying both normal function and disease states with single cell analysis and spatial gene expression solutions.

Uncover molecular insights of neural cell function and disease mechanisms

Gain insight into the diverse, complex cellular and signaling networks that control the function of the CNS with unbiased, high-resolution approaches.

Delve deeper into the intricacies of cellular phenotypes and signaling pathways within the CNS with single cell gene expression analysis. Study the epigenetic mechanisms of gene regulation with single cell epigenomics, offering insights into the regulatory landscape of the neural genome and transcriptome.

Interrogate the molecular mechanisms that underlie normal development, neural function, disease, and injury. Access an integrated cellular view of RNA and protein expression with multiomics approaches.

Examine the dynamic nature of gene expression patterns and regional gene expression alterations, and how they may contribute to normal brain function or neurodegenerative disorders, with spatial gene expression.

Gain a deeper understanding of neural cell identity

Explore neural cell identity and discover a comprehensive, unbiased view of the nervous system at single cell resolution.

Access enhanced cellular phenotyping at the transcriptome level based on distinct gene expression patterns with single cell gene expression profiling. Use these molecular signatures to classify individual cells into major cell types in the brain and catalog neural cell populations.

Reveal the full complexity of neural diversity

Characterize complex populations and reveal rare cell populations, as well as new biomarkers for cellular phenotypes and cell states, with single cell transcriptomics.

Simultaneously decipher gene expression patterns and location with spatial gene expression profiling for high-throughput analysis of transcriptome profiles in situ.

References

1. SM Carpanini et al., Analysis of gene expression in the nervous system identifies key genes and novel candidates for health and disease. *Neurogenetics.* 18, 81–95 (2017).
2. SH Bae et al., Decipher reliable biomarkers of brain aging by integrating literature-based evidence with interactome data. *Exp Mol Med.* 50, 28 (2018).
3. X Wang, MJ Cairns, Understanding complex transcriptome dynamics in schizophrenia and other neurological diseases using RNA sequencing. *Int Rev Neurobiol.* 116, 127–52 (2014).
4. IY Iourov, SG Vorsanova, YB Yurov, Single cell genomics of the brain: Focus on neuronal diversity and neuropsychiatric diseases. *Curr Genomics.* 13, 477–488 (2012).

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