The Company of **Biologists**

Single-cell RNA-sequencing of mammalian brain development: insights and future directions

Elin Vinsland* and Sten Linnarsson*

ABSTRACT

Understanding human brain development is of fundamental interest but is also very challenging. Single-cell RNA-sequencing studies in mammals have revealed that brain development is a highly dynamic process with tremendous, previously concealed, cellular heterogeneity. This Spotlight discusses key insights from these studies and their implications for experimental models. We survey published single-cell RNA-sequencing studies of mouse and human brain development, organized by anatomical regions and developmental time points. We highlight remaining gaps in the field, predominantly concerning human brain development. We propose future directions to fill the remaining gaps, and necessary complementary techniques to create an atlas integrated in space and time of human brain development.

Introduction

Early in development, secreted morphogens define spatial domains within the brain that express distinct transcription factor combinations. These transcription factors, in turn, specify thousands of transcriptionally distinct neural cell types in the adult brain (reviewed by Silbereis et al., 2016). However, this cellular complexity is likely exceeded several-fold by the number of distinct transient cell states that exist during development. Understanding brain development thus requires extraordinarily sensitive and scalable methods.

Single-cell RNA-sequencing (scRNA-seq) has emerged as the predominant technique for discovering the transcriptional cell type composition of complex tissues. Advances in miniaturization and multiplexing have reduced costs and increased throughput, enabling the generation of gene expression profiles of many thousands of cells per experiment (Svensson et al., 2018). Following the first scRNA-seq experiment reported on mouse blastomeres (Tang et al., 2009), the most studied tissue has been the brain, with over 250 publications to date. Here, we review studies of mammalian brain development, focusing on post-gastrulation events. Using a database of scRNA-seq publications (Svensson et al., 2020), catalogs of scRNA-seq datasets [NeMo and the University of California, Santa Cruz (UCSC) Cell browser] and PubMed, we manually identified 89 studies of developing mammalian brain and nervous system that used primary tissues (Table 1). To illustrate the scope of the article, we summarize mouse and human studies by anatomical regions and developmental time points. We found that, in recent years, scRNA-seq has been scaled to whole mouse embryos and brain samples across the full timeline of mouse

Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Solnavägen 9, 171 65 Stockholm, Sweden.

*Authors for correspondence (elin.vinsland@ki.se; sten.linnarsson@ki.se)

E.V., 0000-0001-9695-9192; S.L., 0000-0002-3491-3444

prenatal brain development (Fig. 1). Together with advanced spatial techniques, a full 3D cellular map of mouse brain development seems within reach. However, owing to limitations of human tissue collection and experimentation, studies on human brain development remain less comprehensive (Fig. 2). We highlight important findings in the field so far and their implications for experimental models. Finally, we discuss remaining gaps in the field and suggest future research directions and technologies necessary to understand human brain development.

Atlases and cell heterogeneity

scRNA-seq studies that include data from multiple regions and time points provide the most complete view of cell-type heterogeneity during mammalian brain development. For example, one atlas comprised almost 300,000 sequenced cells from all major regions of the mouse brain during the entire timespan of prenatal brain development [embryonic day (E)7-E18] (La Manno et al., 2021). Eight main cell classes (Fig. 3) and nearly 800 clusters were identified in total, covering the onset and timeline of gastrulation, the emergence of the neural crest and neural tube, neurogenesis, early gliogenesis, and the branching of the meningeal lineage from the neural crest or mesenchyme. This atlas revealed that, although 87 radial glia subtypes were identified, most neuronal diversity was generated in postmitotic neuroblasts (171 subtypes) and maturing neurons (306 subtypes). The number of cell types identified depends on the protocol used (Mereu et al., 2020), the number of cells and depth sequenced (Svensson et al., 2020), as well as clustering methods (Kiselev et al., 2019). Comprehensive atlases are, therefore, the most accurate resources to compare cell heterogeneity between brain regions and time points. Such datasets are also the most accurate way to identify markers that can distinguish cell types globally across the brain or entire body (Box 1). Below, we explore cellular heterogeneity in the mammalian brain revealed by scRNA-seq and single-cell multi-omics.

Diversity in neuronal lineages

Progenitor cells

A study of first-trimester human telencephalon found nine clusters of neuroepithelial and radial glial cells. The gradual transition of these cells from early to more mature progenitors was defined by gene expression gradients (Eze et al., 2021). Three major classes of radial glia were identified in humans: ventricular, outer and truncated radial glia, appearing in early-to-late development (Nowakowski et al., 2017). Cell cycle and differentiation genes were mostly responsible for the transcriptional variation seen in outer radial glia (Pollen et al., 2015). Furthermore, regional transcription factors [e.g. HOX genes (hindbrain); PAX7 (midbrain); GBX2 (thalamus); FOXG1 (cerebral cortex)] were expressed as early as Carnegie stage (CS) 13, but cell type-defining genes were mostly conserved across different brain regions
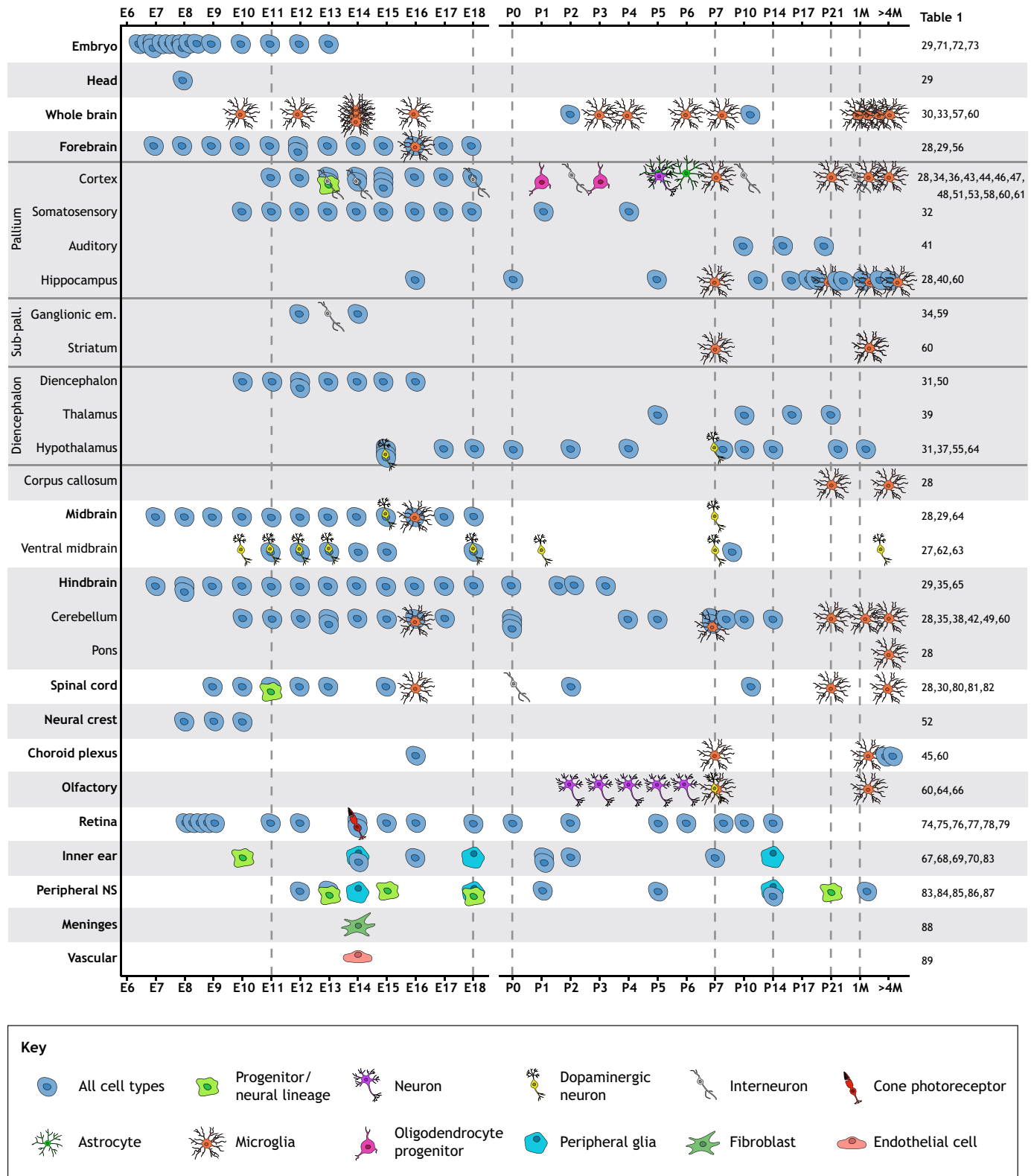
Fig. 1. See next page for legend.

(Eze et al., 2021). Combined scRNA-seq and single-cell assay for transposase-accessible chromatin-sequencing (scATAC-seq) data showed that chromatin activity of a set of genes in cycling cells precedes lineage-specific gene expression, suggesting that progenitors entering the cell cycle were primed towards specific lineages (Trevino et al., 2021). RNA-velocity analysis of the early human brain (CS 12-13) indicated examples of radial glia giving rise to neurons, suggestive of direct neurogenesis (Eze et al., 2021).

Fig. 1. Illustration of available scRNA-seq datasets of mouse nervous system development. An scRNA-seq database of publications up to October 2021 (Svensson et al., 2020), catalogs of scRNA-seq datasets (NeMo and the UCSC Cell browser) and PubMed were used to identify studies of mammalian nervous system development, using keywords for developing nervous system components ('autonomic', 'brain', 'dorsal root ganglia', 'embryo', 'enteric', 'inner ear', 'nasal', 'neural tube', 'olfactory', 'peripheral nervous system', 'retina', 'sensory', 'spinal cord', 'sympathetic'). Publications were manually inspected and included in Table 1 if they covered post-gastrulation time points. Postnatal data was only included if the inclusion criteria were met, or if the region or cell type of interest develops postnatally. Only publications that generated their own data were included. Our curated list was also sent to V. Svensson (Vesalius Therapeutics, Cambridge, MA, USA). The figure shows scRNA-seq datasets from mouse studies by anatomical regions and developmental time points. Half embryonic days were rounded to the lower integer (e.g. E13.5 is plotted as E13), except for more precisely timed experiments (references 72 and 76 in Table 1). Colors indicate whether all cell types or cell-type-specific isolation and sequencing were performed. If studies sequenced the same region at the same time point, symbols overlap along the y-axis (e.g. whole brain at E14, and cerebellum at P0). A second y-axis (right) indicates which publications the data are derived from per row (see Table 1 for matching numbers). E, embryonic day; Ganglionic em., ganglionic eminence; m, month; NS, nervous system; P, postnatal day; Sub-Pall., subpallium.

Intermediate progenitor populations appeared at ∼CS 18, implying the beginning of stereotypical (indirect) neuronal differentiation (Eze et al., 2021). However, the expression of TBR2 (also known as EOMES), which is the basis for intermediate progenitor definition, was also found in postmitotic neurons, such as Cajal-Retzius cells (Eze et al., 2021). In addition, a novel mesenchymal progenitor was described that expressed LUM and ALX1, potentially supporting telencephalic development before the radial glial scaffold emerges (Eze et al., 2021). These cells may be the early mesenchymal progenitors destined to form the meninges, as recently described (La Manno et al., 2021).

Excitatory and glutamatergic neurons

In the mouse, forebrain excitatory neurons were a clear transcriptional outgroup distinct from the other neuronal lineages (La Manno et al., 2021), supporting data from adolescent animals (Zeisel et al., 2018). In humans, by mid-gestation there were populations of migrating, differentiating and maturing excitatory neurons (Zhong et al., 2018; Polioudakis et al., 2019). There were also area- and layer-specific pyramidal cell subtypes in the human cortex, which emerged during peak neurogenesis. These results differ from early models of a cortical 'column' consisting of similar cell types and lamination patterns irrespective of topographical location in the cortical sheet (Nowakowski et al., 2017; Zhong et al., 2018; Bhaduri et al., 2020). Multi-ome data defined a developmental trajectory for glutamatergic neurons, revealing a continuous progression of transcription factor motif activities associated with neuronal specification and migration (Trevino et al., 2021). Furthermore, a comparison of scRNA-seq and scATAC-seq profiles of human excitatory lineage cells revealed chromatin state signatures of cortical arealization already in intermediate progenitor populations, preceding that of areal transcriptomic signatures in excitatory neurons (Ziffra et al., 2021).

Cortical inhibitory neurons

Mouse and human forebrain inhibitory neurons develop from the ganglionic eminences and migrate tangentially into the neocortex. The precise origins and development of these cells have been the subject of intense study (Lim et al., 2018). Precursors located in the mouse caudal, lateral and medial ganglionic eminences clearly expressed distinct transcription factors that bias their fates upon

Box 1. Improved markers and targets
More precise markers enable the continued investigation of heterogenous cell populations and can improve basic experimental readouts, evolutionary biology and specificity in gene manipulations for model systems. Canonical markers are often not specific enough to distinguish subtypes of major cell classes from each other, or similar cells present in different tissues (e.g. AIF1 is used for both macrophages and microglia, making ontological studies difficult). Canonical markers are especially unhelpful in developmental datasets where progenitors express overlapping genes that only become specific in more differentiated states (e.g. SOX10 is expressed by neural crest and later the oligodendrocyte lineage). By analyzing the single-cell expression of ligand-receptor pairs it is also possible to predict cell-cell interactions (e.g. Dani et al., 2021; Efremova et al., 2020) and target specific cells for drug development.

differentiation (Mayer et al., 2018; Mi et al., 2018), but whether the full set of mature interneuron types is prespecified in the eminences remains unclear. During parvalbumin and somatostatin interneuron development, combined scRNA-seq and scATAC-seq revealed a predominantly shared chromatin architecture of general interneuron identity from E13 to postnatal day (P)2, followed by the expression of specific transcription factors that initiated terminal differentiation and cell type diversification (Allaway et al., 2021). There were also conflicting reports concerning the existence of interneuron progenitors in the human neocortex (Zhong et al., 2018; Polioudakis et al., 2019). Recent findings indicate that most of the diversity of interneuron types is generated in the ganglionic eminences (Shi et al., 2021). However, genetic fate mapping revealed a shared origin for human excitatory and inhibitory cortical neurons (Delgado et al., 2022). Interestingly, the same was not true in mice (Bandler et al., 2022).

Diversity in glia

Astrocytes and oligodendrocytes

A common progenitor for astrocytes and oligodendrocytes, and two additional astrocyte precursor states, were found in humans (Zhong et al., 2018; Polioudakis et al., 2019). In mice, transitional intermediate states in astroglial and oligodendroglial lineages persist in postnatal gliogenesis and were altered in gliomagenesis (Weng et al., 2019). A population of late radial glial cells (glioblasts) was identified, that gradually lost proliferative capacity and switched to gliogenesis (La Manno et al., 2021). Oligodendrocytes were established as a transcriptionally heterogeneous lineage with twelve clusters identified in P21-P60 mice (Marques et al., 2016), whereas three subpopulations of early progenitor, late progenitor (myelinating) and mature oligodendrocytes were identified in humans (Perlman et al., 2020). Overall, oligodendrocytes were consistently found to be much less heterogeneous than neuronal or astrocyte lineages.

Microglia

Microglia have a yolk sac origin (i.e. non-neural) and migrate into the human brain by gestational week (GW) 8 (Zhong et al., 2018). Their development involves three distinct temporal stages in synchrony with the homeostatic needs of the developing brain, and displays heterogeneity from embryonic to adult ages (Matcovitch-Natan et al., 2016; Li et al., 2019; Kracht et al., 2020). Interestingly, human microglia may be more heterogeneous than five other mammals surveyed (macaque, marmoset, sheep, mouse and hamster) (Geirsdottir et al., 2019).
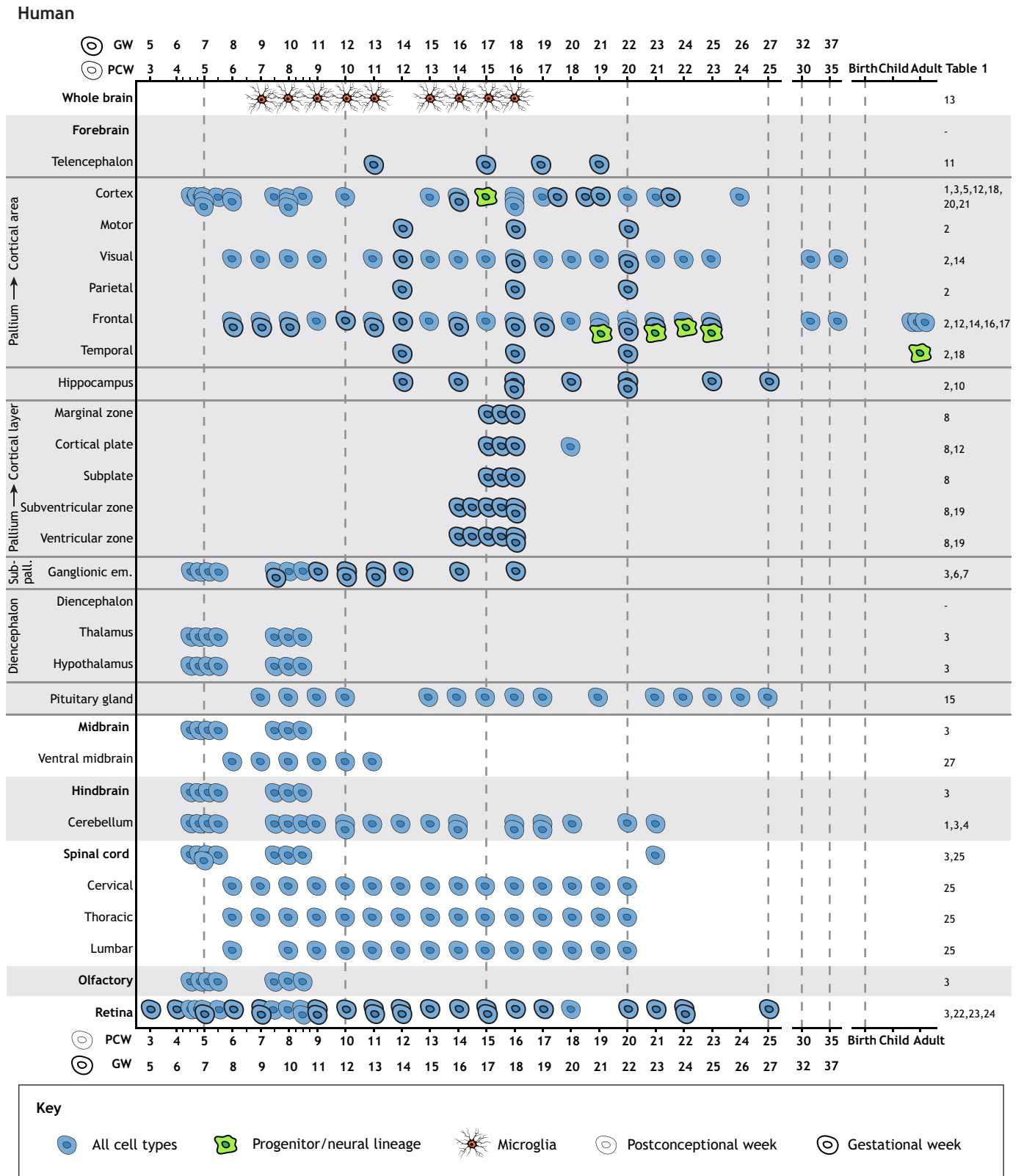
Fig. 2. See next page for legend.
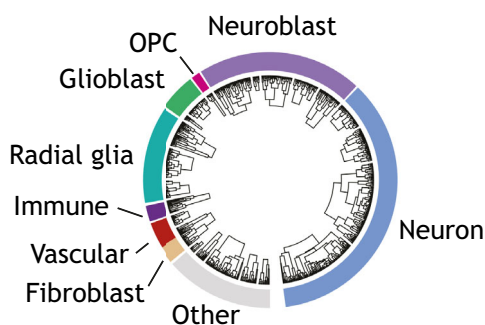
Temporal transcriptomes

Neurodevelopment is a highly dynamic process with precise spatiotemporal regulation of the transcriptome. To understand the sequence of events during development, and the origin of adult cell types, samples can be collected over time. One such study analyzed various brain regions from embryonic, fetal, adolescent and adult

Fig. 2. Illustration of available scRNA-seq datasets of human nervous system development. An scRNA-seq database of publications up to October 2021 (Svensson et al., 2020), catalogs of scRNA-seq datasets (NeMo and the UCSC Cell browser) and PubMed were used to identify studies of mammalian nervous system development, using keywords for developing nervous system components ('autonomic', 'brain', 'dorsal root ganglia', 'embryo', 'enteric', 'inner ear', 'nasal', 'neural tube', 'olfactory', 'peripheral nervous system', 'retina', 'sensory', 'spinal cord', 'sympathetic'). Publications were manually inspected and included in Table 1 if they covered post-gastrulation time points. Postnatal data was only included if the inclusion criteria were met, or if the region or cell type of interest develops postnatally. Only publications that generated their own data were included. Our curated list was also sent to V. Svensson (Vesalius Therapeutics, Cambridge, MA, USA). The figure shows scRNA-seq datasets from human studies by anatomical regions and developmental time points. Half weeks were plotted accordingly, whereas other decimals were rounded to the nearest integer (e.g. 16.5 is plotted in between 16 and 17, and 5.85 at 6). Colors indicate whether all cell types or cell-type-specific isolation and sequencing was performed. If studies sequenced the same region at the same time point, symbols overlap along the y-axis [e.g. two studies sequenced gestational week (GW) 22 hippocampus]. Parallel x-axes are used to show postconceptional week (PCW; solid cell border) and GW (dashed cell border). We defined GW as PCW+2 weeks, but GWs are sometimes ambiguous in the original publications. PCW 4-9 contain extra ticks for Carnegie stages (CS; see Table 1). A second y-axis (right) indicates which publications the data are derived from per row (see Table 1 for matching numbers). Ganglionic em., ganglionic eminence; Sub-Pall., subpallium.

human brains using bulk and scRNA-seq and found a global temporal transcriptomic pattern (Li et al., 2018). This, and the molecular signatures of topographical gene expression and bilaterality in the developing human brain, were previously uncovered using bulk RNA-sequencing alone (Pletikos et al., 2014). During embryonic to mid-fetal stages, there was high transcriptomic variation between brain regions (Li et al., 2018). At late fetal stages, there was a sharp decrease in regional differences but an increase in genes related to neuronal maturation, synaptogenesis and myelination. High intra- and inter-regional variation resumed in childhood-adolescence. Alignment of prenatal and adult data revealed that putative embryonic and fetal excitatory neurons clustered near their adult counterparts, whereas fetal cells in the oligodendrocyte lineage did not, indicating a distinctly different maturation status (Li et al., 2018).

Focusing on a single region over time facilitates detailed sub-anatomical analysis of timing, patterning events and the effects of gene mutations. For example, in mice, it was shown that the neurogenic-to-gliogenic switch occurred between E12 and E16, that nearly all neuronal lineages passed through an early neuroblast state, and that the earliest astrocytes appeared at ~E15 (La Manno et al.,

Fig. 3. Major classes of cells in the mammalian developing brain. The figure shows the dendrogram of cell types from La Manno et al. (2021), with colored sectors indicating major classes of cell types. Immune cells include macrophages and microglia. Fibroblasts include those that form the meninges. OPC, oligodendryte precursor cell.

2021). In mouse hypothalamus development, *Nkx2-1* was identified as a potential positive regulator of ventral hypothalamic identity while also suppressing prethalamic identity, which was not predicted by the prosomeric model for forebrain organization (Puelles, 2009; Ferran et al., 2015), highlighting an unexpected developmental connection between the hypothalamus and prethalamus (Kim et al., 2020). Another study generated an atlas of cortical development in *Fezf2* mutant mice (Di Bella et al., 2021). *Fezf2* is a crucial transcription factor for forebrain development, the absence of which causes a loss of sub-cerebral projection neurons (reviewed by Molyneaux et al., 2007). At E13.5, postmitotic projection neurons were transcriptionally more like projection neurons of later time points. Thus, although *Fezf2* was expressed in progenitors, its role in sub-cerebral projection neuron specification was primarily postmitotic (Di Bella et al., 2021). In addition, other specific regions that were studied over time include the mouse developing cerebellum (Carter et al., 2018), dentate gyrus (Hochgerner et al., 2018), as well as the human hippocampus (Zhong et al., 2020) and pituitary (Zhang et al., 2020a) (Table 1).

Computational tools, such as Monocle (Trapnell et al., 2014), URD (Farrell et al., 2018), RNA velocity (La Manno et al., 2018) and scVelo (Bergen et al., 2020), can be used to infer differentiation trajectories, model transcriptional dynamics and predict the direction of differentiation (reviewed by Saelens et al., 2019). This information can guide complementary experiments such as genetic lineage tracing, to prove the predicted lineages and clonal relationships (Wagner and Klein, 2020).

Evolutionary conservation of cell types

Evolutionary conservation can reveal the function of cell types and can be used to transfer knowledge about these functional properties from model organisms to humans. For example, little is known about the electrophysiological properties of human neurons, but such properties can be inferred from the corresponding mouse or non-human primate cell types. At the same time, non-conserved cell types or states may reveal human-specific traits and disorders. For example, one study comparing human cortical development with mice showed that the human cortex developed more slowly and certain human cell populations were absent in early mouse development (Eze et al., 2021).

A study using bulk and scRNA-seq compared human and macaque brain development and found numerous shared and divergent transcriptomic features. In the hippocampus, striatum and cerebellar cortex, differentially expressed genes were enriched in oligodendrocyte progenitors, medium spiny neurons, external granular layer neurons and microglia, indicating specific cell-type differences between human and macaque. In the prenatal dorsolateral prefrontal cortex, all human cell types had a close homologue in the macaque. However, there was a prolonged neurogenic period in humans with an enrichment for intermediate progenitor cells, suggesting that the duration of neurogenesis may differ between the two species (Zhu et al., 2018). These data highlight that distinct models for early human cortical development are necessary. Drawing correlations with genes known to be implicated in neuropsychiatric diseases, five heterotopic clusters specific to schizophrenia-associated genes were found. Three of them displayed exclusive species differences prenatally. One schizophrenia-associated gene, GRIA1 (an AMPA receptor subunit), was expressed in neocortical areas earlier in humans. Instead, autism spectrum disorder-associated genes (SHANK2 and SHANK3) were expressed earlier in macaque than human neocortex (Zhu et al., 2018). These findings are relevant for *in vitro* models of neurodevelopment and non-human primate

models of neuropsychiatric disease, such as the SHANK3-deficient macaque model (Zhao et al., 2017).

Another study compared adult human, marmoset and mouse motor cortices and found that most cell types were homologous between the three species. Surprisingly, however, most cell-type-specific genes in each species were not conserved in the other species (Bakken et al., 2021). The same was observed in a study comparing interneurons in three primates (human, macaque and marmoset), mice and ferrets (Krienen et al., 2020). Evolutionary comparisons can, therefore, both reveal conserved genes likely to support the core function of each cell type, but also pinpoint divergent genes that have evolved recently in each species to serve species-specific adaptations.

Taken together, this suggests that interspecies differences can result from variations in cell-type diversity and abundance, more so than the transcriptional divergence between cell types. Interestingly, a new classification system based on an evolutionary definition of cell type was proposed (Arendt et al., 2016). The proposal can be tested with new tools, such as SAMap, which maps cell atlas manifolds across species and identifies homologous cell types (Tarashansky et al., 2021). We, therefore, stress the importance of studies on evolutionary conservation.

Gaps in the field
Understudied neuroanatomical regions
To fully understand brain development, data from all neuroanatomical regions and developmental time points is necessary. Regions of the nervous system and time points that have been studied in mice and humans are shown in Figs 1 and 2, respectively. Here, we clarify the remaining gaps in the field.

In mouse
Whole mouse embryos were sequenced from E6 to E13 using scRNA-seq (Cao et al., 2019; Pijuan-Sala et al., 2019). In the central nervous system, the forebrain, midbrain, hindbrain, cerebellum and retina were sequenced almost throughout prenatal development, and the latter three during the first postnatal week as well. The spinal cord had almost no scRNA-seq data from the second half of prenatal development. The forebrain was studied the most, although with missing time points for subdivisions of the forebrain. Some neuroanatomical regions develop late, which may explain the absence of sequencing data on certain time points (e.g. the cerebellum develops from E10 through to adolescence) (Fig. 1; Table 1).

Peripheral nervous system (PNS) development was not as well covered across development. We found publications for neural crest-derived autonomic and sensory neurons, the enteric nervous system, the inner ear, sciatic nerve and dorsal root ganglia (Fig. 1; Table 1). More studies on dorsal root ganglia were found in adult mice (Usoskin et al., 2015; Li et al., 2016; Wang et al., 2021).

Although the sequencing coverage for mouse neurodevelopment was high, most regions and time points were only sequenced once (and a maximum of four times, e.g. E13-E15 cortex). Of 61 studies, 18 isolated specific cell types for sequencing [e.g. microglia from the whole brain (Hammond et al., 2019) and midbrain dopaminergic neurons (Tiklová et al., 2019)]. Furthermore, other than by inclusion in a larger dataset (La Manno et al., 2021), no study focused on analyzing all cell types in the developing hindbrain, thalamus or pons (Fig. 1; Table 1).

In human
There were roughly half as many publications on human nervous system development compared with mice (Table 1). Several regions

Box 2. Limitations to human tissue sampling
All prenatal stages of development have been sampled in mouse (Fig. 1). However, in humans there are major age gaps in sample collections due to ethical restrictions on human research and legal limits on abortions. Acquiring tissue from third trimester (28-40 weeks) and half of the second trimester (14-27 weeks) is particularly difficult. These time periods cover many developmental events, such as neuronal migration, gliogenesis, synaptogenesis, synaptic pruning and myelination. Such challenges motivate our proposed investments into advancing in vitro model systems, such as long-term explant culturing and organoids. Furthermore, work on postnatal stages is restricted to post-mortem tissue or rare surgical resections, underlining the importance of integration between single-cell techniques carried out on fresh versus frozen tissues.

have been sequenced at almost all prenatal weeks up until birth: the cortex, pituitary gland and retina, and the spinal cord and cerebellum have also been covered extensively over prenatal time points (Fig. 2). Although it is not a trivial undertaking, data could be integrated across publications to create a full atlas of human cortical development. Comparing this to the atlas of mouse cortical development could yield interesting evolutionary findings and implications for experimental models. Notably, two studies covering wide age ranges and ten (Eze et al., 2021) or six (Zhu et al., 2018) different brain regions, mostly focused their analysis on the cortex, leaving avenues for interesting post hoc analyses.

Not many other regions were sequenced and analyzed in-depth, other than the hippocampus (Zhong et al., 2020) and ventral midbrain (La Manno et al., 2016). Only 5/30 studies isolated one specific cell type for sequencing (Table 1). We found no studies on PNS development in humans. The gaps in human studies are partly due to limitations on tissue acquisition (Box 2). Furthermore, the influence of other tissues on brain development are also important (Box 3).

Perspectives
The growth of scRNA-seq and the vast amount of data produced have raised a few considerations: how many cells and what sequencing depth is optimal to capture all cell types and distinguish them from each other can we define cell type diversity during brain development with similar datasets and methods as the adult brain, considering that rare and transient cell states vary between regions and time points during development; how do we create a common taxonomy of cell types (is a 'glioblast' in one study the same as 'glial progenitor' in another); can we bridge different sequencing techniques (single nucleus/cell sequencing, fresh/fixed tissue, chemistry A or B, multimodal sequencing assays, etc.)? To answer these questions and more, and to integrate single-cell data, international consortia, such as the Human Cell Atlas, have been formed. Its mission is to map every cell in the human body (a 'Periodic Table' of human cells) using single-cell and spatial genomics and computational techniques (Regev et al., 2017; Haniffa et al., 2021; Rozenblatt-Rosen et al., 2021). International consortia, together with integrative and interactive databases, and standardization efforts for data analysis (Luecken and Theis, 2019) are essential to combine findings and shape productive future directions.

In addition, although the most used single-cell method is scRNA-seq, methylation, chromatin accessibility, genetic variation and protein abundance can be measured using related techniques. Single-cell biology has entered a multi-omics age (Perkel, 2021), and key scATAC-seq work during mammalian development is already emerging (Preissl et al., 2018; Domcke et al., 2020; Ziffra et al., 2021).

DEVELOPMENT

The combination of scRNA-seq and scATAC-seq is particularly powerful for developmental studies because it can simultaneously reveal transcriptional cell states and the gene regulatory elements – enhancers and promoters – that drive those states. Furthermore, cell-type-specific enhancer elements are powerful experimental tools for genetic targeting of cell types and states, such as by using viral vectors *in vivo*, in explants or in organoids (Mich et al., 2021).

Some important neurodevelopmental questions cannot be addressed by single-cell analyses alone. For example, scRNA-seq can reveal which cells express genes involved in brain patterning, but not their spatial location, as this information is lost upon tissue dissociation. Spatial-omics techniques are, therefore, under tremendous technical development and usage (Rao et al., 2021). For example, sci-Space allows the construction of spatially resolved single-cell atlases, recently mapping whole transcriptomes of 120,000 nuclei in whole E14.5 mouse embryos at single-cell resolution (Srivatsan et al., 2021). Comparing the transcriptome and location of cell types can also elucidate whether the transcriptome of a particular cell type is defined before or after migrating to its terminal niche. Data integration from scRNA-seq and spatial transcriptomics has been reviewed elsewhere (Longo et al., 2021).

Future directions

One of the main goals in neuroscience is to understand human brain development and the mechanisms underlying neurodevelopmental disorders. A hurdle to achieving this – unlike other model organisms – is the difficulty of acquiring human samples at all developmental stages (Box 2) and the impossibility of *in vivo* experimentation. To a large extent, rodents and non-human primates serve as models of human biology. However, further development of advanced *in vitro* systems, such as *ex vivo* brain explant culturing and organoids, is essential to make progress in the field. As human

brain development is slow compared with rodents, focusing on long-term culturing is necessary to investigate inaccessible time points. Single-cell and spatial-omics techniques can be used to verify the extent to which *in vitro* systems recapitulate normal development (Camp et al., 2015; Velasco et al., 2019; Bhaduri et al., 2020; Fleck et al., 2021), which can subsequently be used for live-tissue experiments, such as lineage tracing, live imaging, gene perturbations and other functional tests. Future integration of long-term time-lapse imaging with single-cell and spatial-omics could directly link cell behavior, lineage trajectories and molecular cell states. We believe data integration across organs is important to place brain development into the context of the whole body, as demonstrated by recent work on organogenesis and diverse fetal tissues (Cao et al., 2019, 2020; Pijuan-Sala et al., 2019; Lohoff et al., 2022). No model system is ideal for the study of all biological questions, but the integrated combination of existing models can eventually lead to a common atlas over space and time, with a coordinate framework of human brain development.

References

Aldinger, K. A., Thomson, Z., Phelps, I. G., Haldipur, P., Deng, M., Timms, A. E., Hirano, M., Santpere, G., Roco, C., Rosenberg, A. B. et al. (2021). Spatial and cell type transcriptional landscape of human cerebellar development. *Nat. Neurosci.* **24**, 1163-1175. doi:10.1038/s41593-021-00872-y

Allaway, K. C., Gabitto, M. I., Wapinski, O., Saldi, G., Wang, C. Y., Bandler, R. C., Wu, S. J., Bonneau, R. and Fishell, G. (2021). Genetic and epigenetic coordination of cortical interneuron development. *Nature* **597**, 693-697. doi:10.1038/s41586-021-03933-1

Arendt, D., Musser, J. M., Baker, C. V. H., Bergman, A., Cepko, C., Erwin, D. H., Pavlicev, M., Schlosser, G., Widder, S., Laubichler, M. D. et al. (2016). The origin and evolution of cell types. *Nat. Rev. Genet.* **17**, 744-757. doi:10.1038/nrg.2016.127

Bakken, T. E., Jorstad, N. L., Hu, Q., Lake, B. B., Tian, W., Kalmbach, B. E., Crow, M., Hodge, R. D., Krienen, F. M., Sorensen, S. A. et al. (2021). Comparative cellular analysis of motor cortex in human, marmoset and mouse. *Nature* **598**, 111-119. doi:10.1038/s41586-021-03465-8

Bandler, R. C., Vitali, I., Delgado, R. N., Ho, M. C., Dvoretskova, E., Ibarra Molinas, J. S., Frazel, P. W., Mohammadkhani, M., Machold, R., Maedler, S. et al. (2022). Single-cell delineation of lineage and genetic identity in the mouse brain. *Nature* **601**, 404-409. doi:10.1038/s41586-021-04237-0

Bergen, V., Lange, M., Peidli, S., Wolf, F. A. and Theis, F. J. (2020). Generalizing RNA velocity to transient cell states through dynamical modeling. *Nat. Biotechnol.* **38**, 1408-1414. doi:10.1038/s41587-020-0591-3

Bhaduri, A., Andrews, M. G., Mancia Leon, W., Jung, D., Shin, D., Allen, D., Jung, D., Schmunk, G., Haeussler, M., Salma, J. et al. (2020). Cell stress in cortical organoids impairs molecular subtype specification. *Nature* **578**, 142-148. doi:10.1038/s41586-020-1962-0

Buenaventura, D. F., Corseri, A. and Emerson, M. M. (2019). Identification of genes with enriched expression in early developing mouse cone photoreceptors. *Invest. Ophthalmol. Vis. Sci.* **60**, 2787-2799. doi:10.1167/iovs.19-26951

Burns, J. C., Kelly, M. C., Hoa, M., Morell, R. J. and Kelley, M. W. (2015). Single-cell RNA-Seq resolves cellular complexity in sensory organs from the neonatal inner ear. *Nat. Commun.* **6**, 8557. doi:10.1038/ncomms9557

Camp, J. G., Badsha, F., Florio, M., Kanton, S., Gerber, T., Wilsch-Bräuninger, M., Lewitus, E., Sykes, A., Hevers, W., Lancaster, M. et al. (2015). Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. *Proc. Natl. Acad. Sci. USA* **112**, 15672-15677. doi:10.1073/pnas.1520760112

Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D. M., Hill, A. J., Zhang, F., Mundlos, S., Christiansen, L., Steemers, F. J. et al. (2019). The single-cell transcriptional landscape of mammalian organogenesis. *Nature* **566**, 496-502. doi:10.1038/s41586-019-0969-x

Cao, J., O'Day, D. R., Pliner, H. A., Kingsley, P. D., Deng, M., Daza, R. M., Zager, M. A., Aldinger, K. A., Blecher-Gonen, R., Zhang, F. et al. (2020). A human cell atlas of fetal gene expression. *Science* **370**, eaba7721. doi:10.1126/science.aba7721

DEVELOPMENT

Carter, R. A., Bihannic, L., Rosencrance, C., Hadley, J. L., Tong, Y., Phoenix, T. N., Natarajan, S., Easton, J., Northcott, P. A. and Gawad, C. (2018). A single-cell transcriptional atlas of the developing murine cerebellum. *Curr. Biol.* **28**, 2910-2920.e2. doi:10.1016/j.cub.2018.07.062

Clark, B. S., Stein-O'Brien, G. L., Shiau, F., Cannon, G. H., Davis-Marcisak, E., Sherman, T., Santiago, C. P., Hoang, T. V., Rajaii, F., James-Esposito, R. E. et al. (2019). Single-cell RNA-seq analysis of retinal development identifies NFI factors as regulating mitotic exit and late-born cell specification. *Neuron* **102**, 1111-1126.e5. doi:10.1016/j.neuron.2019.04.010

Couturier, C. P., Ayyadhury, S., Le, P. U., Nadaf, J., Monlong, J., Riva, G., Allache, R., Baig, S., Yan, X., Bourgey, M. et al. (2020). Single-cell RNA-seq reveals that glioblastoma recapitulates a normal neurodevelopmental hierarchy. *Nat. Commun.* **11**, 3406. doi:10.1038/s41467-020-17186-5

Dani, N., Herbst, R. H., McCabe, C., Green, G. S., Kaiser, K., Head, J. P., Cui, J., Shipley, F. B., Jang, A., Dionne, D. et al. (2021). A cellular and spatial map of the choroid plexus across brain ventricles and ages. *Cell* **184**, 3056-3074.e21. doi:10.1016/j.cell.2021.04.003

Darmanis, S., Sloan, S. A., Zhang, Y., Enge, M., Caneda, C., Shuer, L. M., Hayden Gephart, M. G., Barres, B. A. and Quake, S. R. (2015). A survey of human brain transcriptome diversity at the single cell level. *Proc. Natl. Acad. Sci. USA* **112**, 7285-7290. doi:10.1073/pnas.1507125112

Delgado, R. N., Allen, D. E., Keefe, M. G., Mancia Leon, W. R., Ziffra, R. S., Crouch, E. E., Alvarez-Buylla, A. and Nowakowski, T. J. (2022). Individual human cortical progenitors can produce excitatory and inhibitory neurons. *Nature* **601**, 397-403. doi:10.1038/s41586-021-04230-7

Delile, J., Rayon, T., Melchionda, M., Edwards, A., Briscoe, J. and Sagner, A. (2019). Single cell transcriptomics reveals spatial and temporal dynamics of gene expression in the developing mouse spinal cord. *Development* **146**, dev173807. doi:10.1242/dev.173807

DeSisto, J., O'Rourke, R., Jones, H. E., Pawlikowski, B., Malek, A. D., Bonney, S., Guimiot, F., Jones, K. L. and Siegenthaler, J. A. (2020). Single-cell transcriptomic analyses of the developing meninges reveal meningeal fibroblast diversity and function. *Dev. Cell* **54**, 43-59.e4. doi:10.1016/j.devcel.2020.06.009

Di Bella, D. J., Habibi, E., Stickels, R. R., Scalia, G., Brown, J., Yadollahpour, P., Yang, S. M., Abbate, C., Biancalani, T., Macosko, E. Z. et al. (2021). Molecular logic of cellular diversification in the mouse cerebral cortex. *Nature* **595**, 554-559. doi:10.1038/s41586-021-03670-5

Dickel, D. E., Ypsilanti, A. R., Pla, R., Zhu, Y., Barozzi, I., Mannion, B. J., Khin, Y. S., Fukuda-Yuzawa, Y., Plajzer-Frick, I., Pickle, C. S. et al. (2018). Ultraconserved Enhancers Are Required for Normal Development. *Cell* **172**, 491-499.e15. doi:10.1016/j.cell.2017.12.017

Domcke, S., Hill, A. J., Daza, R. M., Cao, J., O'Day, D. R., Pliner, H. A., Aldinger, K. A., Pokholok, D., Zhang, F., Milbank, J. H. et al. (2020). A human cell atlas of fetal chromatin accessibility. *Science* **370**, eaba7612. doi:10.1126/science.aba7612

Durruthy-Durruthy, R., Gottlieb, A., Hartman, B. H., Waldhaus, J., Laske, R. D., Altman, R. and Heller, S. (2014). Reconstruction of the mouse otocyst and early neuroblast lineage at single-cell resolution. *Cell* **157**, 964-978. doi:10.1016/j.cell.2014.03.036

Efremova, M., Vento-Tormo, M., Teichmann, S. A. and Vento-Tormo, R. (2020). CellPhoneDB: inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes. *Nat. Protoc.* **15**, 1484-1506. doi:10.1038/s41596-020-0292-x

Eze, U. C., Bhaduri, A., Haeussler, M., Nowakowski, T. J. and Kriegstein, A. R. (2021). Single-cell atlas of early human brain development highlights heterogeneity of human neuroepithelial cells and early radial glia. *Nat. Neurosci.* **24**, 584-594. doi:10.1038/s41593-020-00794-1

Fabra-Beser, J., Alves Medeiros de Araujo, J., Marques-Coelho, D., Goff, L. A., Costa, M. R., Müller, U. and Gil-Sanz, C. (2021). Differential expression levels of Sox9 in early neocortical radial glial cells regulate the decision between stem cell maintenance and differentiation. *J. Neurosci.* **41**, 6969-6986. doi:10.1523/JNEUROSCI.2905-20.2021

Farmer, D. T., Mlcochova, H., Zhou, Y., Koelling, N., Wang, G., Ashley, N., Bugacov, H., Chen, H.-J., Parvez, R., Tseng, K.-C. et al. (2021). The developing mouse coronal suture at single-cell resolution. *Nat. Commun.* **12**, 4797. doi:10.1038/s41467-021-24917-9

Farrell, J. A., Wang, Y., Riesenfeld, S. J., Shekhar, K., Regev, A. and Schier, A. F. (2018). Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis. *Science* **360**, eaar3131. doi:10.1126/science.aar3131

Fazel Darbandi, S., Robinson Schwartz, S. E., Pai, E. L., Everitt, A., Turner, M. L., Cheyette, B. N. R., Willsey, A. J., State, M. W., Sohal, V. S. and Rubenstein, J. L. R. (2020). Enhancing WNT signaling restores cortical neuronal spine maturation and synaptogenesis in Tbr1 mutants. *Cell Rep.* **31**, 107495. doi:10.1016/j.celrep.2020.03.059

Ferran, J. L., Puelles, L. and Rubenstein, J. L. (2015). Molecular codes defining rostrocaudal domains in the embryonic mouse hypothalamus. *Front Neuroanat.* **9**, 46. doi:10.3389/fnana.2015.00046

Fleck, J. S., Sanchís-Calleja, F., He, Z., Santel, M., Boyle, M. J., Camp, J. G. and Treutlein, B. (2021). Resolving organoid brain region identities by mapping single-cell genomic data to reference atlases. *Cell Stem Cell* **28**, 1148-1159.e8. doi:10.1016/j.stem.2021.02.015

Fu, Y., Yang, M., Yu, H., Wang, Y., Wu, X., Yong, J., Mao, Y., Cui, Y., Fan, X. and Wen, L. (2021). Heterogeneity of glial progenitor cells during the neurogenesis-to-gliogenesis switch in the developing human cerebral cortex. *Cell Rep.* **34**, 108788. doi:10.1016/j.celrep.2021.108788

Garcia, F. J., Sun, N., Lee, H., Godlewski, B., Galani, K., Mantero, J., Bennett, D. A., Sahin, M., Kellis, M. and Heiman, M. (2021). Single-cell dissection of the human cerebrovasculature in health and disease. *bioRxiv*. doi:10.1101/2021.04.26.440975

Geirsdottir, L., David, E., Keren-Shaul, H., Weiner, A., Bohlen, S. C., Neuber, J., Balic, A., Giladi, A., Sheban, F., Dutertre, C.-A. et al. (2019). Cross-species single-cell analysis reveals divergence of the primate microglia program. *Cell* **179**, 1609-1622.e16. doi:10.1016/j.cell.2019.11.010

Gerber, D., Pereira, J. A., Gerber, J., Tan, G., Dimitrieva, S., Yánguez, E. and Suter, U. (2021). Transcriptional profiling of mouse peripheral nerves to the single-cell level to build a sciatic nerve ATlas (SNAT). *Elife* **10**, e58591. doi:10.7554/eLife.58591

Guo, Q. and Li, J. Y. H. (2019). Defining developmental diversification of diencephalon neurons through single cell gene expression profiling. *Development* **146**, dev174284. doi:10.1242/dev.174284

Hammond, T. R., Dufort, C., Dissing-Olesen, L., Giera, S., Young, A., Wysoker, A., Walker, A. J., Gergits, F., Segel, M., Nemesh, J. et al. (2019). 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. doi:10.1016/j.immuni.2018.11.004

Hanchate, N. K., Kondoh, K., Lu, Z., Kuang, D., Ye, X., Qiu, X., Pachter, L., Trapnell, C. and Buck, L. B. (2015). Single-cell transcriptomics reveals receptor transformations during olfactory neurogenesis. *Science* **350**, 1251-1255. doi:10.1126/science.aad2456

Haniffa, M., Taylor, D., Linnarsson, S., Aronow, B. J., Bader, G. D., Barker, R. A., Camara, P. G., Camp, J. G., Chédotal, A., Copp, A. et al. (2021). A roadmap for the human developmental cell Atlas. *Nature* **597**, 196-205. doi:10.1038/s41586-021-03620-1

Hayashi, M., Hinckley, C. A., Driscoll, S. P., Moore, N. J., Levine, A. J., Hilde, K. L., Sharma, K. and Pfaff, S. L. (2018). Graded arrays of spinal and supraspinal V2a interneuron subtypes underlie forelimb and hindlimb motor control. *Neuron* **97**, 869-884.e5. doi:10.1016/j.neuron.2018.01.023

Hochgerner, H., Zeisel, A., Lönnerberg, P. and Linnarsson, S. (2018). Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing. *Nat. Neurosci.* **21**, 290-299. doi:10.1038/s41593-017-0056-2

Honnell, V., Norrie, J. L., Patel, A. G., Ramirez, C., Zhang, J., Lai, Y.-H., Wan, S. and Dyer, M. A. (2022). Identification of a modular super-enhancer in murine retinal development. *Nat. Commun.* **13**, 253. doi:10.1038/s41467-021-27924-y

Hook, P. W., McClymont, S. A., Cannon, G. H., Law, W. D., Morton, A. J., Goff, L. A. and McCallion, A. S. (2018). Single-cell RNA-seq of mouse dopaminergic neurons informs candidate gene selection for sporadic parkinson disease. *Am. J. Hum. Genet.* **102**, 427-446. doi:10.1016/j.ajhg.2018.02.001

Hu, Y., Wang, X., Hu, B., Mao, Y., Chen, Y., Yan, L., Yong, J., Dong, J., Wei, Y., Wang, W. et al. (2019). Dissecting the transcriptome landscape of the human fetal neural retina and retinal pigment epithelium by single-cell RNA-seq analysis. *PLoS Biol.* **17**, e3000365. doi:10.1371/journal.pbio.3000365

Huisman, C., Cho, H., Brock, O., Lim, S. J., Youn, S. M., Park, Y., Kim, S., Lee, S.-K., Delogu, A. and Lee, J. W. (2019). Single cell transcriptome analysis of developing arcuate nucleus neurons uncovers their key developmental regulators. *Nat. Commun.* **10**, 3696. doi:10.1038/s41467-019-11667-y

Hupe, M., Li, M. X., Kneitz, S., Davydova, D., Yokota, C., Kele, J., Hot, B., Stenman, J. M. and Gessler, M. (2017). Gene expression profiles of brain endothelial cells during development at bulk and single-cell levels. *Sci. Signal.* **10**, eaag2476. doi:10.1126/scisignal.aag2476

Ibarra-Soria, X., Jawaid, W., Pijuan-Sala, B., Ladopoulos, V., Scialdone, A., Jörg, D. J., Tyser, R. C. V., Calero-Nieto, F. J., Mulas, C., Nichols, J. et al. (2018). Defining murine organogenesis at single-cell resolution reveals a role for the leukotriene pathway in regulating blood progenitor formation. *Nat. Cell Biol.* **20**, 127-134. doi:10.1038/s41556-017-0013-z

Kalish, B. T., Cheadle, L., Hrvatin, S., Nagy, M. A., Rivera, S., Crow, M., Gillis, J., Kirchner, R. and Greenberg, M. E. (2018). Single-cell transcriptomics of the developing lateral geniculate nucleus reveals insights into circuit assembly and refinement. *Proc. Natl. Acad. Sci. USA* **115**, E1051-E1060. doi:10.1073/pnas.1717871115

Kalish, B. T., Barkat, T. R., Diel, E. E., Zhang, E. J., Greenberg, M. E. and Hensch, T. K. (2020). Single-nucleus RNA sequencing of mouse auditory cortex reveals critical period triggers and brakes. *Proc. Natl. Acad. Sci. USA* **117**, 11744-11752. doi:10.1073/pnas.1920433117

Kee, N., Volakakis, N., Kirkeby, A., Dahl, L., Storvall, H., Nolbrant, S., Lahti, L., Björklund, Å. K., Gillberg, L., Joodmardi, E. et al. (2017). Single-cell analysis reveals a close relationship between differentiating dopamine and subthalamic nucleus neuronal lineages. *Cell Stem Cell* **20**, 29-40. doi:10.1016/j.stem.2016.10.003

Kim, D. W., Washington, P. W., Wang, Z. Q., Lin, S. H., Sun, C., Ismail, B. T., Wang, H., Jiang, L. and Blackshaw, S. (2020). The cellular and molecular landscape of hypothalamic patterning and differentiation from embryonic to late postnatal development. *Nat. Commun.* **11**, 4360. doi:10.1038/s41467-020-18231-z

Kiselev, V. Y., Andrews, T. S. and Hemberg, M. (2019). Challenges in unsupervised clustering of single-cell RNA-seq data. *Nat. Rev. Genet.* **20**, 273-282. doi:10.1038/s41576-018-0088-9

Klum, S., Zaouter, C., Alekseenko, Z., Björklund, Å. K., Hagey, D. W., Ericson, J., Muhr, J. and Bergsland, M. (2018). Sequentially acting SOX proteins orchestrate astrocyte- and oligodendrocyte-specific gene expression. *EMBO Rep.* **19**, e46635. doi:10.15252/embr.201846635

Kolla, L., Kelly, M. C., Mann, Z. F., Anaya-Rocha, A., Ellis, K., Lemons, A., Palermo, A. T., So, K. S., Mays, J. C., Orvis, J. et al. (2020). Characterization of the development of the mouse cochlear epithelium at the single cell level. *Nat. Commun.* **11**, 2389. doi:10.1038/s41467-020-16113-y

Kracht, L., Borggrewe, M., Eskandar, S., Brouwer, N., Chuva de Sousa Lopes, S. M., Laman, J. D., Scherjon, S. A., Prins, J. R., Kooistra, S. M., and Eggen, B. J. L. (2020). Human fetal microglia acquire homeostatic immune-sensing properties early in development. *Science* **369**, 530-537. doi:10.1126/science.aba5906

Krienen, F. M., Goldman, M., Zhang, Q., C H Del Rosario, R., Florio, M., Machold, R., Saunders, A., Levandowski, K., Zaniewski, H. and Schuman, B. (2020). Innovations present in the primate interneuron repertoire. *Nature* **586**, 262-269. doi:10.1038/s41586-020-2781-z

La Manno, G., Gyllborg, D., Codeluppi, S., Nishimura, K., Salto, C., Zeisel, A., Borm, L. E., Stott, S. R. W., Toledo, E. M., Villaescusa, J. C. et al. (2016). Molecular diversity of midbrain development in mouse, human, and stem cells. *Cell* **167**, 566-580.e19. doi:10.1016/j.cell.2016.09.027

La Manno, G., Soldatov, R., Zeisel, A., Braun, E., Hochgerner, H., Petukhov, V., Lidschreiber, K., Kastriti, M. E., Lönnerberg, P., Furlan, A. et al. (2018). RNA velocity of single cells. *Nature* **560**, 494-498. doi:10.1038/s41586-018-0414-6

La Manno, G., Siletti, K., Furlan, A., Gyllborg, D., Vinsland, E., Mossi Albiach, A., Mattsson Langseth, C., Khven, I., Lederer, A. R., Dratva, L. M. et al. (2021). Molecular architecture of the developing mouse brain. *Nature* **596**, 92-96. doi:10.1038/s41586-021-03775-x

Lasrado, R., Boesmans, W., Kleinjung, J., Pin, C., Bell, D., Bhaw, L., McCallum, S., Zong, H., Luo, L., Clevers, H. et al. (2017). Lineage-dependent spatial and functional organization of the mammalian enteric nervous system. *Science* **356**, 722-726. doi:10.1126/science.aam7511

Lau, S. T., Li, Z., Pui-Ling Lai, F., Nga-Chu Lui, K., Li, P., Munera, J. O., Pan, G., Mahe, M. M., Hui, C.-C., Wells, J. M. et al. (2019). Activation of hedgehog signaling promotes development of mouse and human enteric neural crest cells, based on single-cell transcriptome analyses. *Gastroenterology* **157**, 1556-1571.e5. doi:10.1053/j.gastro.2019.08.019

Li, C.-L., Li, K.-C., Wu, D., Chen, Y., Luo, H., Zhao, J.-R., Wang, S.-S., Sun, M.-M., Lu, Y.-J., Zhong, Y.-Q. et al. (2016). Somatosensory neuron types identified by high-coverage single-cell RNA-sequencing and functional heterogeneity. *Cell Res.* **26**, 83-102. doi:10.1038/cr.2015.149

Li, M., Santpere, G., Imamura Kawasawa, Y., Evgrafov, O. V., Gulden, F. O., Pochareddy, S., Sunkin, S. M., Li, Z., Shin, Y., Zhu, Y. et al. (2018). Integrative functional genomic analysis of human brain development and neuropsychiatric risks. *Science* **362**, eaat7615. doi:10.1126/science.aat7615

Li, Q., Cheng, Z., Zhou, L., Darmanis, S., Neff, N. F., Okamoto, J., Gulati, G., Bennett, M. L., Sun, L. O., Clarke, L. E. et al. (2019). Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. *Neuron* **101**, 207-223.e10. doi:10.1016/j.neuron.2018.12.006

Li, Z., Tyler, W. A., Zeldich, E., Santpere Baró, G., Okamoto, M., Gao, T., Li, M., Sestan, N. and Haydar, T. F. (2020). Transcriptional priming as a conserved mechanism of lineage diversification in the developing mouse and human neocortex. *Sci. Adv.* **6**, eabd2068. doi:10.1126/sciadv.abd2068

Lim, L., Mi, D., Llorca, A. and Marín, O. (2018). Development and functional diversification of cortical interneurons. *Neuron* **100**, 294-313. doi:10.1016/j.neuron.2018.10.009

Liu, S. J., Nowakowski, T. J., Pollen, A. A., Lui, J. H., Horlbeck, M. A., Attenello, F. J., He, D., Weissman, J. S., Kriegstein, A. R., Diaz, A. A. et al. (2016). Single-cell analysis of long non-coding RNAs in the developing human neocortex. *Genome Biol.* **17**, 67. doi:10.1186/s13059-016-0932-1

Lo Giudice, Q., Leleu, M., La Manno, G. and Fabre, P. J. (2019). Single-cell transcriptional logic of cell-fate specification and axon guidance in early-born retinal neurons. *Development* **146**, dev178103. doi:10.1242/dev.178103

Lohoff, T., Ghazanfar, S., Missarova, A., Koulena, N., Pierson, N., Griffiths, J. A., Bardot, E. S., Eng, C.-L., Tyser, R. C. V., Argelaguet, R. et al. (2022). Integration of spatial and single-cell transcriptomic data elucidates mouse organogenesis. *Nat. Biotechnol.* **40**, 74-85. doi:10.1038/s41587-021-01006-2

Longo, S. K., Guo, M. G., Ji, A. L. and Khavari, P. A. (2021). Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. *Nat. Rev. Genet.* **22**, 627-644. doi:10.1038/s41576-021-00370-8

Loo, L., Simon, J. M., Xing, L., McCoy, E. S., Niehaus, J. K., Guo, J., Anton, E. S. and Zylka, M. J. (2019). Single-cell transcriptomic analysis of mouse neocortical development. *Nat. Commun.* **10**, 134. doi:10.1038/s41467-018-08079-9

Lu, Y., Shiau, F., Yi, W., Lu, S., Wu, Q., Pearson, J. D., Kallman, A., Zhong, S., Hoang, T., Zuo, Z. et al. (2020). Single-cell analysis of human retina identifies evolutionarily conserved and species-specific mechanisms controlling development. *Dev. Cell* **53**, 473-491.e9. doi:10.1016/j.devcel.2020.04.009

Luecken, M. D. and Theis, F. J. (2019). Current best practices in single-cell RNA-seq analysis: a tutorial. *Mol. Syst. Biol.* **15**, e8746. doi:10.15252/msb.20188746

Marques, S., Zeisel, A., Codeluppi, S., van Bruggen, D., Mendanha Falcão, A., Xiao, L., Li, H., Häring, M., Hochgerner, H., Romanov, R. A. et al. (2016). Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science* **352**, 1326-1329. doi:10.1126/science.aaf6463

Masuda, T., Sankowski, R., Staszewski, O., Böttcher, C., Amann, L., Sagar, Nessler, S., Kunz, P., and, van Loo, G. (2019). Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature* **566**, 388-392. doi:10.1038/s41586-019-0924-x

Matcovitch-Natan, O., Winter, D. R., Giladi, A., Vargas Aguilar, S., Spinrad, A., Sarrazin, S., Ben-Yehuda, H., David, E., Zelada González, F., Perrin, P., et al. (2016). Microglia development follows a stepwise program to regulate brain homeostasis. *Science* **353**, aad8670. doi:10.1126/science.aad8670

Mayer, C., Hafemeister, C., Bandler, R. C., Machold, R., Batista Brito, R., Jaglin, X., Allaway, K., Butler, A., Fishell, G. and Satija, R. (2018). Developmental diversification of cortical inhibitory interneurons. *Nature* **555**, 457-462. doi:10.1038/nature25999

Mereu, E., Lafzi, A., Moutinho, C., Ziegenhain, C., McCarthy, D. J., Álvarez-Varela, A., Batlle, E., Sagar, Grün, D., Lau, J. K. et al. (2020). Benchmarking single-cell RNA-sequencing protocols for cell atlas projects. *Nat. Biotechnol.* **38**, 747-755. doi:10.1038/s41587-020-0469-4

Mi, D., Li, Z., Lim, L., Li, M., Moissidis, M., Yang, Y., Gao, T., Hu, T. X., Pratt, T., Price, D. J. et al. (2018). Early emergence of cortical interneuron diversity in the mouse embryo. *Science* **360**, 81-85. doi:10.1126/science.aar6821

Mich, J. K., Graybuck, L. T., Hess, E. E., Mahoney, J. T., Kojima, Y., Ding, Y., Somasundaram, S., Miller, J. A., Kalmbach, B. E., Radaelli, C. et al. (2021). Functional enhancer elements drive subclass-selective expression from mouse to primate neocortex. *Cell Rep.* **34**, 108754. doi:10.1016/j.celrep.2021.108754

Molyneaux, B. J., Arlotta, P., Menezes, J. R. and Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nat. Rev. Neurosci.* **8**, 427-437. doi:10.1038/nrn2151

Morarach, K., Mikhailova, A., Knoflach, V., Memic, F., Kumar, R., Li, W., Ernfors, P. and Marklund, U. (2021). Diversification of molecularly defined myenteric neuron classes revealed by single-cell RNA sequencing. *Nat. Neurosci.* **24**, 34-46. doi:10.1038/s41593-020-00736-x

Nowakowski, T. J., Bhaduri, A., Pollen, A. A., Alvarado, B., Mostajo-Radji, M. A., Di Lullo, E., Haeussler, M., Sandoval-Espinosa, C., Liu, S. J., Velmeshev, D. et al. (2017). Spatiotemporal gene expression trajectories reveal developmental hierarchies of the human cortex. *Science* **358**, 1318-1323. doi:10.1126/science.aap8809

Peng, J., Sheng, A. L., Xiao, Q., Shen, L., Ju, X. C., Zhang, M., He, S. T., Wu, C. and Luo, Z. G. (2019). Single-cell transcriptomes reveal molecular specializations of neuronal cell types in the developing cerebellum. *J. Mol. Cell Biol.* **11**, 636-648. doi:10.1093/jmcb/mjy089

Perkel, J. M. (2021). Single-cell analysis enters the multiomics age. *Nature* **595**, 614-616. doi:10.1038/d41586-021-01994-w

Perlman, K., Couturier, C. P., Yaqubi, M., Tanti, A., Cui, Q. L., Pernin, F., Stratton, J. A., Ragoussis, J., Healy, L., Petrecca, K. et al. (2020). Developmental trajectory of oligodendrocyte progenitor cells in the human brain revealed by single cell RNA sequencing. *Glia* **68**, 1291-1303. doi:10.1002/glia.23777

Pijuan-Sala, B., Griffiths, J. A., Guibentif, C., Hiscock, T. W., Jawaid, W., Calero-Nieto, F. J., Mulas, C., Ibarra-Soria, X., Tyser, R. C. V., Ho, D. L. L. et al. (2019). A single-cell molecular map of mouse gastrulation and early organogenesis. *Nature* **566**, 490-495. doi:10.1038/s41586-019-0933-9

Pletikos, M., Sousa, A. M., Sedmak, G., Meyer, K. A., Zhu, Y., Cheng, F., Li, M., Kawasawa, Y. I. and Šestan, N. (2014). Temporal specification and bilaterality of human neocortical topographic gene expression. *Neuron* **81**, 321-332. doi:10.1016/j.neuron.2013.11.018

Polioudakis, D., de la Torre-Ubieta, L., Langerman, J., Elkins, A. G., Shi, X., Stein, J. L., Vuong, C. K., Nichterwitz, S., Gevorgian, M., Opland, C. K. et al. (2019). A single-cell transcriptomic Atlas of human neocortical development during mid-gestation. *Neuron* **103**, 785-801.e8. doi:10.1016/j.neuron.2019.06.011

Pollen, A. A., Nowakowski, T. J., Shuga, J., Wang, X., Leyrat, A., Lui, J. H., Li, N., Szpankowski, L., Fowler, B., Chen, P. et al. (2014). Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex. *Nat. Biotechnol.* **32**, 1053-1058. doi:10.1038/nbt.2967

Pollen, A. A., Nowakowski, T. J., Chen, J., Retallack, H., Sandoval-Espinosa, C., Nicholas, C. R., Shuga, J., Liu, S. J., Oldham, M. C., Diaz, A. et al. (2015). Molecular identity of human outer radial glia during cortical development. *Cell* **163**, 55-67. doi:10.1016/j.cell.2015.09.004

Preissl, S., Fang, R., Huang, H., Zhao, Y., Raviram, R., Gorkin, D. U., Zhang, Y., Sos, B. C., Afzal, V., Dickel, D. E. et al. (2018). Single-nucleus analysis of accessible chromatin in developing mouse forebrain reveals cell-type-specific transcriptional regulation. *Nat. Neurosci.* **21**, 432-439. doi:10.1038/s41593-018-0079-3

Puelles, L. (2009). Forebrain development: prosomere model. *Encycl. Neurosci.* **2009**, 315-319. doi:10.1016/b978-008045046-9.01076-7

DEVELOPMENT

Rao, A., Barkley, D., França, G. S. and Yanai, I. (2021). Exploring tissue architecture using spatial transcriptomics. *Nature* **596**, 211-220. doi:10.1038/s41586-021-03634-9

Regev, A., Teichmann, S. A., Lander, E. S., Amit, I., Benoist, C., Birney, E., Bodenmiller, B., Campbell, P., Carninci, P., Clatworthy, M. et al. (2017). Science forum: The human cell atlas. *eLife* **6**, e27041. doi:10.7554/eLife.27041

Romanov, R. A., Tretiakov, E. O., Kastriti, M. E., Zupancic, M., Häring, M., Korchynska, S., Popadin, K., Benevento, M., Rebernik, P., Lallemend, F. et al. (2020). Molecular design of hypothalamus development. *Nature* **582**, 246-252. doi:10.1038/s41586-020-2266-0

Rosenberg, A. B., Roco, C. M., Muscat, R. A., Kuchina, A., Sample, P., Yao, Z., Graybuck, L. T., Peeler, D. J., Mukherjee, S., Chen, W. et al. (2018). Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. *Science* **360**, 176-182. doi:10.1126/science.aam8999

Ross, J. M., Kim, C., Allen, D., Crouch, E. E., Narsinh, K., Cooke, D. L., Abla, A. A., Nowakowski, T. J. and Winkler, E. A. (2020). The expanding cell diversity of the brain vasculature. *Front. Physiol.* **11**, 600767. doi:10.3389/fphys.2020.600767

Rozenblatt-Rosen, O., Shin, J. W., Rood, J. E., Hupalowska, A., Human Cell Atlas Standards and Technology Working Group, Regev, A., and Heyn, H. (2021). Building a high-quality Human Cell Atlas. *Nat. Biotechnol.* **39**, 149-153. doi:10.1038/s41587-020-00812-4

Saelens, W., Cannoodt, R., Todorov, H. and Saeys, Y. (2019). A comparison of single-cell trajectory inference methods. *Nat. Biotechnol.* **37**, 547-554. doi:10.1038/s41587-019-0071-9

Shi, Y., Wang, M., Mi, D., Lu, T., Wang, B., Dong, H., Zhong, S., Chen, Y., Sun, L., Zhou, X. et al. (2021). Mouse and human share conserved transcriptional programs for interneuron development. *Science* **374**, eabj6641. doi:10.1126/science.abj6641

Silbereis, J. C., Pochareddy, S., Zhu, Y., Li, M. and Sestan, N. (2016). The cellular and molecular landscapes of the developing human central nervous system. *Neuron* **89**, 248-268. doi:10.1016/j.neuron.2015.12.008

Soldatov, R., Kaucka, M., Kastriti, M. E., Petersen, J., Chontorotzea, T., Englmaier, L., Akkuratova, N., Yang, Y., Häring, M., Dyachuk, V. et al. (2019). Spatiotemporal structure of cell fate decisions in murine neural crest. *Science* **364**, eaas9536. doi:10.1126/science.aas9536

Sridhar, A., Hoshino, A., Finkbeiner, C. R., Chitsazan, A., Dai, L., Haugan, A. K., Eschenbacher, K. M., Jackson, D. L., Trapnell, C., Bermingham-McDonogh, O. et al. (2020). Single-cell transcriptomic comparison of human fetal retina, hPSC-derived retinal organoids, and long-term retinal cultures. *Cell Rep.* **30**, 1644-1659.e4. doi:10.1016/j.celrep.2020.01.007

Srivatsan, S. R., Regier, M. C., Barkan, E., Franks, J. M., Packer, J. S., Grosjean, P., Duran, M., Saxton, S., Ladd, J. J., Spielmann, M. et al. (2021). Embryo-scale, single-cell spatial transcriptomics. *Science* **373**, 111-117. doi:10.1126/science.abb9536

Svensson, V., Vento-Tormo, R. and Teichmann, S. A. (2018). Exponential scaling of single-cell RNA-seq in the past decade. *Nat. Protoc.* **13**, 599-604. doi:10.1038/nprot.2017.149

Svensson, V., da Veiga Beltrame, E. and Pachter, L. (2020). A curated database reveals trends in single-cell transcriptomics. *Database*, **2020**, baaa073. doi:10.1093/database/baaa073

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B. B., Siddiqui, A. et al. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. *Nat. Methods* **6**, 377-382. doi:10.1038/nmeth.1315

Tarashansky, A. J., Musser, J. M., Khariton, M., Li, P., Arendt, D., Quake, S. R. and Wang, B. (2021). Mapping single-cell atlases throughout Metazoa unravels cell type evolution. *Elife* **10**, e66747. doi:10.7554/eLife.66747

Tasdemir-Yilmaz, O. E., Druckenbrod, N. R., Olukoya, O. O., Dong, W., Yung, A. R., Bastille, I., Pazyra-Murphy, M. F., Sitko, A. A., Hale, E. B., Vigneau, S. et al. (2021). Diversity of developing peripheral glia revealed by single-cell RNA sequencing. *Dev. Cell* **56**, 2516-2535.e8. doi:10.1016/j.devcel.2021.08.005

Telley, L., Agirman, G., Prados, J., Amberg, N., Fièvre, S., Oberst, P., Bartolini, G., Vitali, I., Cadilhac, C., Hippenmeyer, S. et al. (2019). Temporal patterning of apical progenitors and their daughter neurons in the developing neocortex. *Science* **364**, eaav2522. doi:10.1126/science.aav2522

Their, M. C., Hommerding, O., Panten, J., Pinna, R., García-González, D., Berger, T., Wörsdörfer, P., Assenov, Y., Scognamiglio, R., Przybylla, A. et al. (2019). Identification of embryonic neural plate border stem cells and their generation by direct reprogramming from adult human blood cells. *Cell Stem Cell* **24**, 166-182.e13. doi:10.1016/j.stem.2018.11.015

Tiklová, K., Björklund, Å. K., Lahti, L., Fiorenzano, A., Nolbrant, S., Gillberg, L., Volakakis, N., Yokota, C., Hilscher, M. M., Hauling, T. et al. (2019). Single-cell RNA sequencing reveals midbrain dopamine neuron diversity emerging during mouse brain development. *Nat. Commun.* **10**, 581. doi:10.1038/s41467-019-08453-1

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., Lennon, N. J., Livak, K. J., Mikkelsen, T. S. and Rinn, J. L. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat. Biotechnol.* **32**, 381-386. doi:10.1038/nbt.2859

Trevino, A. E., Müller, F., Andersen, J., Sundaram, L., Kathiria, A., Shcherbina, A., Farh, K., Chang, H. Y., Pa⬚ca, A. M., Kundaje, A. et al. (2021). Chromatin and gene-regulatory dynamics of the developing human cerebral cortex at single-cell resolution. *Cell* **184**, 5053-5069.e23. doi:10.1016/j.cell.2021.07.039

Usoskin, D., Furlan, A., Islam, S., Abdo, H., Lönnerberg, P., Lou, D., Hjerling-Leffler, J., Haeggström, J., Kharchenko, O., Kharchenko, P. V. et al. (2015). Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat. Neurosci.* **18**, 145-153. doi:10.1038/nn.3881

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Del Gaudio, F., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L. et al. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature* **554**, 475-480. doi:10.1038/nature25739

Velasco, S., Kedaigle, A. J., Simmons, S. K., Nash, A., Rocha, M., Quadrato, G., Paulsen, B., Nguyen, L., Adiconis, X., Regev, A. et al. (2019). Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature* **570**, 523-527. doi:10.1038/s41586-019-1289-x

Vladoiu, M. C., El-Hamamy, I., Donovan, L. K., Farooq, H., Holgado, B. L., Sundaravadanam, Y., Ramaswamy, V., Hendrikse, L. D., Kumar, S., Mack, S. C. et al. (2019). Childhood cerebellar tumours mirror conserved fetal transcriptional programs. *Nature* **572**, 67-73. doi:10.1038/s41586-019-1158-7

Wagner, D. E. and Klein, A. M. (2020). Lineage tracing meets single-cell omics: opportunities and challenges. *Nat. Rev. Genet.* **21**, 410-427. doi:10.1038/s41576-020-0223-2

Waldhaus, J., Durruthy-Durruthy, R. and Heller, S. (2015). Quantitative high-resolution cellular map of the organ of corti. *Cell Rep.* **11**, 1385-1399. doi:10.1016/j.celrep.2015.04.062

Wang, K., Wang, S., Chen, Y., Wu, D., Hu, X., Lu, Y., Wang, L., Bao, L., Li, C. and Zhang, X. (2021). Single-cell transcriptomic analysis of somatosensory neurons uncovers temporal development of neuropathic pain. *Cell Res.* **31**, 904-918. doi:10.1038/s41422-021-00479-9

Weng, Q., Wang, J., Wang, J., He, D., Cheng, Z., Zhang, F., Verma, R., Xu, L., Dong, X., Liao, Y. et al. (2019). Single-cell transcriptomics uncovers glial progenitor diversity and cell fate determinants during development and gliomagenesis. *Cell Stem Cell* **24**, 707-723.e8. doi:10.1016/j.stem.2019.03.006

Winkler, E. A., Kim, C. N., Ross, J. M., Garcia, J. H., Gil, E., Oh, I., Chen, L. Q., Wu, D., Catapano, J. S., Raygor, K. et al. (2022). A single-cell atlas of the normal and malformed human brain vasculature. *Science* **375**, eabi7377. doi:10.1126/science.abi7377

Wittmann, M. T., Katada, S., Sock, E., Kirchner, P., Ekici, A. B., Wegner, M., Nakashima, K., Lie, D. C. and Reis, A. (2021). scRNA sequencing uncovers a TCF4-dependent transcription factor network regulating commissure development in mouse. *Development* **148**, dev196022. doi:10.1242/dev.196022

Wizeman, J. W., Guo, Q., Wilion, E. M. and Li, J. Y. (2019). Specification of diverse cell types during early neurogenesis of the mouse cerebellum. *eLife* **8**, e42388. doi:10.7554/eLife.42388

Yamada, R., Oguri, A., Fujiki, K., Shirahige, K., Takezoe, H., Takahashi, N. and Kanai, Y. (2021). Single-cell transcriptional analysis reveals developmental stage-dependent changes in retinal progenitors in the murine early optic vesicle. *Biochem. Biophys. Res. Commun.* **543**, 80-86. doi:10.1016/j.bbrc.2021.01.043

Yu, Y., Zeng, Z., Xie, D., Chen, R., Sha, Y., Huang, S., Cai, W., Chen, W., Li, W., Ke, R. et al. (2021). Interneuron origin and molecular diversity in the human fetal brain. *Nat. Neurosci.* **24**, 1745-1756. doi:10.1038/s41593-021-00940-3

Yuzwa, S. A., Borrett, M. J., Innes, B. T., Voronova, A., Ketela, T., Kaplan, D. R., Bader, G. D. and Miller, F. D. (2017). Developmental emergence of adult neural stem cells as revealed by single-cell transcriptional profiling. *Cell Rep.* **21**, 3970-3986. doi:10.1016/j.celrep.2017.12.017

Zarkada, G., Howard, J. P., Xiao, X., Park, H., Bizou, M., Leclerc, S., Künzel, S. E., Boisseau, B., Li, J., Cagnone, G. et al. (2021). Specialized endothelial tip cells guide neuroretina vascularization and blood-retina-barrier formation. *Dev. Cell* **56**, 2237-2251.e6. doi:10.1016/j.devcel.2021.06.021

Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., Häring, M., Braun, E., Borm, L. E., La Manno, G. et al. (2018). Molecular architecture of the mouse nervous system. *Cell* **174**, 999-1014.e22. doi:10.1016/j.cell.2018.06.021

Zhang, S., Cui, Y., Ma, X., Yong, J., Yan, L., Yang, M., Ren, J., Tang, F., Wen, L., Qiao, J. et al. (2020a). Single-cell transcriptomics identifies divergent developmental lineage trajectories during human pituitary development. *Nat. Commun.* **11**, 5275. doi:10.1038/s41467-020-19012-4

Zhang, Y., Liu, G., Guo, T., Liang, X. G., Du, H., Yang, L., Bhaduri, A., Li, X., Xu, Z., Zhang, Z. et al. (2020b). Cortical neural stem cell lineage progression is regulated by extrinsic signaling molecule sonic hedgehog. *Cell Rep.* **30**, 4490-4504.e4. doi:10.1016/j.celrep.2020.03.027

Zhang, Q., Wu, X., Fan, Y., Jiang, P., Zhao, Y., Yang, Y., Han, S., Xu, B., Chen, B., Han, J. et al. (2021). Single-cell analysis reveals dynamic changes of neural cells in developing human spinal cord. *EMBO Rep.* **22**, e52728. doi:10.15252/embr.202152728

Zhao, H., Tu, Z., Xu, H., Yan, S., Yan, H., Zheng, Y., Yang, W., Zheng, J., Li, Z., Tian, R. et al. (2017). Altered neurogenesis and disrupted expression of synaptic proteins in prefrontal cortex of SHANK3-deficient non-human primate. *Cell Res.* **27**, 1293-1297. doi:10.1038/cr.2017.95

Zhong, S., Zhang, S., Fan, X., Wu, Q., Yan, L., Dong, J., Zhang, H., Li, L., Sun, L., Pan, N. et al. (2018). A single-cell RNA-seq survey of the developmental landscape of the human prefrontal cortex. *Nature* **555**, 524-528. doi:10.1038/nature25980

DEVELOPMENT

Zhong, S., Ding, W., Sun, L., Lu, Y., Dong, H., Fan, X., Liu, Z., Chen, R., Zhang, S., Ma, Q. et al. (2020). Decoding the development of the human hippocampus. *Nature* **577**, 531-536. doi:10.1038/s41586-019-1917-5

Zhu, Y., Sousa, A. M. M., Gao, T., Skarica, M., Li, M., Santpere, G., Esteller-Cucala, P., Juan, D., Ferrández-Peral, L., Gulden, F. O. et al.

(2018). Spatiotemporal transcriptomic divergence across human and macaque brain development. *Science* **362**, eaat8077. doi:10.1126/science.aat8077

Ziffra, R. S., Kim, C. N., Ross, J. M., Wilfert, A., Turner, T. N., Haeussler, M., Casella, A. M., Przytycki, P. F., Keough, K. C., Shin, D. et al. (2021). Single-cell epigenomics reveals mechanisms of human cortical development. *Nature* **598**, 205-213. doi:10.1038/s41586-021-03209-8

Table 1. Publications using scRNA-seq on developing human and mouse nervous system tissues

No.	Reference	Reported cells/nuclei	Species	Cell source, neuroanatomical region	Cell type/lineage sequenced	Developmental time point (for sc-seq)
1	Cao et al. (2020)	4,062,980	Human	Adrenal gland, brain (cerebrum and cerebellum), eye, heart, intestine, kidney, liver, lung, muscle, pancreas, placenta, spleen, stomach, thymus	All cell types	PCD 72-129 (around 90, 100, 110, 120 day, plotted as PCW 10, 13, 14, 16, 17)
2	Bhaduri et al. (2020)	424,530	Human	Brain: cortex [central (motor), V1, parietal, frontal, temporal], hippocampus	All cell types	CS 13, 22; GW 14, 18, 22
3	Eze et al. (2021)	289,000	Human	Brain: cortex, ganglionic eminences, thalamus, hypothalamus, midbrain, hindbrain, cerebellum, retina, SC, olfactory placode	All cell types	CS 12, 13, 14, 15, 19, 20, 22
4	Aldinger et al. (2021)	69,174	Human	Brain: cerebellum	All cell types	PCW 9, 10, 11, 12, 14, 16, 17, 18, 20, 21
5	Trevino et al. (2021)	66,849	Human	Brain: cortex	All cell types	PCW 16, 20, 21, 24
6	Shi et al. (2021)	56,412	Human	Brain: ganglionic eminences	All cell types	GW 9, 12, 13, 16, 18
7	Yu et al. (2021)	40,572	Human	Brain: subpallium (ganglionic eminences)	All cell types	GW 9, 10, 11, 12
8	Polioudakis et al. (2019)	40,000	Human	Brain: ventricular zone, subventricular zone, subplate, cortical plate, marginal zone	All cell types	GW 17, 17.5, 18
9	Perlman et al. (2020)	37,985	Human	Brain: white matter cortical resections, fetal telencephalon	All cell types	Second-trimester fetal*. 2, 13, 58, 62 year
10	Zhong et al. (2020)	30,416	Human	Brain: hippocampus	All cell types	GW 16, 18, 20, 22, 25, 27
11	Couturier et al. (2020)	22,637	Human	Brain: telencephalon	All cell types; Progenitors [CD133 (PROM1)]	GW 13,17,19, 21
12	Li et al. (2018)	18,288	Human	Brain: pallium (cortex), frontoparietal plate (CP), DFC (frontal cortex)	All cell types	Cortex: PCW 5, 6, 8, 16; CP: 125 days (PCW 18); DFC: PCW 19, 20‡. 18.9 (plotted as 19), 36, 64 year
13	Kracht et al. (2020)	16,563	Human	Brain: whole brain	Microglia	GW 9, 10, 11, 12, 13, 15, 16, 17 ,18
14	Nowakowski et al. (2017)	4261	Human	Brain: prefrontal cortex, visual cortex	All cell types	PCW 5.85, 7, 8, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 32, 37
15	Zhang et al. (2020a)	4113	Human	Brain: pituitary gland	All cell types	PCW 7, 8, 9, 10, 13, 14, 15, 16, 17, 19, 21, 22, 23, 24, 25
16	Fu et al. (2021)	3355	Human	Brain: frontal cortex	Neural progenitors (EGFR)	GW 21, 23, 24, 25
17	Zhong et al. (2018)	2394	Human	Brain: prefrontal cortex	All cell types	GW 8, 9, 10, 12, 13, 16, 19, 23, 26
18	Darmanis et al. (2015)	466	Human	Brain: cortex, adult temporal lobe	Neural cells	GW 16-18 (unspecified, plotted as 17 weeks); Adult 21, 22, 37, 47, 54, 63 year
19	Pollen et al. (2015)	393	Human	Brain: ventricular zone, (outer) subventricular zone	All cell types	GW 16, 16.5, 18
20	Pollen et al. (2014)	301	Human	Brain: neocortex, primary cortex	All cell types	GW 16, 21
21	Liu et al. (2016)	226	Human	Brain: neocortex	All cell types	GW 19.5, 20.5, 23.5
22	Lu et al. (2020)	113,999	Human	Eye: retina	All cell types	GW 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 22, 24, 27
23	Sridhar et al. (2020)	39,768	Human	Eye: retina	All cell types	Fetal day 59, 82, 125 (around PCW 8.5, 12, 18)
24	Hu et al. (2019)	2421	Human	Eye: retina and retinal pigment epithelium	All cell types	GW 5, 6, 7, 8, 9, 11, 13, 17, 23, 24
25	Zhang et al. (2021)	827,265	Human	SC: whole SC, cervical (C), thoracic (T), lumbar (L)	All cell types	Whole SC: GW 7, 23; C, T: GW 9; C, T, L: GW 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22

Table 1. Continued

No.	Reference	Reported cells/nuclei	Species	Cell source, neuroanatomical region	Cell type/lineage sequenced	Developmental time point (for sc-seq)
26	Zhu et al. (2018)	113,274	Macaque, (Human)	Brain: dorsal frontal cortex, hippocampus, amygdala, striatum, mediodorsal nucleus of thalamus, cerebellar cortex	All cell types	Macaque E110 [Human tissue from Li et al. (2018) for comparison, not included in the figure]
27	La Manno et al. (2016)	6100	Human, Mouse	Brain: midbrain (V)	All cell types	Human: PCW 6, 7, 8, 9, 10, 11; Mouse: E11.5, 12.5, 13.5, 14.5, 15.5, 18.5. P21
28	Masuda et al. (2019)	2966	Human, Mouse	Brain (human): healthy temporal/frontal versus multiple sclerosis parietal cortex; Brain (mouse): forebrain, cortex, hippocampus, corpus callosum, midbrain, cerebellum, pons facial nucleus, SC	Microglia	Human: 22-54 year§; Mouse: forebrain, midbrain: E16.5; cerebellum, SC: E16.5, 3 week, 16 week; Corpus callosum, cortex, hippocampus: 3 week, 16 week; Pons: 16 week
29	La Manno et al. (2021)	292,495	Mouse	Embryo: head; Brain: hindbrain, midbrain (D/V), forebrain (V-thalamic, V-lateral, D/V)	All cell types	Embryo: E7; Head: E8; Brain regions: E9, 10, 11, 12, 13, 14, 15, 16, 17, 18
30	Rosenberg et al. (2018)	150,049	Mouse	Brain: whole brain, spinal cord	All cell types	P2, 11
31	Kim et al. (2020)	129,151	Mouse	Brain: diencephalon (prethalamus and hypothalamus), hypothalamus	All cell types	Diencephalon: E10, 11, 12, 13, 14, 15, 16; Hypothalamus: E18. P4, 8, 14, 45
32	Di Bella et al. (2021)	98,047	Mouse	Brain: somatosensory cortex	All cell types	E10.5, 11.5, 12.5, 13.5, 14.5, 15.5, 16.5, 17.5, 18.5. P1, 4
33	Hammond et al. (2019)	76,149	Mouse	Brain: whole brain	Microglia	E14.5. P4, 30, 100, 540
34	Allaway et al. (2021)	64,524	Mouse	Brain: medial ganglionic eminence, cortex	Interneurons (Dlx6)	Medial ganglionic eminence: E13.5; Cortex: E18. P2, 10, 28
35	Vladoiu et al. (2019)	60,000	Mouse	Brain: hindbrain, mesial cerebellum	All cell types	Hindbrain: E10, 12; Cerebellum: E14, 16, 18. P0, 5, 7, 14
36	Mayer et al. (2018)	60,000	Mouse	Brain: cortex	Inhibitory interneurons	E13.5, 14.5
37	Romanov et al. (2020)	51,199	Mouse	Brain: hypothalamus	All cell types	E15.5, 17.5. P0, 2, 10, 23
38	Carter et al. (2018)	39,245	Mouse	Brain: cerebellum	All cell types	E10, 11, 12, 13, 14, 15, 16, 17. P0, 4, 7, 10
39	Kalish et al. (2018)	35,000	Mouse	Brain: lateral geniculate nucleus (thalamus)	All cell types	P5, 10, 16, 21
40	Hochgerner et al. (2018)	33,939	Mouse	Brain: dentate gyrus (hippocampus)	All cell types	E16.5. P0, 5, 12, 16, 18, 19, 23, 24, 35, 120, 132
41	Kalish et al. (2020)	31,293	Mouse	Brain: auditory cortex A1	All cell types	P10, 15, 20 (tonotopic critical period)
42	Peng et al. (2019)	21,119	Mouse	Brain: cerebellum	All cell types	P0, 8
43	Loo et al. (2019)	18,545	Mouse	Brain: neocortex	All cell types	E14.5. P0
44	Zhang et al. (2020b)	15,805	Mouse	Brain: cortex	All cell types	E16.5
45	Dani et al. (2021)	15,620	Mouse	Brain: choroid plexus	All cell types	E16.5. 4, 20 month
46	Wittmann et al. (2021)	14,196	Mouse	Brain: neocortex	All cell types	E18.5
47	Fazel Darbandi et al. (2020)	11,070	Mouse	Brain: cortex	L5 prefrontal neurons	P5
48	Yuzwa et al. (2017)	9972	Mouse	Brain: cortex	All cell types	E11.5, 13.5, 15.5, 17.5
49	Wizeman et al. (2019)	9400	Mouse	Brain: cerebellum	All cell types	E13.5
50	Guo and Li (2019)	7365	Mouse	Brain: diencephalon	All cell types	E12.5
51	Weng et al. (2019)	6792	Mouse	Brain: cortex	Oligodendrocyte progenitors (PDGFRα); astrocyte (GFAP)	P1, 3 (PDGFRα), P5, 6 (GFAP)
52	Soldatov et al. (2019)	6124	Mouse	Brain: neural crest	All cell types	E8.5, 9.5, 10.5
53	Li et al. (2020)	5777	Mouse	Brain: neocortex	All cell types	E15.5
54	Marques et al. (2016)	5072	Mouse	Brain: somatosensory cortex, striatum, dentate gyrus, hippocampus, corpus callosum, amygdala, hypothalamus, zona incerta, substantia nigra ventral tegmental area, SC (D)	All cell types	P21-30*, P60
55	Huisman et al. (2019)	5038	Mouse	Brain: hypothalamic arcuate nucleus	All cell types	E15
56	Dickel et al. (2018)	4723	Mouse	Brain: forebrain	All cell types	E12.5
57	Matcovitch-Natan et al. (2016)	2831	Mouse	Brain: whole brain	Microglia	E10.5, 12.5, 14.5, 16.5. P3, 6, 9. 8 weeks
58	Telley et al. (2019)	2756	Mouse	Brain: neocortex	All cell types	E12, 13, 14, 15
59	Mi et al. (2018)	2003	Mouse	Brain: caudal ganglionic eminence, medial ganglionic eminence (D/V)	All cell types	E12.5, 14.5

Continued

Table 1. Continued

No.	Reference	Reported cells/nuclei	Species	Cell source, neuroanatomical region	Cell type/lineage sequenced	Developmental time point (for sc-seq)
60	Li et al. (2019)	1816	Mouse	Brain: whole brain; Brain regions: cortex, cerebellum, hippocampus, striatum, OB, choroid plexus	Microglia	Whole brain: E14.5; Brain regions: P7, 60
61	Fabra-Beser et al. (2021)	1115	Mouse	Brain: neocortex	Radial glial cells (neural progenitors)	E12.5+16 h (plotted as E13)
62	Tiklová et al. (2019)	1106	Mouse	Brain: ventral midbrain	Dopaminergic neurons	E13.5, 15.5, 18.5. P1, 7, 90
63	Kee et al. (2017)	550	Mouse	Brain: ventral midbrain	Dopaminergic neurons	E10.5, 11.5, 12.5, 13.5
64	Hook et al. (2018)	473	Mouse	Brain: forebrain (hypothalamus), midbrain, OB	Dopaminergic neurons	Hypothalamus and midbrain: E15.5; OB, hypothalamus and midbrain: P7
65	Thier et al. (2019)	173	Mouse	Brain: mid-hindbrain area	All cell types	E8.5
66	Hanchate et al. (2015)	93	Mouse	Brain: olfactory epithelium	Neurons	P2, 3, 4, 5, 6
67	Kolla et al. (2020)	30,000	Mouse	Ear: cochlea	All cell types	E14, 16. P1,7
68	Durruthy-Durruthy et al. (2014)	382	Mouse	Ear: otocyst and early neuroblast	All cell types	E10.5
69	Waldhaus et al. (2015)	808	Mouse	Ear: organ of Corti (cochlea)	All cell types	P2
70	Burns et al. (2015)	301	Mouse	Ear: utricle and cochlea	All cell types	P1
71	Cao et al. (2019)	2,058,652	Mouse	Embryos	All cell types	E9.5, 10.5, 11.5, 12.5, 13.5
72	Pijuan-Sala et al. (2019)	116,312	Mouse	Embryos	All cell types	E6.5, 6.75, 7.0, 7.25, 7.5, 7.75, 8.0, 8.25, 8.5
73	Ibarra-Soria et al. (2018)	27,000	Mouse	Embryos	All cell types	E8.5
74	Clark et al. (2019)	121,551	Mouse	Eye: retina	All cell types	E11, 12, 14, 16, 18. P0, 2, 5, 8, 14
75	Zarkada et al. (2021)	22,933	Mouse	Eye: retina	All cell types; Endothelial [CD31 (Pecam1)]	P6,10
76	Yamada et al. (2021)	7989	Mouse	Eye: optic vesicle (retinal progenitors)	All cell types	E8.5-E9.5 (5 time points: 12-, 16-, 20-, 24- and 26-somite stages)
77	Honnell et al. (2022)	WT: 5932; KO: 35,697	Mouse	Eye: retina	All cell types	E14.5
78	Lo Giudice et al. (2019)	5348	Mouse	Eye: retina	All cell types	E15.5
79	Buenaventura et al. (2019)	4000	Mouse	Eye: retina	Retinal cells	E14.5
80	Delile et al. (2019)	38,975	Mouse	Neural tube	All cell types	E9.5, 10.5, 11.5, 12.5, 13.5
81	Hayashi et al. (2018)	949	Mouse	SC: C and L segments	V2a interneurons	P0
82	Klum et al. (2018)	350	Mouse	SC: T segment	Neural (CD133); All cell types	Neural: E11.5; All: E15.5
83	Tasdemir-Yilmaz et al. (2021)	52,323	Mouse	PNS: sciatic nerve, lumbar dorsal root ganglia, spiral ganglia, organ of Corti (cochlea)	Glia (Plp1)	E14, 18. P14
84	Gerber et al. (2021)	23,767	Mouse	PNS: sciatic nerve	All cell types; Schwann cells (Mpz)	All: P1, 5, 14, 60; Schwann cells: P1, 60
85	Morarach et al. (2021)	9141	Mouse	PNS: ENS (gastrointestinal tract)	Neural lineage [Baf53b (Actl6b)] and neural crest (Wnt1)	E15.5, 18.5. P21
86	Lau et al. (2019)	7671	Mouse	PNS: ENS	Enteric neural crest [p75 (Ngfr) and Wnt1]	E13.5
87	Lasrado et al. (2017)	120	Mouse	PNS: ENS	All cell types	E12.5, 13
88	DeSisto et al. (2020)	8581	Mouse	Meninges	Fibroblasts (Col1a1)	E14
89	Hupe et al. (2017)	80	Mouse	Vascular	Endothelial cells	E14.5

Publications are arranged first by human, human and mouse, and mouse primary tissue; then by anatomical region (divided by horizontal lines); and finally the number of single cells/nuclei sequenced in primary healthy tissue in descending order. The information about the number of cells/nuclei sequenced per sample, anatomical regions and ages was not always clear in the manuscripts (see below), and were found either in the main text, figures, figure legends, methods section or supplementary material. Despite our best effort to document correct information about samples, there may therefore be some errors. Some studies contain data from other regions and time points obtained using other methods (e.g. bulk RNA- and ATAC-seq), that are not included in this table. The anatomical regions, cell types and developmental time points were used to make Fig. 1 (mouse) and Fig. 2 (human). The numbers in the left column are matched between this table and the figures for cross reference purposes. Ages: m, months; w, weeks; y, years. Human-specific ages: CS, Carnegie stage; GW, gestational week; PCD, postconceptional day; PCW, postconceptional week. Carnegie stages 1-4 (PCW 1), 5-6 (PCW 2), 7-8 (PCW 3), 10-13 (PCW 4), 14-15 (PCW 5), 16-17 (PCW 6), 18-19 (PCW 7), 20-23 (PCW 8). Mouse-specific ages: E, embryonic day (also macaque); P, postnatal day. C, cervical; CP, cortical plate; D, dorsal; DFC, dorsofrontal cortex; ENS, enteric nervous system; L, lumbar; OB, olfactory bulb; PNS, peripheral nervous system; SC, spinal cord; T, thoracic; V: ventral.
*Fetal age not specified; not in figure.
‡Unclear information, used Tables S3 and S4.
§Only mean age and range reported; not in figure.