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# Single cell genomics for cellular phenotyping: applications in brain disorders

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**Abstract**

This article explore the avenues opened by single cell genomics in medical research and clinical practice, with focus on brain and neurodevelopmental disorders such as autism.

**Key words:**

single cell sequencing, brain transcriptomics, autism, human cell atlas, precision medicine

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## Cell identity and defining parameters

Cell identity is a challenging and elusive concept defined with regard to cell type, cell lineage and cell state. Central to cell identity definitions are the phenotype and function that allow categorization into cell types. A cell's phenotypic individuality is confered by the unique set of molecules it contains, such as DNA and RNA, proteins, metabolites and a wide range of other molecules. While cell identity is stable during the lifetime of a normal cell, the state of a cell is a dynamic set of different phenotypes that occur in response to cell intrinsic and extrinsic factors (Kosik, 2010; Mincarelli et al., 2018).

Cells have been traditionally classified by their shape and size (morphology), the anatomical/tissue location and types of cell-to-cell interactions. The technological advances in molecular biology, namely interrogation of the genome, transcriptome, epigenome and proteome etc., lead to a new understanding of the molecular structure and cell's physiological functions. Among these approaches, transcriptome-wide gene expression profiling is the most widely employed for cell classification, while offering a comprehensive understanding of biological processes in health and disease (Naumova, 2013; Xia and Yanai, 2019; Morris, 2019). Until recently, the majority of profiling studies have analyzed large populations of input cells, such approaches masking the cellular heterogeneity. This cell-to-cell variability can be observed even in similar cell types as the result of differences between cell sub-types, homeostatically regulated and/or stochastic process variations. The field of single-cell genomics, including profiling of RNA, DNA or proteins, has advanced rapidly in the last few years and has generated new insights in many fields including neurobiology, cancer and developmental biology. The tehnological progress has enabled high-throughput single cell or single nucleus sequencing of thousands of individual cells in a single experiment, allowing the study of cell diversity in a sample without the loss of information that occurs analyzing bulk tissue samples. Since the first single cell RNA sequencing (scRNA-seq) study was published (Tang et al., 2009), the number of studies increased, and new methods for single-cells isolation and expression profiling from low amounts of RNA were developed.

scRNA-seq is a powerfull tool to characterize heterogeneous, complex and rare cell populations, to understand the relationships among cells during different processes, and the functional state of individual cells (Hwang et al., 2018). Furthermore, as cell identity is defined not only by its transcriptome, but also by the genome, epigenome, proteome etc., the multidimensional / multi-omics analysis allows a comprehensive understanding of single cell functions (Macaulay et al., 2017; Song et al., 2019). Considering the multiple phenotypic layers that contribute to a cell's identity, the ability to dissect and accurately describe the individual cell's genome, epigenome, transcriptome, proteome, metabolome etc, separately or combined in multi-omics assays, is essential for understanding complex biological systems (Kosik, 2010; Mincarelli et al., 2018; Morris, 2019).

## Brain transcriptomics

The mammalian brain stands out as one the most complex and challenging tissue; it contains a large number of cells, categorized into a wide range of cell types and subtypes, with highly specialized or support functions. Traditionally, brain cells have been classified by a combination of features such as anatomical location, cell morphology, electrophysiological properties, connectivity and gene expression (Cuevas-Diaz Duran et al., 2017; Mu et al., 2019). Gene expression studies, starting with targeted assays based on PCR and FISH and expanding to transcriptome-wide assays such as microarray and RNA sequencing, greatly advance the mechanistic understanding of human brain functionality in health and disease. Transcriptomic studies revealed that brain gene expression processes are characterized by several particuliarities that make brain unique among other tissues and organs. Firstly, the number of genes expressed is very high, around 80–95% of protein-coding genes being expressed in the human brain (Bae et al., 2015). Secondly, the brain RNA populations are more diverse compared to other tissues; also, there is a high level of alternative splicing (de la Grange et al., 2010; Naumova et al., 2013). Thirdly, the brain transcriptome varies across different brain regions reflecting the functional and anatomical differ-

ences among these structures. The patterns of gene expression define cell types and neuroanatomical structures, and highlight molecular pathways with critical roles for the development and function of the brain (Bae et al., 2015). Single-cell analysis is critical for understanding the heterogeneity of brain cells, their function and state, as well as the regulatory networks within brain cells (Cuevas-Diaz Duran et al., 2017). Systematic gene expression profilling studies hold the promise of a better understanding of molecular mechanisms involved in the control of cognitive and behavioral functions, both in neurotypic individuals and in disease states (Naumova et al., 2013).

## Brain genomics

The human brain is a highly heterogeneous collection of neuronal and non-neuronal cells. Gene expression changes, in response to external stimuli or during development, have been intensely studied in order to decipher the complexity of the brain. In addition, somatic genomic differences among cells has also been suggested to play a role in the functional diversity of brain cells (Evrony, 2016).

Genetic variation within an organ or tissue defines somatic mosaicism. The phenomenon is well recognized in healthy individuals in immune compartment cells involved in generating diverse immune responses to antigens (O'Huallachain et al., 2012) or in disease conditions, such as cancer. Age related structural genomic variation has been described in human brain consisting of aneuploidy and retrotransposition events (Yurov et al., 2005; Baillie et al., 2011). Single cell genomic studies further contributed by unveiling a high degree of genome variation in human brain due to DNA copy number variants (CNVs) and single nucleotide sequence variants (SNVs), in normal and diseased tissues (Evrony et al., 2012; Cai et al., 2014; Lodato et al., 2015). Mature neurons in the adult cortex of normal individuals have an estimated 1500 somatic SNVs per cell, enriched in neurodevelopmental genes actively transcribed (Lodato et al., 2015).

## Neurodevelopmental disorders exemplified by autism spectrum disorders

Autism spectrum disorders (ASDs) are complex neurodevelopmental conditions characterized by impairments in communication and social interaction, and restricted, repetitive patterns of behavior and interests (Lai et al., 2014). ASD patients often present with co-morbidities such as gastrointestinal problems, epilepsy, intellectual disability, and motor abnormalities (Lai et al., 2014). ASD is thus, not only a neuropsychiatric disorder, but a multiple systems condition. As a neurodevelopmental disorder, different neuroanatomical anomalies have been reported in ASD. Such patients often display an increased size of the cortex during the toddler period followed, in childhood and adolescence, by a reduction of the brain growth. Also, a larger minicolumn width in younger children with ASD has been reported (Donovan and Basson, 2017).

ASD has a strong genetic component and one of the highest heritability among neuropsychiatric disorders (Smalley et al., 1988). The genetic architecture of ASD is extremely heterogenous in terms of alleles frequencies, patterns of inheritance and variant types (De Rubeis and Buxbaum, 2015; Ramaswami and Geschwind, 2018). During decades of genetic studies, that started with karyotyping and progressed toward genome-wide chromosomal microarrays, whole exome and whole genome sequencing, a wide spectrum of genetic defects were identified, from large chromosomal rearrangements and CNVs to small insertions/deletions (indels), and SNVs. The combination of chromosomal microarrays and whole exome sequencing revealed many ASD risk genes and highlighted the role played by rare de novo mutations, detected in 10-20% of patients, in ASD susceptibility (Jeste and Geshwind, 2014). Monogenic ASD-related disorders, such as Rett syndrome, fragile X syndrome, tuberous sclerosis characterize one end of the ASD genetic spectrum, where a single gene mutation or a CNV affecting one gene have a major phenotypic effect (Rylaarsdam and Guemez-Gamboa, 2019). The opposite end of the spectrum is the polygenic model that recognizes the contribution of multiple, small effect, common variants (Iakoucheva et al. 2019). The oligogenic model applies to ASDs with two or more genetic defects, the joint effects of known pathogenic variants and other rare variants leading to clinical heterogeneity in term of severity and disease manifestations (Iakoucheva et al. 2019). Boyle et al. (2017) proposes another complex genetic model, the omnigenic model,

which involves highly interconnected cell regulatory networks. In this model, the disturbance of „peripheral" gene expression impairs the normal regulation and function of „core" genes.

Somatic SNVs affecting known risk genes for psychiatric disorders, such as autism, identified in postmortem brains of affected individuals indicate that this type of genetic alteration may be associated with disease development (D'Gama et al., 2015; Nishioka et al., 2019), although further research is needed. It has also been proposed that somatic mutations may act as modifiers of germline variants, influencing disease expression or penetrance (Nishioka et al., 2019). Moreover, in a recent study, Rodin et al. found an increased frequency of somatic mutations in neural enhancers in ASD patients cortex versus controls and hypothesize that mosaic enhancer mutations are potential contributors to ASD development (Rodin et al., 2020).

Taking into account the genetic heterogeneity of ASDs, efforts have been made to identify commonly affected pathways in order to advance the understanding of disease pathomechanisms for larger patients groups and to pinpoint potential therapeutic targets. The analysis of genetic defects in ASD and the functions of the affected proteins converged towards proteins involved in synaptic development and function and transcription regulation and chromatin remodeling (De Rubeis et al., 2014).

Whole-tissue RNA microarray and sequencing studies revealed gene expression changes in the brain of ASD patients in comparison to control samples (Voineagu et al., 2011; Parikshak et al., 2016). Co-expression analyses of transcriptomic data by Parikshak et al showed ASD associated changes in gene co-expression modules, with upregulation of modules involved in development, inflammation and glial function and downregulation of modules involved in synaptic and neuronal function (Parikshak et al., 2016; Bray, 2017). In brain samples of young ASD patients, downregulation of genes involved in brain structure was noted. On the other hand, in the brain of the adult patients with ASD, the genes involved in cellular diferentiation are dysregulated, explaining the reduced brain size in the ASD adult patients (Donovan and Basson, 2017). Furthermore, ASD individuals show a reduction of gene expression differences between distinct brain regions (Parikshak et al., 2016; Donovan and Basson, 2017). However, these results reflect the gene expression of bulk tissues consisting of diverse cell populations and do not describe the expression of specific genes in specific neuronal cell types and sub-types. This limitation has been overcomed by studying the transcriptomes of single brain cells in ASD (Velmeshev et al., 2019). Velmeshev et al applied single-nucleus RNA sequencing to analyse neurons and glia from postmortem cortical tissue. The most affected pathways in studied cells were those involved in regulation of synapse function, neuronal outgrowth and migration. The study revealed that synaptic signaling of upper-layer cortical projection neurons and microglia were affected in ASD patients. Furthermore, the data suggest that dysregulation of specific sets of genes in cortico-cortical projection neurons is correlated with clinical severity of the disease. The authors also highlight the importance of single cell multi-omics assays that will allow, for example, the analysis of brain cell transcriptomic profile in the context of the cellular genomic landscape, in order to better understand the mechanisms of ASD and design specific treatments targeting affected cell types (Velmeshev et al., 2019).

## Medical and research applications of single cell approaches

Single cell assays brought an unprecedented level of understanding of cell identity, its processes and functions as well as communication and interactions between cells. The challange of single cell era is now the translation of this wealth of information to clinical practice.

Single-cell transcriptomics has made a substantial contribution to the development of comprehensive cell type atlases of different tissues /organs. Great research efforts were dedicated to identifying and characterizing cells in complex organs such as mouse brain (Carter RA et al., 2018; Rosenberg et al., 2018; Saunders et al., 2018; Zeisel et al., 2018.). In 2018, Han et al. created Mouse Cell Atlas derived from comprehensive single-cell transcriptomic analysis. This Atlas can be used for different applications, one example being the Mouse Cell Network Atlas created by Suo et al. (2018), which can be use for the identification of regulatory network structure of different cells and characterisation of critical regulators for cell identity.

The creation of a human brain cell atlas that incorporates well known parameters used for cell type identification (anatomical location, morphological and electrophysiological data, synaptic and conectivity properties) alongside information regarding their molecular identity, developmental lineage, and contribution to brain disease has proved challenging (Mahfouz et al., 2017; Mu et al., 2019). A comprehensive map of human and mouse brain gene expression has been developed by the Allen Institute for Brain Science (Pollock et al., 2014), many data being provided by microarray and more recently single-cell RNA seq gene expression studies. This resource also provides information about the spatial and anatomic location of analysed cells. To reduce the paucity of relevant human samples, datasets of previous studies were integrated in Allen Human Brain Atlas; therefore healthy and disease sample results are available for multiple brain regions. Single cell RNA sequencing data in corroboration with results obtained by molecular profiling of genes in bulk tissue samples, and data regarding morphology, connectivity and physiology of cells are essential for a correct characterization of brain cell types. Data comparison of normal and disease samples from the same brain regions would help to understand the functions and interactions of brain cells, providing insights into the affected pathways in different cell types, including the role of distinct cell types in disease. The Human Cell Atlas, represents yet another ambitious research endeavor that parallels The Human Genome Project, and aims to provide a reference map of human healthy and diseased tissues (Regev et al., 2017; Rozenblatt-Rosen et al., 2017). The generation of the Human Cell Atlas will allow comparisons of diseased tissues with a standard reference. New opportunities are thus created for early accurate diagnosis and precision medicine approaches (Strzelecka et al., 2018).

The use of combined single-cell omics technologies to study brain tissue will generate information which can lead to identification of candidate pathways involved in neurodevelopment diseases, facilitating discovery of novel biomarkers and creating new opportunities for targeted therapies development. For example, it is well known that many ASD genes are involved directly or indirectly in synaptic structure and function, leading to the concept of ASD as a "synaptopathy" (Zoghbi and Bear, 2012). For these patients, the development of a gene therapy to modulate the expression of targeted proteins within this network could be an efficient approach to regain the normal function of synapses.

Alongside a multitude of promises, the single cell genomics field also faces many challenges and has limitations that need to be addressed. There are technical issues, statistical issues, and biological factors which generate a high variability of single cell sequencing data. The quality of the results depends on the design, implementation, assembly and interpretation of the data, all of which raise challenges for protocol type selection, computational processing and data analyses. With the technological advances for single cell isolation, -omics approaches and data integration, many of these issues will be overcome. For some of the single cell genomics limitations the solution lies in using more than one -omic approach. For example, the limitation in mutation detection sensitivity by RNA sequencing, can be surpassed by integration of multiple data sets from the same cell, for example RNA and DNA sequencing. The limitations in resolving spatial and time resolution can be solved by using in situ sequencing and single cell approaches of organoids, respectively.

Overall, it is to be hoped that multi-omics profiling of single cells will advance precision medicine, in accurate diagnostic assays and better targeted therapies (Strzelecka et al., 2018).

## Conclusions

Single-cell sequencing technologies, by accurately studying multiple phenotypic levels of individual cells, enable researchers to refine cell type classifications and to construct maps of cells within different tissues and organs up to the entire organism. Single cell studies reveal a level of genomic and transcriptomic heterogeneity previously unrecognized.

Thus, the intimate understanding at single cell level, in health and in disease, becomes critical in the quest for new diagnostic, prognostic or predictive biomarkers and molecular therapeutic targets.

**References:**

**Bae BI, Jayaraman D, Walsh CA** (2016) Genetic changes shaping the human brain. Dev Cell. 32(4):423-434. doi: 10.1016/j.devcel.2015.01.035

**Baillie JK, Barnett MW, Upton KR, et al** (2011) Somatic retrotransposition alters the genetic landscape of the human brain. Nature. 479(7374):534–537. doi: 10.1038/nature10531

**Boyle EA, Li YI, Pritchard JK** (2017) An expanded view of complex traits: from polygenic to omnigenic. Cell. 169(7):1177–1186. doi: 10.1016/j.cell.2017.05.038

**Bray N** (2017) Converging on autism spectrum disorder. Nat Rev Neurosci 18(2):67. doi: 10.1038/nrn.2016.184

**Cuevas-Diaz Duran R, Wei H, Wu JQ** (2017) Single-cell RNA-sequencing of the brain. Clin Trans Med. 6(1):20. doi: 10.1186/s40169-017-0150-9

**Cai X, Evrony GD, Lehmann HSet al** (2014) Single-cell, genome-wide sequencing identifies clonal somatic copy-number variation in the human brain. Cell reports. 8(5):1280–1289. doi: 10.1016/j.celrep.2014.07.043

**Carter RA, Bihannic L, Rosencrance C, et al** (2018) A single-cell transcriptional Atlas of the developing murine cerebellum. Curr Biol. ;28(18):2910-2920.e2. doi: 10.1016/j.cub.2018.07.062

**de la Grange P, Gratadou L, Delord M, et al** (2010) Splicing factor and exon profilingacross human tissues. Nucleic Acids Research. 2010;38(9):2825–2838. doi: 10.1093/nar/gkq008

**D'Gama AM, Pochareddy S, Li M, et al** (2015). Targeted DNA sequencing from autism spectrum disorder brains implicates multiple genetic mechanisms. Neuron.88(5):910–917. doi: 10.1016/j.neuron.2015.11.009.

**De Rubeis S, He X, Goldberg AP, et al** (2014) Synaptic, transcriptional and chromatin genes disrupted in autism. Nature. 515:209–215. doi: 10.1038/nature13772

**De Rubeis S, Buxbaum JD** (2015) Genetics and genomics of autism spectrum disorder: embracing complexity. Hum Mol Genet. 24(R1):R24–31. doi: 10.1093/hmg/ddv273

**Donovan APA, Basso, MA** (2017) The neuroanatomy of autism – a developmental perspective. J. Anat. 230(1):4-15. doi: 10.1111/joa.12542

**Evrony GD, Cai X, Lee E et al** (2012) Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain. Cell. 151(3):483–496. doi: 10.1016/j.cell.2012.09.035

**Evrony GD, Lee E, Park PJ et al** (2016) Resolving rates of mutation in the brain using single-neuron genomics. Elife. 5:e12966. doi: 10.7554/eLife.12966

**Geschwind DH, Paulson HL, Klein C** (eds) (2018), Handbook of Clinical Neurology, Vol. 147 Neurogenetics, Part I, 3rd series. Ramaswami G and Geschwind DH. Chapter 21 Genetics of autism spectrum disorder. doi: 10.1016/B978-0-444-63233-3.00021-X

**Han X, Wang R, Zhou Y, et al** (2018) Mapping the mouse cell atlas by microwell-seq. Cell. 172(5):1091–1107. doi: 10.1016/j.cell.2018.02.001

**Hwang B, Lee JH, Bang D** (2018) Single-cell RNA sequencing technologies and bioinformatics pipelines. Exp Mol Med. 50(8):96. doi: 10.1038/s12276-018-0071-8

**Jeste SS, Geschwind DH** (2014) Disentangling the heterogeneity of autism spectrum disorder through genetic findings. Nature reviews. Neurology. 10(2):74–81. doi: 10.1038/nrneurol.2013.278

**Iakoucheva LM, Muotri AR, Sebat J** (2019) Getting to the Cores of Autism. Cell. 178:1287-1298. doi:10.1016/j.cell.2019.07.037

**Kosik K** (2010) MicroRNA an cellular phenotypy. Cell. 143(1):21-26. doi: 10.1016/j.cell.2010.09.008

**Lai MC, Lombardo MV, Baron-Cohen S** (2014). Autism. Lancet. 383(9920):896–910. doi: 10.1016/S0140-6736(13)61539-1

**Lodato MA., Woodworth MB, Lee S, et al** (2015) Somatic mutation in single human neurons tracks developmental and transcriptional history. Science. 350(6256):94–98. doi: 10.1126/science.aab1785

**Macaulay IC, Ponting CP, Voet T** (2017) Single-cell multiomics: multiple measurements for single cells. Trends Genet. 33(2):155-168. doi: 10.1016/j.tig.2016.12.003

**Mahfouz A, Huisman SMH, Lelieveldt BPF** (2017) Brain transcriptome atlases: a computational perspective.Brain Struct Funct. 222(4):1557–1580. doi: 10.1007/s00429-016-1338-2

**Mincarelli L, Lister A, Lipscombe J, et al** (2018) Defining cell identity with single-cell omics. Proteomics. 18(18):e1700312. doi:10.1002/pmic.201700312

**Morris SA** (2019) The evolving concept of cell identity in the single cell era. Development 146(12):dev169748. doi:10.1242/dev.169748

**Mu Q, Chen Y, Wang J** (2019) Deciphering brain complexity using single-cell sequencing. Genomics Proteomics Bioinformatics. 17(4:344-366. doi: 10.1016/j.gpb.2018.07.007

**Naumova OY, Lee M, Rychkov SY, et al** (2013). Gene expression in the human brain: the current state of the study of specificity and spatiotemporal dynamics. Child Development. 84(1):76– 88. doi: 10.1111/cdev.12014

**Nishioka M, Bundo M, Iwamoto K et al** (2019) Somatic mutations in the human brain: implications for psychiatric research. Mol Psychiatry. 24(6):839–856 doi.: 10.1038/s41380-018-0129-y

**O'Huallachain M, Karczewski KJ, Weissman SM et al** (2012) Extensive genetic variation in somatic human tissues. Proc Natl Acad Sci U S A. 109(44):18018-18023. doi: 10.1073/pnas.1213736109

**Parikshak NN, Swarup V, Belgard TG, et al** (2016) Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. Nature. 540(7633):423-427. doi:10.1038/nature20612

**Pollock JD, Wu DY, Satterlee JS** (2014) Molecular neuroanatomy: a generation of progress. Trends Neurosci. 37(2):106–123. doi: 10.1016/j.tins.2013.11.001

**Regev A, Teichmann SA, Lander ES et al** (2017) The human cell atlas. Elife 6, e27041. doi:10.7554/eLife.27041

**Rodin RE., Dou Y, Kwon M, et al** (2020) The landscape of mutational mosaicism in autistic and normal human cerebral cortex. bioRxiv 2020.02.11.944413. doi: 10.1101/2020.02.11.944413

**Rosenberg AB, Roco CM, Muscat RA, et al** (2018) Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. Science. 360(6385):176–182. doi: 10.1126/science.aam8999

**Rozenblatt-Rosen O, Stubbington M, Regev A, Teichmann S** (2017) The Human Cell Atlas: From vision to reality. Nature. 550(7677):451-453. doi:10.1038/550451a.

**Rylaarsdam L, Guemez-Gamboa A** (2019) Genetic causes and modifiers of autism spectrum disorder. Front Cell Neurosci. 13:385. doi: 10.3389/fncel.2019.00385

**Saunders A, Macosko EZ, Wysoker A, et al.** (2018). Molecular diversity and specializations among the cells of the adult mouse brain. Cell. 174(4):1015–1030. doi: 10.1016/j.cell.2018.07.028

**Smalley SL, Asarnow RF, Spence MAArch Gen Psychiatry** (1988) Autism and genetics. A decade of research. Arch Gen Psichiatry. 45(10):953-961. doi: 10.1001/archpsyc.1988.01800340081013

**Song Y, Xu X, Wang W, et al** (2019) Single cell transcriptomics: moving towards multi-omics. Analyst. 144(10):3172-3189. doi:10.1039/c8an01852a

**Strzelecka PM, Ranzoni AM, Cvejic A** (2018) Dissecting human disease with single-cell omics: application in model systems and in the clinic. Dis Model Mech. 11(11):dmm036525. doi: 10.1242/dmm.036525

**Suo S, Zhu Q, Saadatpour A, et al** (2018) Revealing the critical regulators of cell identity in the Mouse Cell Atlas. Cell Rep. 25(6):1436-1445.e3. doi: 10.1016/j.celrep.2018.10.045

**Tang F, Barbacioru C, Wang Y, et al** (2009) mRNA-Seq whole-transcriptome analysis of a single cell. Nat Methods. 6(5):377-382. doi:10.1038/nmeth.1315

**Velmeshev D, Schirmer L, Jung D et al** (2019) Single-cell genomics identifies cell type–specific molecular changes in autism. Science. 364(6441):685–689. doi: 10.1126/science.aav8130

**Voineagu I, Wang X, Johnston P, et al** (2011) Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature. 25;474(7351):380-384. doi: 10.1038/nature10110.

**Xia B, Yanai I** (2019) A periodic table of cell types. Development. 146(12):dev169854. doi: 10.1242/dev.169854

**Yurov YB, Iourov IY, Monakhov VV et al** (2005) The variation of aneuploidy frequency in the developing andadult human brain revealed by an interphase FISH study. J Histochem Cytochem. 53(3):385–390. doi: 10.1369/jhc.4A6430.2005

**Zeisel A, Hochgerner H, Lönnerberg P, et al** (2018). Molecular architecture of the mouse nervous system. Cell. 174(4):999–1014.e22. doi: 10.1016/j.cell.2018.06.021

**Zoghbi HY, Bear MF** (2012) Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. Cold Spring Harb Perspect Biol. 2012. 4(3):a009886. doi: 10.1101/cshperspect.a009886