HHS Public Access

Author manuscript

Brain Res. Author manuscript; available in PMC 2020 December 15.

Published in final edited form as:

Brain Res. 2019 December 15; 1725: 146470. doi:10.1016/j.brainres.2019.146470.

"Human Brain Development through the Lens of Cerebral Organoid Models"

Madeline G. Andrews[1,2,*], **Tomasz J. Nowakowski**[3,4,5,*]

[1]Department of Neurology, University of California, San Francisco, CA, USA

[2]The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA, USA

[3]Department of Anatomy, University of California, San Francisco, CA, USA

[4]Department of Psychiatry, University of California, San Francisco, CA, USA

[5]Chan Zuckerberg Biohub, San Francisco, CA, USA

Abstract

The brain is one of the most complex organs in the body, which emerges from a relatively simple set of basic 'building blocks' during early development according to complex cellular and molecular events orchestrated through a set of inherited instructions. Innovations in stem cell technologies have enabled modelling of neural cells using two- and three- dimensional cultures. In particular, cerebral ('brain') organoids have taken the center stage of brain development models that have the potential for providing meaningful insight into human neurodevelopmental and neurological disorders. We review the current understanding of cellular events during human brain organogenesis, and the events occurring during cerebral organoid differentiation. We highlight the strengths and weaknesses of this experimental model system. In particular, we explain evidence that organoids can mimic many aspects of early neural development, including neural induction, patterning, and broad neurogenesis and gliogenesis programs, offering the opportunity to study genetic regulation of these processes in a human context. Several shortcomings of the current culture methods limit the utility of cerebral organoids to spontaneously give rise to many important cell types, and to model higher order features of tissue organization. We suggest that future studies aim to improve these features in order to make them better models for the study of laminar organization, circuit formation and how disruptions of these processes relate to disease.

Neural Development

Brain Region Specification

During early embryogenesis, a portion of the ectoderm becomes specified by the underlying mesoderm to become the neural plate ('neural induction'). The neural plate then invaginates

*Correspondence: Madeline.Andrews@ucsf.edu and Tomasz.Nowakowski@ucsf.edu.

and closes forming the neural tube. At the midline, and at the edges of the neural plate, organizing centers form and begin to secrete morphogens that induce the expression of brain-region specific transcription factors in the neuroepithelial stem cells. As a consequence, the emerging neural tissue becomes broadly divided into the following domains along the rostro-caudal axis: forebrain (or prosencephalon), which consists of telencephalon and diencephalon, midbrain (or mesencephalon), hindbrain (rhombencephalon; containing the metencephalon and myelencephalon) and spinal cord, respectively (Figure 1). Neural domains are progressively restricted by expression of transcription factors where a border of morphogens, Wnt1 and Fgf8, divide the midbrain and hindbrain to induce expression of Otx2 rostral to or En caudal to that border. Activity of these signals induces the expression of forebrain marker Foxgl and Six3, the Midbrain markers Irx3 and En1/2, and the hindbrain Gbx2 and Hoxb genes (Lupo et al., 2014). Although these regions develop adjacent to one another, they give rise to anatomically and functionally distinct regions in the adult brain. The telencephalon develops into the cerebral cortex, basal ganglia, hippocampus and amygdala, the diencephalon contains the hypothalamus and thalamus, the mesencephalon connects adjacent rostral and caudal regions together and contains the tectum and cerebral aqueduct, and the rhombencephalon includes the cerebellum, medulla and pons. Different cell types reside within each of these structures and have distinct developmental trajectories to form the appropriate synaptic connections and to support specific functional activities. In order to understand the processes that result in the final complexity of the human brain we need accurate models of human brain development.

Model Systems to Study Brain Development

Animal model systems have been an essential tool in establishing our understanding of neural induction and the processes that regulate formation of different brain structures. Classic experiments using *Xenopus* embryos established the role of organizers in body axis and neuroectoderm formation and the role of BMP signaling inhibition in neural induction (Spemann and Mangold, 1924). Drosophila have been a key genetic model system to decode rostral-caudal axis formation and body plan initiation by the Hox genes (Hales et al., 2015; Nüsslein-Volhard and Wieschaus, 1980). The developmental lineage of the entire nervous system has been traced in the nematode, *Caenorhabditis elegans,* making it an excellent model to evaluate the role of specific neurons and circuits and their functional output (Brenner, 1974; Hobert, 2016; Singhvi and Shaham, 2019). Zebrafish (*Danio rerio*) due their embryonic transparency, rapid development and embryogenesis outside of the mother, are also a good model system to evaluate developmental processes (Dooley and Zon, 2000; Kizil et al., 2012). Embryonic chickens (*Gallus gallus*) are a particularly tractable system because development can be observed *in ovo*, genetic modifications introduced during early neurogenesis and then consequences evaluated after an extended period (Davey et al., 2018; Pourquié, 2018).

However, in order to approach mammalian neural development, mouse models have become the gold standard model system, in part, due to the availability of excellent genetic tools. The ability to delete specific genes either constitutively or conditionally in particular tissues, or cell types and observe their requirement for molecular development has been a

remarkable advance (Friedel et al., 2011; Skarnes et al., 2011). We now have defined many of the relevant cell types, processes that regulate their developmental trajectory and key features of neural development in great level of detail (Foong, 2016; Guido, 2018; Seabrook et al., 2017). Although all of the described models have been key in establishing our understanding of how the nervous system develops and functions, features that have been challenging to approach are human-specific development and insights into neurological disease. While the mouse has been a key tool for neuroscientific discovery, there are major differences between the mouse and human brain. The size, number of cells and cell type composition are unique in the human. In particular regions, like the cerebral cortex, the level of expansion is dramatically different (Florio and Huttner, 2014; Lui et al., 2011; Rakic, 2009). Mouse models, despite their robustness in providing genetic and molecular mechanisms underlying neurodevelopmental processes, are not always reliable models when approaching disease. Although many therapeutics have excellent results in mice, there is little predictive capacity of how well the treatment will do in humans (Denayer et al., 2014; Mak et al., 2014; Young et al., 2011). Many have asserted that in order to study the human brain and approach human disease, it is important to study human cells behaving in an accurate context.

Modelling brain development using cerebral organoids

Organoids are stem-cell derived three-dimensional suspension cultures that resemble features of organ development. Pioneering work from the labs of Yoshiki Sasai (Eiraku et al., 2008; Kadoshima et al., 2013) and Juergen Knoblich (Lancaster et al., 2013) innovated what we now refer to as the three-dimensional cerebral (or brain) organoid (or spheroid) by demonstrating the capacity of stem cells to self-organize and resemble structural features of the developing human brain. Brain organoids have provided an experimentally tractable system to study development and disease of the human nervous system. To differentiate brain organoids, adherent human pluripotent cells, such as embryonic or induced pluripotent stem cells, are placed in suspension and exposed to media components that promote neural induction. After aggregation, cell clusters are placed in larger volumes of media with or without matrigel embedding. Later, after a few weeks of static culture, organoids are often cultured with agitation through use of orbital shakers or spinning bioreactors (Qian et al., 2016). These methods promote oxygen diffusion to the center of the organoid and better perfusion of media. Alternatively, incubators containing higher concentrations of oxygen have also been used to achieve this goal. Organoids are routinely kept in suspension for many months and can be collected over various time points to evaluate neurodevelopmental processes and model neural disease.

There are two broad categories of brain organoid protocols, undirected whole brain organoids (Lancaster et al., 2013), or directed regional brain organoids (Kadoshima et al., 2013). Whole brain organoid protocols rely on the intrinsic signaling potential of the cells within the culture resulting in a variety of brain regions and CNS structures (Camp et al., 2015; Lancaster et al., 2013; Quadrato et al., 2017). Whole brain organoids do not contain all brain regions, but rather have the potential to develop into multiple neural structures. As the exact sequence and timing of signals required for a given developmental process are unknown, this method provides the potential to observe patterning of adjacent neural

structures, cell-cell interactions throughout different brain regions, characterization of cellular morphologies and synapse formation. However, the composition of whole brain organoids are inconsistent across experiments making the study of biologically relevant phenomenon technically difficult. Whole brain organoids, which often vary in size and contain a variety of cell types with different regional identities in mixed organization, prompted interest in using directed differentiation protocols to pattern particular neural structures.

Directed differentiation protocols utilize small molecules to promote neural induction, typically through inhibition of the BMP/TGFB signaling pathways, and through manipulation of other relevant signaling pathways to produce organoids that resemble different neural structures (Figure 1). Brain organoids have now been patterned using additional factors to resemble distinct brain regions including the cerebral cortex (Kadoshima et al., 2013; Qian et al., 2016), hypothalamus (Qian et al., 2016), hippocampus (Sakaguchi et al., 2015), thalamus (Xiang et al., 2019), cerebellum (Muguruma et al., 2015) and ganglionic eminences (Bagley et al., 2017; Birey et al., 2017; Xiang et al., 2017).

While modeling of individual brain regions using organoids may reveal region-specific developmental processes, many questions regarding synapse formation, neuronal circuitry and electrophysiological activity may not be best suited for organoids resembling only one region. In addition to the use of whole brain organoids for these questions, another method to study the interactions between brain regions is to differentiate organoids with different areal identities and then culture together, forming the co-called “assembloids”. Recently, several groups differentiated dorsal and ventral forebrain organoids or spheroids and then fused together after initial patterning (Bagley et al., 2017; Birey et al., 2017; Xiang et al., 2017). Cellular migration was observed from ventral to dorsal, as *in vivo*, and connections made between aggregates with different cellular and regional identities. Similarly, cortical and thalamic organoids were directed separately and fused together to model corticothalamic interactions (Xiang et al., 2019), which are vital for the regulation of sensory processing. Axonal projections were observed in cellular-appropriate domains and reciprocal connections made across the fused organoids suggesting that physiologically relevant connectivity patterns may one day be modelled using these approaches.

The various approaches to organoid culture have begun to provide tractable experimental tools to study relevant processes of human brain development, but not without limitations. There is a tradeoff between reproducibility across experiments and artificiality of the introduced signaling mechanisms into the culture system. As such, the scientific question should dictate which type of culture system is best suited for a particular set of experiments. Questions on patterning and regional and topographical organization may be best suited for whole brain undirected organoids where intrinsic inductive properties can be observed. Whereas questions on cell fate specification, differentiation programs and lineage trajectory analyses within a particular CNS region will likely be better modeled with directed differentiation protocols. Questions regarding cellular interactions across areas like axon guidance and synapse formation may be best suited for fused organoid culture. However, many different cellular processes may be dysregulated in disease and thus taking a multifaceted approach to organoid culture by using different types of culture systems, in

addition to in vivo model systems, may be the most rigorous method to approach a complex scientific question. In the next sections, we will review the current understanding of some of the critical developmental processes that give rise to cerebral cortex, and comment on how well these processes are recapitulated in cerebral organoids.

Neurogenesis

Excitatory neurons (ENs) of the cerebral cortex are generated only during prenatal development. In humans, neurogenesis is restricted to a time-window during midgestation (Rakic and Sidman, 1968). Despite some regional and temporal differences, the basic developmental principles of neurogenesis are conserved throughout the cortex (Bystron et al., 2008). First, radial glia, a class of cortical stem cells, reside within the ventricular zone (lining the lateral ventricle) where they undergo cell division (proliferation). Their daughter cells migrate away from the ventricle towards the cortical plate where they give rise to the differentiated neurons (Miyata et al., 2001; Noctor et al., 2001). Intermediate progenitor cells are derived from radial glia and are fate-restricted transit amplifying cells that contribute to neurogenesis via symmetric cell divisions (Haubensak et al., 2004; Noctor et al., 2004). Subsequent to the neurogenic phase, progenitor cells contribute to gliogenesis where they produce astrocytes and oligodendrocytes (deAzevedo et al., 2003; Rash et al., 2019). Although the sequence of developmental events and the general contributing populations have been identified in the mouse, many of these processes have not been characterized in a region and cell-type specific context, especially in the human. A better understanding of these events will not only provide information at a molecular scale, but may also contribute broader insight into brain structure, function and behavior and the contribution of abnormal development in disease. For the purposes of this review we focus predominantly on neurogenesis in the cerebral cortex because of its importance in human cognitive function, the particular complexity of human cortical disease, and because it has been the subject of many organoid studies.

Cortical Cell Types

The cerebral cortex develops from cortical radial glia, which undergo an initial expansion from neuroepithelial stem cells (Misson et al., 1988). Three main subtypes of radial glia, each with a distinct morphology and transcriptional identity, have been described. Cell bodies of the ventricular radial glia (vRGs) closely line the surface of the lateral ventricle, form apical end-feet that directly contact the ventricle, and establish a long ‘basal fiber’ that extends to the pial surface (Noctor et al., 2001; Rakic, 1972). In rodents, vRGs are the main population of neural stem cells, which differentiate first into the deep layer cortical neurons and later upper layer neurons, before giving rise to glial populations, including astrocytes and oligodendrocytes.

In the human cortex neuroepithelial stem cells give rise first to vRGs (Subramanian et al., 2017), which later differentiate into outer radial glia cells (oRGs, also known as basal radial glia)(Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011; Smart et al., 2002). The oRGs are morphologically distinct, retain the basal fiber, but lose apical contact, and their cell bodies translocate into the outer subventricular zone (oSVZ). The oRGs can be characterized by a distinct transcriptional signature compared with vRG cells. They also

divide in a unique manner, called mitotic somal translocation (MST) where cells jump prior to division (Hansen et al., 2010). A third population of radial glia, called truncated radial glia (tRGs), develops later in neurogenesis, around gestational week (GW) 16 (Nowakowski et al., 2016b). Cell bodies of tRG cells reside near the ventricular surface and possess basal processes that do not reach the pial surface and are, therefore, “truncated”. All populations of radial glia give rise to transient amplifying neurogenic intermediate progenitor cells (IPCs) that contribute to increased output of cortical neurons from radial glia (Hansen et al., 2010). During early neurogenesis, at the beginning of the second trimester, the vRGs and IPCs give rise to the neurons which are present in the deep layers. In contrast, oRG cells emerge later in development, give rise to later-born IPCs, and differentiate into upper layer neurons. The six cortical laminae are comprised of multiple molecularly-defined subtypes of ENs. These cells connect intracortically to regulate synaptic activity inside the cortex, as well as subcortically to provide executive regulation of sensory and motor activity (Greig et al., 2013; Molyneaux et al., 2007).

To balance the input and output of circuit activity the inhibitory interneurons (INs), which are generated in the ventral forebrain (Ganglionic Eminences), migrate tangentially towards the cortex (Anderson et al., 1997; de Carlos et al., 1996). Although these cells are born outside of the cortex, they play a vital role in cortical development and function. Recent work on the third trimester and newborns, identified interneurons generated late in development from a presumptive source of these cells in the arch (Paredes et al., 2016), although the precise developmental origin and function of that population is unknown. Similarly, several studies have suggested that interneurons may also be generated locally in the dorsal telencephalon during neurogenesis. However, evidence for such population is limited, and has been discussed in several recent reviews (Al-Jaberi et al., 2015; Clowry, 2015; Hansen et al., 2013; Letinic et al., 2002; Zhong et al., 2018).

At the end of neurogenesis, radial glia produce astrocytes and oligodendrocytes (deAzevedo et al., 2003; Rash et al., 2019). The oRGs, which express some of the same molecular markers of astrocytes, produce a variety of astrocyte subtypes including protoplasmic and fibrous which play vital roles in synaptic formation, syapse pruning and circuit function. Additionally, oRGs may give rise to oligodendrocyte precursor cells (OPCs)(Rash et al., 2019), which mature into myelinating oligodendrocytes. Oligodendrocytes myelinate neuronal axons and are vital for action potential transduction and synaptic communication (Barateiro et al., 2016; Goldman and Kuypers, 2015).

Another population of glial cells are microglia. Although microglia are categorized under the same cellular class, they are actually non-neural in lineage. Microglia are the resident macrophages of the central nervous system that migrate into the developing brain from the yolk sac (Alliot et al., 1999) where they are believed to engulf dysfunctional synapses and infectious agents (Paolicelli et al., 2011). A variety of cell types associated with the nascent vasculature have also been identified and may play a vital role in normal blood brain barrier formation (Bautch and James, 2009). In particular, these cells likely support appropriate nutrient supply and immune isolation from the rest of the body. Interplay between neural and non-neural cell types is likely necessary for normal development and organization of the

cerebral cortex (Javaherian and Kriegstein, 2009; Stubbs et al., 2009), although it is rarely studied.

Cortical Cells in the Organoid

After neural induction, neural stem cells rapidly proliferate, organize into rosettes that later expand into germinal zone-like structures, and express markers of cycling neuroepithelial stem cells. In the subsequent weeks, different neurogenic progenitor populations arise (Figure 2). In cortical organoids, both vRG-like and oRG-like cells emerge emerge around week 8 of culture (Bershteyn et al., 2017). Even though the organoid cultures lack a pial surface, limited studies visualizing radial glia cell morphology and behavior suggest that these cell types nonetheless establish long fiber processes that do not retract through cell division. Organoid-derived oRGs express markers of this population, such as HOPX, and they exhibit functional behaviors, like MST divisions (Bershteyn et al., 2017). The transit amplifying progenitors, the IPCs, are also present at this time. Notably, however, organoids contain only about one-fifth the number of IPCs and they do not express the full complement of genes observed in the endogenous developing brain (Camp et al., 2015; Pollen et al., 2019).

Landmark studies of *in vitro* differentiation have suggested that iPS-derived neural stem cells sequentially generate first deep then upper layer neurons, and it has been suggested that the emergence of the major cell types may roughly follow *in vivo* timetable (Espuny-Camacho et al., 2013; Kadoshima et al., 2013; Shi et al., 2012). As brain organoids are composed of human neural cells, the culture and developmental processes occur on a human time-scale, which means they divide over the course of days, proliferate for many weeks and differentiate over the course of months. This property of *in vitro* organoids, combined with the possibility to control human genetic background, suggests that organoids could serve as one of the models for elucidating the consequences of disease insults (genetic or environmental) on human brain development. As progenitor cells undergo division, they begin to differentiate into neurons which express markers of deep layer identity, such as BCL11B, and the organoid emulates the cortical ‘inside out’ developmental lineage trajectory. After the birth of deep layer neurons, the remaining RGs and IPCs give rise to neurons that express markers of the superficial layers, like SATB2. Recent studies have begun to address similarities between organoid cells and their *in vivo* counterparts (Amiri et al., 2018; Camp et al., 2015; Pollen et al., 2019; Sloan et al., 2017; Xiang et al., 2019). Single cell RNA sequencing (sc-seq) approaches have provided evidence that organoid cells have broad similarity to their *in vivo* counterparts, but also that remarkable differences in gene expression persist. Overall, although organoid cell types can be viewed as similar to primary cells (Camp et al., 2015), transcriptome-wide comparisons reveal that only 70-80% gene expression levels correlate when organoid and primary cell types are compared (Pollen et al., 2019; Velasco et al., 2019). Additionally, many of these comparisons cannot be confidently performed at the level of neuronal subtypes due to the lack of subtype specification of *in vitro* cell types. In addition to transcriptomic comparisons, more studies are needed to validate the morphological and electrophysiological properties of organoid cells and directly compared to endogenous human cell types.

As the goal of cortical directed differentiation protocols is to reduce off target regions of the brain from being produced, the ventral-derived inhibitory INs should not emerge. However, a small number of INs are often detected using scRNAseq assays, although their origin is unclear. It is possible that some interneurons may be directed due to lack of stringency in dorsal differentiation protocol, or they could be olfactory bulb INs that are generated from dorsal telencephalic progenitors in late neurogenesis (Huilgol et al., 2013). However, the few that are present do not reflect the IN diversity observed in the cortex, making the organoid a difficult model to study the balance of excitatory and inhibitory activity. Other populations of dorsally-derived neurons, such as Cajal-Retzius cells (CRCs), which play a significant role in cortical organization and appropriate cellular signaling, are rarely observed in the organoid. Although, some protocols produce reelin expressing cells (Kadoshima et al., 2013), a marker of CRCs, single cell sequencing has yet to validate the presence of a bona fide Cajal-Retzius cells in the organoid.

The neurogenic to gliogenic transition is preserved in the organoid; after many months *in vitro,* gliogenesis occurs where astrocytes are observed to develop at a similar rate and express markers similar to during prenatal development (Sloan et al., 2017). However, due to the long duration of these experiments, which range from several months to years, organoid studies focused on astrocyte development are labor intensive and technically challenging. Oligodendrocyte precursor cells (OPCs) and oligodendrocytes are rarely observed in typical cortical organoid and spheroid differentiation protocols. However, protocol modifications can be made to support the induction of these important glial cells, whereby they assume a typical cellular morphology and begin to myelinate neuronal axons (Marton et al., 2019). The other major glial subtype, microglia, the immune cells of the nervous system, are completely absent since they are non-neural in lineage. Microglia are incredibly important for the regulation of the healthy nervous system; they act as macrophages phagocytosing infectious microorganisms, pruning redundant synapses and regulating inflammation. Dysregulation of microglia in the aged brain is implicated in many neurodegenerative diseases, including Alzheimer’s and Parkinson’s disease (Hansen et al., 2018; Keren-Shaul et al., 2017; Liu et al., 2019), and without these cells these diseases cannot be accurately modeled.

One significant component absent from brain organoids is vasculature. Although the inadvertent specification of a small number of endothelial cells can be identified through scRNA-seq (Pollen et al., 2019), their affect appears negligible compared the drastic impact vasculature has on the developing brain. By midgestation the cortex is completely innervated by blood vessels, supplying nutrients and oxygen and known to play crucial roles in metabolism and neurogenesis. For example, IPCs associate with blood vessels during cortical development, dividing near blood vessel branch points suggesting that endothelial cells may actively contribute to the neural stem cell niche (Javaherian and Kriegstein, 2009). The absence of endothelial cells may not only contribute to secondary issues like lack of oxygen diffusion but may directly impact neurogenesis in the organoid model. Since the IPC population in the organoid is lower in proportion and transcriptionally inconsistent as compared to primary IPCs, the lack of vasculature may be a significant contributing factor.

Tissue Organization

Cytoarchitecture

The human cerebral cortex is highlighly organized; it not only consists of a variety of cell types, but has diverse cellular interactions that dynamically change over time. Since lissencephalic animal models do not have the same expanded pool of progenitors and number of neurons in the cortical plate as in the human, cerebral organoid cultures may offer the opportunity to study the cellular and molecular correlates of higher order normal developmental processes such as lamination and folding. In cortical organoids progenitors form ventricular zone-like structures in the shape of rosettes within the larger organoid space. Typically, multiple adjacent progenitor rosettes are present within an individual organoid (Figure 2). These VZ-like structures create a radial scaffold, where vRG cells proliferate along the ‘ventricular’ edge (Lancaster et al., 2013). As RG cells give rise to IPCs and early born deep layer neurons, they migrate away from the VZ to form an intermediate zone which encircles the proliferating cells. As the diversity of RG subtypes increases, oRG cells are observed within the rosette which expands to contain an outer subventricular zone (oSVZ)-like space.

Neurons generated from RG-like cells in organoids differentiate and are typically positioned exterior to the rosettes populating the space around the progenitor zones. As development proceeds, increased diversity in neuronal type are observed, first with deep layer neurons and several weeks later, with upper layer neurons. The ratio of neurons increases over progenitors and intermixed populations of neurons from both the deep and upper layers fill the organoid.

Topography

The adult human cortex is defined by dozens of discrete anatomical and functional areas, each controlling complex behavioral output like motor, sensory, visual, and executive functions. However, it is currently unclear when areal differences in cell types and their corresponding connections arise. In the developing human cortex there is evidence of arealization in neuronal populations across the rostral-caudal axis during neurogenesis, where prefrontal cortex (PFC) ENs co-express markers of subcortically and intracortically projecting neurons, while during the same period, neurons in the visual cortex (V1) do not (Nowakowski et al., 2017). The mechanisms driving the molecular differences in areal signatures are not currently well understood. Both secreted molecules and gene signatures drive appropriate areal identity. Signaling molecules, like Wnt7b have been established to play a role, since it regulates neuronal class selection in the caudal cortex (Ozair et al., 2018).

Similar to the manner in which the cellular domains resemble, but do not recapitulate endogenous structure, there are major limitations regarding axial topography in organoids. Whole-brain organoids have the potential to contain many brain regions that emerge either spontaneously or in response to intrinsically-defined organizers. These regional structures can self pattern in appropriate order along the rostral-caudal axis (Lancaster et al., 2013). The use of undirected cerebral organoids, while inconsistent across differentiations, may be

a valuable tool to illuminate the intrinsic mechanisms defining regional identities including gene expression, timing of area formation, and signaling mechanisms.

An alternative method is to impose an organizer (or organizers) within the organoid using either a cluster of cells or bead that secretes a patterning protein. Rather than small molecules bathing the organoid in the media, an organizer creates an endogenous-like morphogen gradient to produce cells with a specific regional identity based on concentration of exposure. Sonic Hedgehog (Shh) secreting iPSCs were embedded at one pole of forebrain-directed spheroids (Cederquist et al., 2019) and adjacent regions were ventralized. As the distance from the organizer increased, and presumably the signal became less concentrated, the cells became correspondingly more dorsal. Culture of mouse embryonic stem cells embedded with cells expressing markers of rostral or caudal genes, like Six3 and Irx3, were also sufficient to organize the axis of the culture (Takata et al., 2017). This method demonstrated increased topographical organization, and a platform to understand interactions between the dorsal/ventral or rostral/caudal cortex in a more endogenous-like system. However, it also highlights the need for a better understanding of the signaling mechanisms that guide early human neurogenesis. Other organizers of the forebrain may rely upon Wnt and BMP signaling activities and modeling the detailed effects of these morphogens on patterning activity, cellular identity and cell-specific difference across cortical areas will be key to improving organoid topography.

Layering

The human cortex is characterized by the development of distinct neuronal populations born in an ‘inside out’ manner to form stratified laminae . In the adult cortex, there are six cytoarchitectonically distinct layers of cortical neurons, designated layer I - VI, where I-IV are the superficial layers and V-VI are the deep layers. In the organoid, despite the progenitor zones resembling aspects of VZ expansion and the ability of differentiating neurons to migrate along the radial fibers, the resulting laminar organization is not conserved. Although the temporal hierarchy is preserved where deep layers are born prior to upper layer neurons, the inside-out organization is not. Neurons with different layer identities are typically intermixed throughout the organoid. Likely due to the multiple numbers of rosettes and the orientation by which neurons differentiate from the progenitors, the laminar organization of the human cortex is not typically well preserved in the organoid model, although some protocols report aspects of laminar-like organization (Qian et al., 2016). Because of the lack of robust and consistent laminar organization, other properties of neuronal development, including axon guidance, synaptogenesis and circuit formation are consequently difficult to study. Interestingly, molecular changes observed in recent sequencing studies of Autism Spectrum Disorder brain tissue suggested that certain layers of the cortex may be differentially affected in the disorder (Parikshak et al., 2013; Velmeshev et al., 2019; Willsey et al., 2013). Identifying methods to more faithfully recapitulate lamination in organoids may be needed to gain meaningful inroads into the disorder using organoid models.

In addition to organoid cytoarchitectural dynamics that may limit the ability of cells to make uniform cortical layers, there is also an absence of layer I Cajal Retzius cells, which

normally reside along the pial surface of the developing neocortex. Cajal Retzius express Reelin, which is necessary for appropriate laminar organization during development. Loss of Reelin function results in disorganized laminar formation in the developing mouse cortex (Ogawa et al., 1995). Additionally, in the organoid there is lack of subcortical input. Reciprocal interactions between the cortex and thalamus are important for appropriate laminar organization, which may also contribute. Notably, corticothalamic fusion organoids did not report increased laminar organization, suggesting that this is likely a more pervasive problem, which may have multiple sources.

Folding

Mechanisms that drive cortical folding *in vivo* continue to be elucidated (Sun and Hevner, 2014). Only a few studies in mouse have profoundly altered neuroepithelial organization and folding. These include genetic mutations that alter patterns of apoptotic cell death (Oppenheim et al., 2001), progenitor cell self-renewal (Chenn and Walsh, 2002; Florio et al., 2015; Matsumoto et al., 2017; Rash et al., 2011; Stahl et al., 2013; Wang et al., 2016), and neuronal migration (Del Toro et al., 2017). Unlike the human cortex, which begins to fold around midgestation and results in many gyri and sulci, cortical organoids do not normally fold, no matter how long they are maintained in culture. One advantage of studying human cells is to gain insight into disease, however organoids, like rodent models, are naturally lissencephalic. The lack of proper cellular expansion, likely both at the progenitor and neuronal level, in the organoid does not allow for appropriate biophysical cellular properties that result in folding. In order to study aspects of cortical folding, different approaches to organoid modification have been taken. Organoids have now been cultured under space constraints, on a chip, to better understand the cellular processes that occur as tissue wrinkles and allow for long-term observations of cell behaviors over time (Karzbrun et al., 2018). Appropriate cytoskeletal contraction and nuclear expansion during cell cycle were required at different locations in the organoid for folding to occur. Folding can also occur in suspension organoids after genetic manipulation. PTEN mutant organoids have a prolonged period of proliferation resulting in increased surface area and convolution at the organoid surface (Li et al., 2017). While these studies highlight methods to induce folding in the organoid model, they do not necessarily clarify the developmental mechanisms responsible for cortical folding in normal human development. Importantly, even in the absence of a full understanding of cortical folding and a complete *in vitro* model, studies investigating individual cell types carrying mutations affecting brain folding in human patients may provide inroads into the cellular and molecular correlates of these processes (Bershteyn et al., 2017).

Expansion

A prominent feature of the human brain is the astonishing expansion of the cerebral cortex. Although the mechanisms underlying human brain expansion have not been fully resolved, the increase in both the numbers of and types of progenitor cells, as well as their differentiation into a larger variety of neuronal types, likely contributes. Human-specific features are a common rationale for utilizing organoids rather than lissencephalic model species, such as the mouse, however, the scale of cortical expansion is markedly decreased in the organoid. Due to the limitations in organoid size, likely because of lack of vasculature

and ability of nutrients to permeate into the organoid, there is a maximum total organoid size of about 4-5mm (Paşca et al., 2015). Thus, progenitor zones cannot expand as *in vivo* to result in the dramatic expansion of the cortical plate. Although many of the appropriate populations of human cells, both progenitors and excitatory neurons, are present in the organoid, in the long-term, they do not reflect the same potential of the cortex to expand. Despite such differences, diseases resulting from inappropriate cortical expansion, like microcephaly, have been successfully modeled in the organoid system (Lancaster et al., 2013). However, the clear limitations of the organoid demonstrate the necessity for continued comparison to *in vivo* models from both gyrencephalic and lissencephalic species, to better understand the mechanisms necessary and sufficient for cortical expansion.

Circuitry

Studies of *in vitro* neural cultures ultimately seek to recapitulate normal circuits and serve as experimental platforms for understanding neural function in a reductionist model. This is especially important in cases where access to primary tissue or live organisms is difficult or impossible, or in cases where hundreds of conditions need to be compared in a systematic and highly controlled manner. Until recently, stem cell-derived neurons could be maintained in culture for some period of time sufficient for the neurons to develop robust synaptic activity (Kirwan et al., 2015; Shi et al., 2012). However, the mature properties of human neurons often take many months to develop (Linaro et al., 2019; Nicholas et al., 2013). Cerebral organoids develop three-dimensional organization of cells that supports longer culture duration and more endogenous-like cytoarchitectural structure, which may overcome some of the limitations of adherent cultures and support more elaborate cell-cell interactions to potentially extend the limit of neuronal maturation.

Recent studies have demonstrated the presence of electrically-active cells and synapses in organoid and spheroid models (Lancaster et al., 2013; Paşca et al., 2015; Quadrato et al., 2017; Watanabe et al., 2017). Using classic electrophysiological techniques, like whole cell patch clamping, it was demonstrated that organoid-derived neurons are electrically active and able to fire action potentials (Paşca et al., 2015). Importantly, all organoid-derived neurons assayed demonstrated similar electrical activity, which was ablated in response to tetrodotoxin, demonstrating bona fide synapses. These important studies demonstrate the capacity of organoid-derived neurons to signal to one another and their potential to be utilized for the modeling of neural circuit formation and function.

Other methods such as calcium imaging and extracellular recordings have also been utilized to assay electrophysiological potential (Lancaster et al., 2013; Quadrato et al., 2017; Trujillo et al., 2018). Whole brain organoids matured to eight months of age exhibit spontaneous electrical activity and retinal photoreceptors, present in these organoids, have evoked responses after exposure to light (Quadrato et al., 2017). While this suggests the ability of organoid cells to both sense stimuli and produce electrical activity as a response , not all organoids tested had these activity patterns due to the variability across the whole brain organoids . Although similar, the timepoint by which electrical activity can be observed is different across scientific groups and appears to depend on organoid differentiation protocol, cell type assessed, and technical method used to assess activity. In another study using a

multi-electrode array to evaluate organoids from two to eight months there was an increase in electrical activity over time including frequencing of firing, spike synchrony and firing rate. Additionally, firing patterns from older organoids appeared similar to that of a preterm neonatal EEG, suggesting an increase in electrophysiological activity, synchrony and circuit strengthening over time (Trujillo et al., 2018).

Although the detection of synaptic and electrophysiological activity is promising, it is unclear whether cell-cell interactions in the organoid recapitulate *in vivo* circuits. As the neuronal populations are disorganized, it is unclear whether the appropriate cell types are forming the correct connections and the robustness of synaptic connections is unclear. Because many neurological and neuropsychiatric disorders may involve changes in synaptic activity or connectivity (Bobilev et al., 2019; Cardozo et al., 2019; Lee et al., 2017; Skaper et al., 2017; Yang et al., 2017), it will be necessary to validate that organoids can accurately model these properties. More studies evaluating the maturation of organoid cells throughout developmental time and how they correspond to normal brain development are required.

In order to assess connections across regions, assembloids made from separately directed aggregates are fused and evaluated for cellular interactions between regions, synaptic formation, and establishment of early circuits. The first series of studies using a dual differentiation model took advantage of the differences between the dorsal and ventral cortex (Bagley et al., 2017; Birey et al., 2017; Xiang et al., 2017) to evaluate interactions between the dorsally-born ENs and the ventrally-derived INs. The appropriate balance of excitatory and inhibitory cells is essential for an equilibrium of electrophysiological activity. Dysregulation of this process has implications in many cortical diseases, such as various forms of epilepsy (Bonansco and Fuenzalida, 2016; Staley, 2015). The findings from dorsal-ventral fusion studies demonstrated not only appropriate migration of INs, but also that synaptic connections were made between INs and ENs. Although it remains unclear whether these interactions form the same types of connections or would serve to balance electrical activity as *in vivo,* assembloids may offer a more complete model of cortical development to evaluate circuitry. Thalamocortical circuits between the cortex and the thalamus are important not only for appropriate development of cell types in both of these regions, but also to regulate complex cognitive and behavioral functions, particularly processing of sensory information. Cells from corticothalamic fusion organoids demonstrated a similar capacity to migrate and form connections across organoids of different regional identities as the dorsal/ventral forebrain organoids (Xiang et al., 2019).

The organoid fusion experiments highlight the importance of studying cortical cells in the appropriate context. The cortex does not exist separately from the rest of the brain and if the greater goal is to model circuitry, there is a need to understand the connections between the cortex and other regions. However, in all organoid fusion experiments, directed brain organoids are differentiated separately and later fused together, but it is unclear if they are being harvested at the appropriate developmental time necessary to evoke the nature of endogenous regional interactions. Additionally, fusion models have been proposed to model circuit dynamics and how these circuits are impacted by disease, but it is currently unclear whether organoid cells and synapses can be matured enough to study such nuanced processes. While there is abundant promise of organoid potential, it is still unknown what

this group of studies collectively teaches us about physiological activity in the developing human brain and the accuracy of the organoids to model electrophysiological activity and circuit dynamics.

Organoid Applications

Disease

Importantly, organoids provide a window into human development and disease that was previously inaccessible. One major advantage of the organoid model system is that iPSC lines can be derived from patients with neurological disorders and then differentiated into neural cells to study the mechanisms of disease in the context of a human model. Several recent studies have utilized brain organoid models to better understand the etiology of neurodevelopmental disorders. To date, studies have used organoids to model lissencephaly (Bershteyn et al., 2017; Karzbrun et al., 2018), genetic microcephaly (Lancaster et al., 2013) and microcephaly as a result of Zika virus infection (Cugola et al., 2016; Nowakowski et al., 2016a; Qian et al., 2016; Watanabe et al., 2017), Timothy syndrome (Birey et al., 2017),Tuberous Sclerosis (Blair et al., 2018) and Autism (Amiri et al., 2018; Mariani et al., 2015).

As early developmental processes appear to be recapitulated most closely in the brain organoid when compared to the complexity of human brain function over time, neurodevelopmental disorders are probably best suited for study in this model system. However, disorders like Autism, while extremely relevant to neural development, may have subtle changes to laminar organization, columnar identity and circuitry which are not well represented in the organoid. Neuropsychiatric disorders, like schizophrenia, that have onset after adolescence and are characterized by behavioral and cognitive symptoms are difficult to study in the organoid. Additionally, although there is increased dopaminergic receptor expression and activity (Breier et al., 1998; Farde et al., 1990; Kegeles et al., 2000; Wong et al., 1986), there are no definitive disease biomarkers, making validation of a schizophrenic organoid model a particular challenge. It remains unclear what phenotypic abnormalities should be evaluated, as well as the relevance of particular observed differences to disease. Aging-related neurodegenerative disorders, like Alzheimer's and Parkison's diseases, are also a challenge to model in the organoid, as these diseases are related to onset after a prolonged period of time, post-development. Organoid maturation states most closely reflect fetal stages (Sloan et al., 2017) and there is little evidence of their ability to mirror postnatal life, or advanced age. Studying many disease-related processes are currently problematic as they require evaluation of aspects of neuronal maturation that are not well represented in the organoid.

Future improvements

Modifications to Cell Culture System—A major issue with the organoid model is the lack of vasculature. One approach to resolve the lack of vascular innervation is the transplantation of organoids into animal models. Recent studies reported that transplantation of a brain organoid into a mouse brain resulted in integration between the donor human cells and the mouse host (Daviaud et al., 2018; Mansour et al., 2018). Within the organoid,

apoptosis was substantially reduced, and the neurons integrated into mouse cortical circuits, with functional connections detected between transplanted human cells and mouse host. In addition, mouse endothelial cells were able to invade the organoid and provide vascular support to the neural tissue. An alternative method is to transplant endothelial cells into the organoid to mimic vascular development. A recent study transplanted umbilical vein endothelial cells into organoids and observed improvement in cortical laminae-like organization and electrophysiological activity (Shi et al., 2019).

Another approach is to culture organoids that have been sliced and cultured at the air liquid interface (Giandomenico et al., 2019). This method rescues the necrosis observed in the core, encourages axonal growth and improves bundle fasciculation. Additionally, cellular maturation is improved, there are fewer reactive astrocytes, and more electrophysiological activity is observed compared to traditional suspension organoids. Promisingly, cells from sliced organoids cultured at the air liquid interface are able to form synaptic connections with co-cultured mouse spinal cord resulting in functional output similar to a corticospinal-like tract. However, neither the transplantation or sectioning of organoids rescue the organizational issues that are still endemic to organoid models. A lack of cytoarchitectural context remains, which is problematic for consistent modeling of neural development and disease.

Cellular Stress—One significant realization from several recently published single cell sequencing studies is that organoid cells have increased expression of genes associated with cellular stress, particularly associated with endoplasmic reticulum dysfunction (Amiri et al., 2018; Pollen et al., 2019; Xiang et al., 2019). These markers may indicate inherent issues with the organoid system that were not easily identifiable until scRNAseq analyses were performed on organoids and compared to developing human cells. As these networks have been independently identified by different scientific groups using various organoid protocols and stem cell lines, this appears to be a problem fundamental to the culturing of brain organoids. The underlying cause is undetermined but issues like lack of oxygen diffusion, necrosis at the organoid core, non-physiological levels of glucose in media composition, and lack of vasculature may all contribute.

Report Card Comparison—There are currently many different published methods for culturing brain organoids and spheroids depending on the regional identity required and question of interest. For modeling cortical neurons, several protocols now exist, although they have been applied mostly to only a handful of iPSC lines each. This lack of benchmarking makes choosing a protocol to follow a significant challenge. Identifying and sharing lines for comparing differentiations and new protocols, and using data driven approaches, such as single cell RNA sequencing, will ultimately help advance this field by providing unbiased benchmarking criteria for reproducibility and robustness of cell type interpretation.

In addition, a thorough comparison of protocols is required to establish not only a best practices of making organoids, but also to establish whether cell types across protocols are equivalent. It’s unclear whether we are currently comparing equivalent model systems or whether some more accurately represent endogenous development. Several groups are now

beginning to characterize their own organoid protocols at a transcriptomic level, as well as measure against data collected from other organoid culture systems (Pollen et al., 2019; Velasco et al., 2019; Yoon et al., 2019). Thus far it appears that the accuracy of cell type correspondence from these protocols are similar, although the reproducibility in making the same proportion of cell types and regional identity vary across protocols. However, these studies should be calibrated against our lack of understanding regarding normal human development. These studies and others have incorporated some comparison to gestational development into their analysis (Amiri et al., 2018; Camp et al., 2015; Pollen et al., 2019; Sloan et al., 2017), however the limited availability of developing human brain tissue has made high resolution comprehensive analysis throughout neurogenesis challenging. In order to determine the extent of organoid translational capacity more studies on postmortem human samples from different developmental stages are required. These studies will greatly inform how well the organoid recapitulates many molecular and cellular aspects of neural development, how well this system can model disease, and contribute insight into how the human brain develops.

Based on our current understanding, brain organoids are a valuable tool that are well utilized to study neural induction, neurogenesis, neural developmental trajectory, neurogenic to gliogenic transition and development of neural cell types. However, organoids lack appropriate topographical and cytoarchitectural organization, many cell types are absent, and there is no vasculature making questions regarding circuit formation and disease challenging to address. The organoid field is growing rapidly and these problems will need to be addressed in order to best utilize this model moving forward.

References

Al-Jaberi N, Lindsay S, Sarma S, Bayatti N, Clowry GJ, 2015 The early fetal development of human neocortical GABAergic interneurons. Cereb. Cortex 25, 631–645. [PubMed: 24047602]

Alliot F, Godin I, Pessac B, 1999 Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res. Dev. Brain Res 117, 145–152. [PubMed: 10567732]

Amiri A, Coppola G, Scuderi S, Wu F, Roychowdhury T, Liu F, Pochareddy S, Shin Y, Safi A, Song L, Zhu Y, Sousa AMM, PsychENCODE Consortium, Gerstein M, Crawford GE, Sestan N, Abyzov A, Vaccarino FM, 2018 Transcriptome and epigenome landscape of human cortical development modeled in organoids. Science 362 10.1126/science.aat6720

Anderson SA, Qiu M, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, Rubenstein JL, 1997 Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. Neuron 19, 27–37. [PubMed: 9247261]

Bagley JA, Reumann D, Bian S, Lévi-Strauss J, Knoblich JA, 2017 Fused cerebral organoids model interactions between brain regions. Nat. Methods 14, 743–751. [PubMed: 28504681]

Barateiro A, Brites D, Fernandes A, 2016 Oligodendrocyte Development and Myelination in Neurodevelopment: Molecular Mechanisms in Health and Disease. Curr. Pharm. Des 22, 656–679. [PubMed: 26635271]

Bautch VL, James JM, 2009 Neurovascular development: The beginning of a beautiful friendship. Cell Adh. Migr 3, 199–204. [PubMed: 19363295]

Bershteyn M, Nowakowski TJ, Pollen AA, Di Lullo E, Nene A, Wynshaw-Boris A, Kriegstein AR, 2017 Human iPSC-Derived Cerebral Organoids Model Cellular Features of Lissencephaly and Reveal Prolonged Mitosis of Outer Radial Glia. Cell Stem Cell 20, 435–449.e4. [PubMed: 28111201]

Birey F, Andersen J, Makinson CD, Islam S, Wei W, Huber N, Fan HC, Metzler KRC, Panagiotakos G, Thom N, O'Rourke NA, Steinmetz LM, Bernstein JA, Hallmayer J, Huguenard JR, Paşca SP, 2017

Assembly of functionally integrated human forebrain spheroids. Nature 545, 54–59. [PubMed: 28445465]

Blair JD, Hockemeyer D, Bateup HS, 2018 Genetically engineered human cortical spheroid models of tuberous sclerosis. Nat. Med 24, 1568–1578. [PubMed: 30127391]

Bobilev AM, Perez JM, Tamminga CA, 2019 Molecular alterations in the medial temporal lobe in schizophrenia. Schizophr. Res 10.1016/j.schres.2019.06.001

Bonansco C, Fuenzalida M, 2016 Plasticity of Hippocampal Excitatory-Inhibitory Balance: Missing the Synaptic Control in the Epileptic Brain. Neural Plast. 2016, 8607038. [PubMed: 27006834]

Breier A, Adler CM, Weisenfeld N, Su TP, Elman I, Picken L, Malhotra AK, Pickar D, 1998 Effects of NMDA antagonism on striatal dopamine release in healthy subjects: application of a novel PET approach. Synapse 29, 142–147. [PubMed: 9593104]

Brenner S, 1974 The genetics of Caenorhabditis elegans. Genetics 77, 71–94. [PubMed: 4366476]

Bystron I, Blakemore C, Rakic P, 2008 Development of the human cerebral cortex: Boulder Committee revisited. Nat. Rev. Neurosci 9, 110–122. [PubMed: 18209730]

Camp JG, Badsha F, Florio M, Kanton S, Gerber T, Wilsch-Bräuninger M, Lewitus E, Sykes A, Hevers W, Lancaster M, Knoblich JA, Lachmann R, Pääbo S, Huttner WB, Treutlein B, 2015 Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. Proc. Natl. Acad. Sci. U. S. A 112, 15672–15677. [PubMed: 26644564]

Cardozo PL, de Lima IBQ, Maciel EMA, Silva NC, Dobransky T, Ribeiro FM, 2019 Synaptic Elimination in Neurological Disorders. Curr. Neuropharmacol 10.2174/1570159X17666190603170511

Cederquist GY, Asciolla JJ, Tchieu J, Walsh RM, Cornacchia D, Resh MD, Studer L, 2019 Specification of positional identity in forebrain organoids. Nat. Biotechnol 37, 436–444. [PubMed: 30936566]

Chenn A, Walsh CA, 2002 Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science 297, 365–369. [PubMed: 12130776]

Clowry GJ, 2015 An enhanced role and expanded developmental origins for gamma-aminobutyric acidergic interneurons in the human cerebral cortex. J. Anat 227, 384–393. [PubMed: 24839870]

Cugola FR, Fernandes IR, Russo FB, Freitas BC, Dias JLM, Guimarães KP, Benazzato C, Almeida N, Pignatari GC, Romero S, Polonio CM, Cunha I, Freitas CL, Brandão WN, Rossato C, Andrade DG, Faria D. de P., Garcez AT, Buchpigel CA, Braconi CT, Mendes E, Sall AA, Zanotto, P.M. de A, Peron JPS, Muotri AR, Beltrão-Braga PCB, 2016 The Brazilian Zika virus strain causes birth defects in experimental models. Nature 534, 267–271. [PubMed: 27279226]

Davey MG, Balic A, Rainger J, Sang HM, McGrew MJ, 2018 Illuminating the chicken model through genetic modification. Int. J. Dev. Biol 62, 257–264. [PubMed: 29616734]

Daviaud N, Friedel RH, Zou H, 2018 Vascularization and Engraftment of Transplanted Human Cerebral Organoids in Mouse Cortex. eNeuro 5 10.1523/ENEURO.0219-18.2018

deAzevedo LC, Fallet C, Moura-Neto V, Daumas-Duport C, Hedin-Pereira C, Lent R, 2003 Cortical radial glial cells in human fetuses: depth-correlated transformation into astrocytes. J. Neurobiol 55, 288–298. [PubMed: 12717699]

de Carlos JA, López-Mascaraque L, Valverde F, 1996 Dynamics of cell migration from the lateral ganglionic eminence in the rat. J. Neurosci 16, 6146–6156. [PubMed: 8815897]

Del Toro D, Ruff T, Cederfjäll E, Villalba A, Seyit-Bremer G, Borrell V, Klein R, 2017 Regulation of Cerebral Cortex Folding by Controlling Neuronal Migration via FLRT Adhesion Molecules. Cell 169, 621–635.e16. [PubMed: 28475893]

Denayer T, Stöhr T, Van Roy M, 2014 Animal models in translational medicine: Validation and prediction. New Horizons in Translational Medicine 2, 5–11.

Dooley K, Zon LI, 2000 Zebrafish: a model system for the study of human disease. Curr. Opin. Genet. Dev 10, 252–256. [PubMed: 10826982]

Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, Wataya T, Nishiyama A, Muguruma K, Sasai Y, 2008 Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell 3, 519–532. [PubMed: 18983967]

Espuny-Camacho I, Michelsen KA, Gall D, Linaro D, Hasche A, Bonnefont J, Bali C, Orduz D, Bilheu A, Herpoel A, Lambert N, Gaspard N, Peron S, Schiffmann SN, Giugliano M, Gaillard A, Vanderhaeghen P, 2013 Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. Neuron 77, 440–456. [PubMed: 23395372]

Farde L, Wiesel FA, Stone-Elander S, Halldin C, Nordström AL, Hall H, Sedvall G, 1990 D2 dopamine receptors in neuroleptic-naive schizophrenic patients. A positron emission tomography study with [11C]raclopride. Arch. Gen. Psychiatry 47, 213–219. [PubMed: 1968328]

Fietz SA, Kelava I, Vogt J, Wilsch-Bräuninger M, Stenzel D, Fish JL, Corbeil D, Riehn A, Distler W, Nitsch R, Huttner WB, 2010 OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. Nat. Neurosci 13, 690–699. [PubMed: 20436478]

Florio M, Albert M, Taverna E, Namba T, Brandl H, Lewitus E, Haffner C, Sykes A, Wong FK, Peters J, Guhr E, Klemroth S, Prüfer K, Kelso J, Naumann R, Nüsslein I, Dahl A, Lachmann R, Pääbo S, Huttner WB, 2015 Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. Science 347, 1465–1470. [PubMed: 25721503]

Florio M, Huttner WB, 2014 Neural progenitors, neurogenesis and the evolution of the neocortex. Development 141,2182–2194. [PubMed: 24866113]

Foong JPP, 2016 Postnatal Development of the Mouse Enteric Nervous System. Adv. Exp. Med. Biol 891, 135–143. [PubMed: 27379641]

Friedel RH, Wurst W, Wefers B, Kühn R, 2011 Generating conditional knockout mice. Methods Mol. Biol 693, 205–231. [PubMed: 21080282]

Giandomenico SL, Mierau SB, Gibbons GM, Wenger LMD, Masullo L, Sit T, Sutcliffe M, Boulanger J, Tripodi M, Derivery E, Paulsen O, Lakatos A, Lancaster MA, 2019 Cerebral organoids at the air-liquid interface generate diverse nerve tracts with functional output. Nat. Neurosci 22, 669–679. [PubMed: 30886407]

Goldman SA, Kuypers NJ, 2015 How to make an oligodendrocyte. Development 142, 3983–3995. [PubMed: 26628089]

Greig LC, Woodworth MB, Galazo MJ, Padmanabhan H, Macklis JD, 2013 Molecular logic of neocortical projection neuron specification, development and diversity. Nat. Rev. Neurosci 14, 755–769. [PubMed: 24105342]

Guido W, 2018 Development, form, and function of the mouse visual thalamus. J. Neurophysiol 120, 211–225. [PubMed: 29641300]

Hales KG, Korey CA, Larracuente AM, Roberts DM, 2015 Genetics on the Fly: A Primer on the Drosophila Model System. Genetics 201, 815–842. [PubMed: 26564900]

Hansen DV, Hanson JE, Sheng M, 2018 Microglia in Alzheimer's disease. J. Cell Biol 217, 459–472. [PubMed: 29196460]

Hansen DV, Lui JH, Flandin P, Yoshikawa K, Rubenstein JL, Alvarez-Buylla A, Kriegstein AR, 2013 Non-epithelial stem cells and cortical interneuron production in the human ganglionic eminences. Nat. Neurosci 16, 1576–1587. [PubMed: 24097039]

Hansen DV, Lui JH, Parker PRL, Kriegstein AR, 2010 Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464, 554–561. [PubMed: 20154730]

Haubensak W, Attardo A, Denk W, Huttner WB, 2004 Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. Proc. Natl. Acad. Sci. U. S. A 101,3196–3201. [PubMed: 14963232]

Hobert O, 2016 A map of terminal regulators of neuronal identity in Caenorhabditis elegans. Wiley Interdiscip. Rev. Dev. Biol 5, 474–498. [PubMed: 27136279]

Huilgol D, Udin S, Shimogori T, Saha B, Roy A, Aizawa S, Hevner RF, Meyer G, Ohshima T, Pleasure SJ, Zhao Y, Tole S, 2013 Dual origins of the mammalian accessory olfactory bulb revealed by an evolutionarily conserved migratory stream. Nat. Neurosci 16, 157–165. [PubMed: 23292680]

Javaherian A, Kriegstein A, 2009 A stem cell niche for intermediate progenitor cells of the embryonic cortex. Cereb. Cortex 19 Suppl 1, i70–7. [PubMed: 19346271]

Kadoshima T, Sakaguchi H, Nakano T, Soen M, Ando S, Eiraku M, Sasai Y, 2013 Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. Proc. Natl. Acad. Sci. U. S. A 110, 20284–20289. [PubMed: 24277810]

Karzbrun E, Kshirsagar A, Cohen SR, Hanna JH, Reiner O, 2018 Human Brain Organoids on a Chip Reveal the Physics of Folding. Nat. Phys 14, 515–522. [PubMed: 29760764]

Kegeles LS, Abi-Dargham A, Zea-Ponce Y, Rodenhiser-Hill J, Mann JJ, Van Heertum RL, Cooper TB, Carlsson A, Laruelle M, 2000 Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. Biol. Psychiatry 48, 627–640. [PubMed: 11032974]

Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, Itzkovitz S, Colonna M, Schwartz M, Amit I, 2017 A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell 169, 1276–1290.e17. [PubMed: 28602351]

Kirwan P, Turner-Bridger B, Peter M, Momoh A, Arambepola D, Robinson HPC, Livesey FJ, 2015 Development and function of human cerebral cortex neural networks from pluripotent stem cells in vitro. Development 142, 3178–3187. [PubMed: 26395144]

Kizil C, Kaslin J, Kroehne V, Brand M, 2012 Adult neurogenesis and brain regeneration in zebrafish. Dev. Neurobiol 72, 429–461. [PubMed: 21595047]

Lancaster MA, Renner M, Martin C-A, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA, 2013 Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379. [PubMed: 23995685]

Lee JH, Kreitzer AC, Singer AC, Schiff ND, 2017 Illuminating Neural Circuits: From Molecules to MRI. J. Neurosci 37, 10817–10825. [PubMed: 29118210]

Letinic K, Zoncu R, Rakic P, 2002 Origin of GABAergic neurons in the human neocortex. Nature 417, 645–649. [PubMed: 12050665]

Linaro D, Vermaercke B, Iwata R, Ramaswamy A, Davis BA, Boubakar L, Libé-Philippot B, Bilheu A, De Bruyne L, Gall D, Conzelmann K, Bonin V, Vanderhaeghen P, 2019 Xenotransplanted human cortical neurons reveal species-specific development and functional integration into mouse visual circuits. bioRxiv. 10.1101/626218

Liu Z, Qiu A-W, Huang Y, Yang Y, Chen J-N, Gu T-T, Cao B-B, Qiu Y-H, Peng Y-P, 2019 IL-17A exacerbates neuroinflammation and neurodegeneration by activating microglia in rodent models of Parkinson's disease. Brain Behav. Immun 10.1016/j.bbi.2019.07.026

Li Y, Muffat J, Omer A, Bosch I, Lancaster MA, Sur M, Gehrke L, Knoblich JA, Jaenisch R, 2017 Induction of Expansion and Folding in Human Cerebral Organoids. Cell Stem Cell 20, 385–396.e3. [PubMed: 28041895]

Lui JH, Hansen DV, Kriegstein AR, 2011 Development and evolution of the human neocortex. Cell 146, 18–36. [PubMed: 21729779]

Lupo G, Bertacchi M, Carucci N, Augusti-Tocco G, Biagioni S, Cremisi F, 2014 From pluripotency to forebrain patterning: an in vitro journey astride embryonic stem cells. Cell. Mol. Life Sci 71, 2917–2930. [PubMed: 24643740]

Mak IW, Evaniew N, Ghert M, 2014 Lost in translation: animal models and clinical trials in cancer treatment. Am. J. Transl. Res 6, 114–118. [PubMed: 24489990]

Mansour AA, Gonçalves JT, Bloyd CW, Li H, Fernandes S, Quang D, Johnston S, Parylak SL, Jin X, Gage FH, 2018 An in vivo model of functional and vascularized human brain organoids. Nat. Biotechnol 36, 432–441. [PubMed: 29658944]

Mariani J, Coppola G, Zhang P, Abyzov A, Provini L, Tomasini L, Amenduni M, Szekely A, Palejev D, Wilson M, Gerstein M, Grigorenko EL, Chawarska K, Pelphrey KA, Howe JR, Vaccarino FM, 2015 FOXG1-Dependent Dysregulation of GABA/Glutamate Neuron Differentiation in Autism Spectrum Disorders. Cell 162, 375–390. [PubMed: 26186191]

Marton RM, Miura Y, Sloan SA, Li Q, Revah O, Levy RJ, Huguenard JR, Païca SP, 2019 Differentiation and maturation of oligodendrocytes in human three-dimensional neural cultures. Nat. Neurosci 22, 484–491. [PubMed: 30692691]

Matsumoto N, Shinmyo Y, Ichikawa Y, Kawasaki H, 2017 Gyrification of the cerebral cortex requires FGF signaling in the mammalian brain. Elife 6 10.7554/eLife.29285

Misson JP, Edwards MA, Yamamoto M, Caviness VS Jr, 1988 Identification of radial glial cells within the developing murine central nervous system: studies based upon a new immunohistochemical marker. Brain Res. Dev. Brain Res 44, 95–108. [PubMed: 3069243]

Miyata T, Kawaguchi A, Okano H, Ogawa M, 2001 Asymmetric inheritance of radial glial fibers by cortical neurons. Neuron 31, 727–741. [PubMed: 11567613]

Molyneaux BJ, Arlotta P, Menezes JRL, Macklis JD, 2007 Neuronal subtype specification in the cerebral cortex. Nat. Rev. Neurosci 8, 427–437. [PubMed: 17514196]

Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y, 2015 Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. Cell Rep. 10, 537–550. [PubMed: 25640179]

Nicholas CR, Chen J, Tang Y, Southwell DG, Chalmers N, Vogt D, Arnold CM, Chen Y-JJ, Stanley EG, Elefanty AG, Sasai Y, Alvarez-Buylla A, Rubenstein JLR, Kriegstein AR, 2013 Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. Cell Stem Cell 12, 573–586. [PubMed: 23642366]

Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR, 2001 Neurons derived from radial glial cells establish radial units in neocortex. Nature 409, 714–720. [PubMed: 11217860]

Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR, 2004 Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat. Neurosci 7, 136–144. [PubMed: 14703572]

Nowakowski TJ, Bhaduri A, Pollen AA, Alvarado B, Mostajo-Radji MA, Di Lullo E, Haeussler M, Sandoval-Espinosa C, Liu SJ, Velmeshev D, Ounadjela JR, Shuga J, Wang X, Lim DA, West JA, Leyrat AA, Kent WJ, Kriegstein AR, 2017 Spatiotemporal gene expression trajectories reveal developmental hierarchies of the human cortex. Science 358, 1318–1323. [PubMed: 29217575]

Nowakowski TJ, Pollen AA, Di Lullo E, Sandoval-Espinosa C, Bershteyn M, Kriegstein AR, 2016a Expression Analysis Highlights AXL as a Candidate Zika Virus Entry Receptor in Neural Stem Cells. Cell Stem Cell 18, 591–596. [PubMed: 27038591]

Nowakowski TJ, Pollen AA, Sandoval-Espinosa C, Kriegstein AR, 2016b Transformation of the Radial Glia Scaffold Demarcates Two Stages of Human Cerebral Cortex Development. Neuron 91, 1219–1227. [PubMed: 27657449]

Nüsslein-Volhard C, Wieschaus E, 1980 Mutations affecting segment number and polarity in Drosophila. Nature. 10.1038/287795a0

Ogawa M, Miyata T, Nakajima K, Yagyu K, Seike M, Ikenaka K, Yamamoto H, Mikoshiba K, 1995 The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. Neuron 14, 899–912. [PubMed: 7748558]

Oppenheim RW, Flavell RA, Vinsant S, Prevette D, Kuan CY, Rakic P, 2001 Programmed cell death of developing mammalian neurons after genetic deletion of caspases. J. Neurosci 21,4752–4760. [PubMed: 11425902]

Ozair MZ, Kirst C, van den Berg BL, Ruzo A, Rito T, Brivanlou AH, 2018 hPSC Modeling Reveals that Fate Selection of Cortical Deep Projection Neurons Occurs in the Subplate. Cell Stem Cell 23, 60–73.e6. [PubMed: 29937203]

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT, 2011 Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456–1458. [PubMed: 21778362]

Paredes MF, James D, Gil-Perotin S, Kim H, Cotter JA, Ng C, Sandoval K, Rowitch DH, Xu D, McQuillen PS, Garcia-Verdugo J-M, Huang EJ, Alvarez-Buylla A, 2016 Extensive migration of young neurons into the infant human frontal lobe. Science 354 10.1126/science.aaf7073

Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, Horvath S, Geschwind DH, 2013 Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. Cell 155, 1008–1021. [PubMed: 24267887]

Paşca AM, Sloan SA, Clarke LE, Tian Y, Makinson CD, Huber N, Kim CH, Park J-Y, O'Rourke NA, Nguyen KD, Smith SJ, Huguenard JR, Geschwind DH, Barres BA, Paşca SP, 2015 Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. Nat. Methods 12, 671–678. [PubMed: 26005811]

Pollen AA, Bhaduri A, Andrews MG, Nowakowski TJ, Meyerson OS, Mostajo-Radji MA, Di Lullo E, Alvarado B, Bedolli M, Dougherty ML, Fiddes IT, Kronenberg ZN, Shuga J, Leyrat AA, West JA, Bershteyn M, Lowe CB, Pavlovic BJ, Salama SR, Haussler D, Eichler EE, Kriegstein AR, 2019

Establishing Cerebral Organoids as Models of Human-Specific Brain Evolution. Cell 176, 743–756.e17. [PubMed: 30735633]

Pourquié O, 2018 Somite formation in the chicken embryo. Int. J. Dev. Biol 62, 57–62. [PubMed: 29616740]

Qian X, Nguyen HN, Song MM, Hadiono C, Ogden SC, Hammack C, Yao B, Hamersky GR, Jacob F, Zhong C, Yoon K-J, Jeang W, Lin L, Li Y, Thakor J, Berg DA, Zhang C, Kang E, Chickering M, Nauen D, Ho C-Y, Wen Z, Christian KM, Shi P-Y, Maher BJ, Wu H, Jin P, Tang H, Song H, Ming G-L, 2016 Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure. Cell 165, 1238–1254. [PubMed: 27118425]

Quadrato G, Nguyen T, Macosko EZ, Sherwood JL, Min Yang S, Berger DR, Maria N, Scholvin J, Goldman M, Kinney JP, Boyden ES, Lichtman JW, Williams ZM, McCarroll SA, Arlotta P, 2017 Cell diversity and network dynamics in photosensitive human brain organoids. Nature 545, 48–53. [PubMed: 28445462]

Rakic P, 2009 Evolution of the neocortex: a perspective from developmental biology. Nat. Rev. Neurosci 10, 724. [PubMed: 19763105]

Rakic P, 1972 Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol 145, 61–83. [PubMed: 4624784]

Rakic P, Sidman RL, 1968 Supravital DNA synthesis in the developing human and mouse brain. J. Neuropathol. Exp. Neurol 27, 246–276. [PubMed: 5646196]

Rash BG, Duque A, Morozov YM, Arellano JI, Micali N, Rakic P, 2019 Gliogenesis in the outer subventricular zone promotes enlargement and gyrification of the primate cerebrum. Proc. Natl. Acad. Sci. U. S. A 116, 7089–7094. [PubMed: 30894491]

Rash BG, Lim HD, Breunig JJ, Vaccarino FM, 2011 FGF signaling expands embryonic cortical surface area by regulating Notch-dependent neurogenesis. J. Neurosci 31, 15604–15617. [PubMed: 22031906]

Reillo I, de Juan Romero C, García-Cabezas MÁ, Borrell V, 2011 A role for intermediate radial glia in the tangential expansion of the mammalian cerebral cortex. Cereb. Cortex 21, 1674–1694. [PubMed: 21127018]

Sakaguchi H, Kadoshima T, Soen M, Narii N, Ishida Y, Ohgushi M, Takahashi J, Eiraku M, Sasai Y, 2015 Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. Nat. Commun 6, 8896. [PubMed: 26573335]

Seabrook TA, Burbridge TJ, Crair MC, Huberman AD, 2017 Architecture, Function, and Assembly of the Mouse Visual System. Annu. Rev. Neurosci 40, 499–538. [PubMed: 28772103]

Shi Y, Kirwan P, Smith J, Robinson HPC, Livesey FJ, 2012 Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. Nat. Neurosci 15, 477–86, S1. [PubMed: 22306606]

Shi Y, Sun L, Liu J, Zhong S, Wang M, Li R, Li P, Guo L, Fang A, Chen R, Ge W-P, Wu Q, Wang X, 2019 Vascularized human cortical organoids model cortical development in vivo. bioRxiv. 10.1101/682104

Singhvi A, Shaham S, 2019 Glia-Neuron Interactions in Caenorhabditis elegans. Annu. Rev. Neurosci 42, 149–168. [PubMed: 30883261]

Skaper SD, Facci L, Zusso M, Giusti P, 2017 Synaptic Plasticity, Dementia and Alzheimer Disease. CNS Neurol. Disord. Drug Targets 16, 220–233. [PubMed: 28088900]

Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, 2011 A conditional knockout resource for the genome-wide study of mouse gene function. Nature 474, 337–342. [PubMed: 21677750]

Sloan SA, Darmanis S, Huber N, Khan TA, Birey F, Caneda C, Reimer R, Quake SR, Barres BA, Pa§ca SP, 2017 Human Astrocyte Maturation Captured in 3D Cerebral Cortical Spheroids Derived from Pluripotent Stem Cells. Neuron 95, 779–790.e6. [PubMed: 28817799]

Smart IHM, Dehay C, Giroud P, Berland M, Kennedy H, 2002 Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. Cereb. Cortex 12, 37–53. [PubMed: 11734531]

Spemann H, Mangold H, 1924 über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. Archiv für Mikroskopische Anatomie und Entwicklungsmechanik. 10.1007/bf02108176

Stahl R, Walcher T, De Juan Romero C, Pilz GA, Cappello S, Irmler M, Sanz-Aquela JM, Beckers J, Blum R, Borrell V, Götz M, 2013 Trnp1 regulates expansion and folding of the mammalian cerebral cortex by control of radial glial fate. Cell 153, 535–549. [PubMed: 23622239]

Staley K, 2015 Molecular mechanisms of epilepsy. Nat. Neurosci 18, 367–372. [PubMed: 25710839]

Stubbs D, DeProto J, Nie K, Englund C, Mahmud I, Hevner R, Molnár Z, 2009 Neurovascular congruence during cerebral cortical development. Cereb. Cortex 19 Suppl 1, i32–41. [PubMed: 19386634]

Subramanian L, Bershteyn M, Paredes MF, Kriegstein AR, 2017 Dynamic behaviour of human neuroepithelial cells in the developing forebrain. Nat. Commun 8, 14167. [PubMed: 28139695]

Sun T, Hevner RF, 2014 Growth and folding of the mammalian cerebral cortex: from molecules to malformations. Nat. Rev. Neurosci 15, 217–232. [PubMed: 24646670]

Takata N, Sakakura E, Eiraku M, Kasukawa T, Sasai Y, 2017 Self-patterning of rostral-caudal neuroectoderm requires dual role of Fgf signaling for localized Wnt antagonism. Nat. Commun 8, 1339. [PubMed: 29109536]

Trujillo CA, Gao R, Negraes PD, Chaim IA, Domissy A, Vandenberghe M, Devor A, Yeo GW, Voytek B, Muotri AR, 2018 Nested oscillatory dynamics in cortical organoids model early human brain network development. bioRxiv. 10.1101/358622

Velasco S, Kedaigle AJ, Simmons SK, Nash A, Rocha M, Quadrato G, Paulsen B, Nguyen L, Adiconis X, Regev A, Levin JZ, Arlotta P, 2019 Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. Nature, 10.1038/s41586-019-1289-x

Velmeshev D, Schirmer L, Jung D, Haeussler M, Perez Y, Mayer S, Bhaduri A, Goyal N, Rowitch DH, Kriegstein AR, 2019 Single-cell genomics identifies cell type-specific molecular changes in autism. Science 364, 685–689. [PubMed: 31097668]

Wang L, Hou S, Han Y-G, 2016 Hedgehog signaling promotes basal progenitor expansion and the growth and folding of the neocortex. Nat. Neurosci 19, 888–896. [PubMed: 27214567]

Watanabe M, Buth JE, Vishlaghi N, de la Torre-Ubieta L, Taxidis J, Khakh BS, Coppola G, Pearson CA, Yamauchi K, Gong D, Dai X, Damoiseaux R, Aliyari R, Liebscher S, Schenke-Layland K, Caneda C, Huang EJ, Zhang Y, Cheng G, Geschwind DH, Golshani P, Sun R, Novitch BG, 2017 Self-Organized Cerebral Organoids with Human-Specific Features Predict Effective Drugs to Combat Zika Virus Infection. Cell Rep. 21, 517–532. [PubMed: 29020636]

Willsey AJ, Sanders SJ, Li M, Dong S, Tebbenkamp AT, Muhle RA, Reilly SK, Lin L, Fertuzinhos S, Miller JA, Murtha MT, Bichsel C, Niu W, Cotney J, Ercan-Sencicek AG, Gockley J, Gupta AR, Han W, He X, Hoffman EJ, Klei L, Lei J, Liu W, Liu L, Lu C, Xu X, Zhu Y, Mane SM, Lein ES, Wei L, Noonan JP, Roeder K, Devlin B, Sestan N, State MW, 2013 Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. Cell 155, 997–1007. [PubMed: 24267886]

Wong DF, Wagner HN Jr, Tune LE, Dannals RF, Pearlson GD, Links JM, Tamminga CA, Broussolle EP, Ravert HT, Wilson AA, Toung JK, Malat J, Williams JA, O'Tuama LA, Snyder SH, Kuhar MJ, Gjedde A, 1986 Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. Science 234, 1558–1563. [PubMed: 2878495]

Xiang Y, Tanaka Y, Cakir B, Patterson B, Kim K-Y, Sun P, Kang Y-J, Zhong M, Liu X, Patra P, Lee S-H, Weissman SM, Park I-H, 2019 hESC-Derived Thalamic Organoids Form Reciprocal Projections When Fused with Cortical Organoids. Cell Stem Cell 24, 487–497.e7. [PubMed: 30799279]

Xiang Y, Tanaka Y, Patterson B, Kang Y-J, Govindaiah G, Roselaar N, Cakir B, Kim K-Y, Lombroso AP, Hwang S-M, Zhong M, Stanley EG, Elefanty AG, Naegele JR, Lee S-H, Weissman SM, Park I-H, 2017 Fusion of Regionally Specified hPSC-Derived Organoids Models Human Brain Development and Interneuron Migration. Cell Stem Cell 21, 383–398.e7. [PubMed: 28757360]

Yang Q, Song D, Qing H, 2017 Neural changes in Alzheimer's disease from circuit to molecule: Perspective of optogenetics. Neurosci. Biobehav. Rev 79, 110–118. [PubMed: 28522119]

Yoon S-J, Elahi LS, Paşca AM, Marton RM, Gordon A, Revah O, Miura Y, Walczak EM, Holdgate GM, Fan HC, Huguenard JR, Geschwind DH, Paşca SP, 2019 Reliability of human cortical organoid generation. Nat. Methods 16, 75–78. [PubMed: 30573846]

Young JW, Henry BL, Geyer MA, 2011 Predictive animal models of mania: hits, misses and future directions. Br. J. Pharmacol 164, 1263–1284. [PubMed: 21410454]

Zhong S, Zhang S, Fan X, Wu Q, Yan L, Dong J, Zhang H, Li L, Sun L, Pan N, Xu X, Tang F, Zhang J, Qiao J, Wang X, 2018 A single-cell RNA-seq survey of the developmental landscape of the human prefrontal cortex. Nature 555, 524–528. [PubMed: 29539641]

This review evaluates the cerebral organoid model system as measured by our current understanding of human brain development, focused on the cerebral cortex. The following are discussed topics in our review:

- Brain organogenesis is a complex and highly orchestrated developmental process regulated by a set of heritable developmental instructions.
- Neural stem cells, also known as radial glia, give rise to the vast majority of cell types in the brain, organized into higher-order tissue cytoarchitecture patterns.
- Cerebral organoids can be derived from pluripotent stem cells and leverage the properties of self-assembly to recapitulate the major cell types of the developing brain.
- Radial glia progenitor diversity and neurogenesis are well represented in the organoid cultures, but emergence of higher order features remain unclear.
- Single cell genomics provide a data-driven approach for unbiased comparisons of organoid cells to in vivo counterparts.

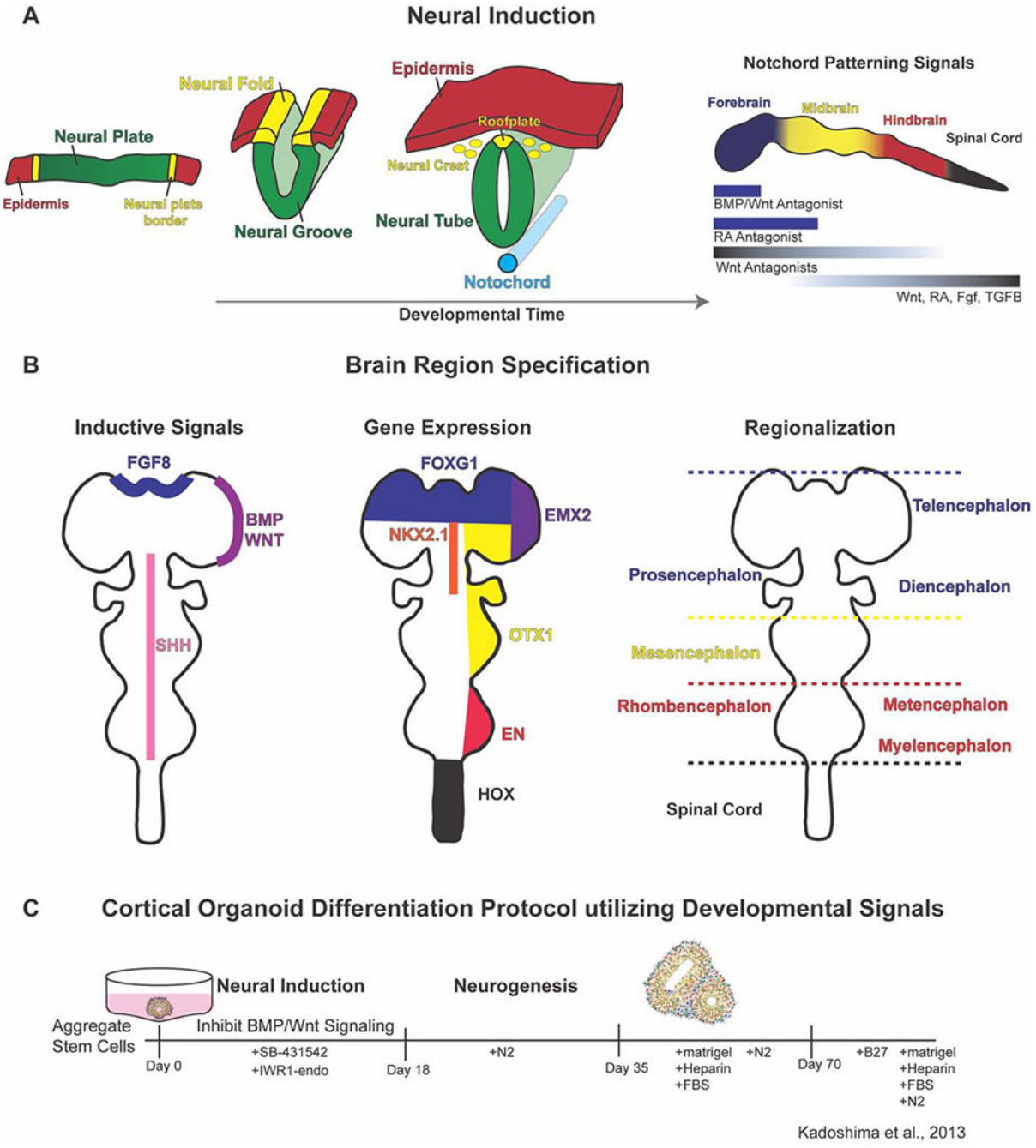

Figure 1: Neural development

A) During early development, notochord cells induce neuroepithelial identity of the overlying epithelium. After neural induction, the neural plate invaginates and closes to form the neural tube. The notochord becomes patterned along the rostral/caudal axis. *B)* Organizers within the nervous system arise and secrete morphogens to pattern neural tissue resulting in the expression of discrete transcription factor domains. These domains develop into distinct regions of the nervous system. **C)** Brain organoid protocols co-opt these

developmental signals to pattern the cells toward particular identities in an attempt to recapitulate early neurodevelopmental events.

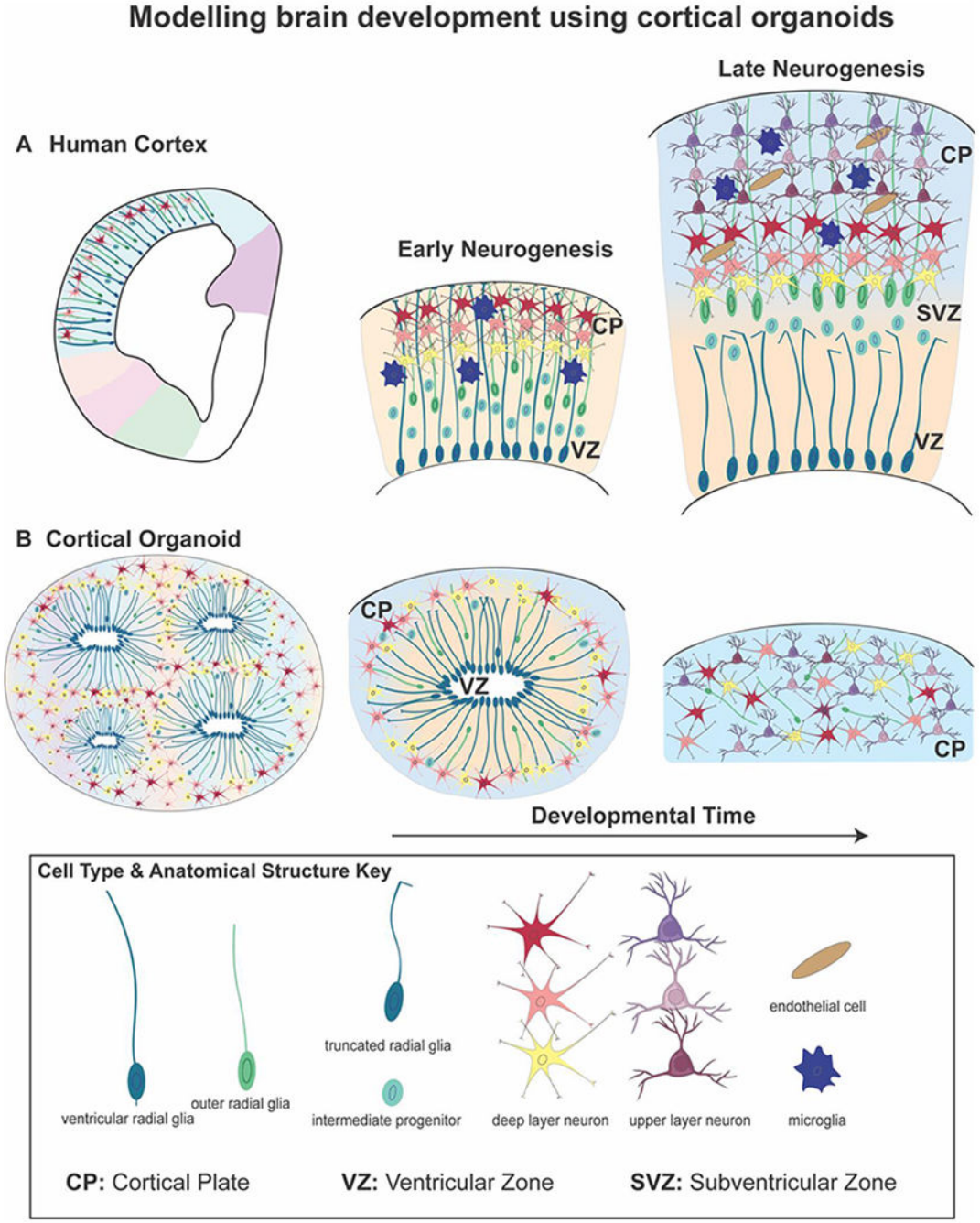

Figure 2: Cortical Development and organoid models

A) During cortical development radial glia arise from a neuroepithelial sheet. Progenitor cells generate deep layer neurons first before producing upper layer neurons, which expand the cortex. **B)** In the cortical organoid, cell types can also be identified using the expression of canonical markers. Organoid cells self-organize typically into multiple progenitor zone-like rosette structures, rather than into a single germinal zone as *in vivo*. Although the VZ-like structure expands, it does not maintain the same cytoarchitectural organization over

time. After a period of culturing, a mix of neuronal populations reside on the exterior of the organoid.