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REVIEW

Neuronal migration in the CNS during development and disease: insights from *in vivo* and *in vitro* models

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ABSTRACT

Neuronal migration is a fundamental process that governs embryonic brain development. As such, mutations that affect essential neuronal migration processes lead to severe brain malformations, which can cause complex and heterogeneous developmental and neuronal migration disorders. Our fragmented knowledge about the aetiology of these disorders raises numerous issues. However, many of these can now be addressed through studies of *in vivo* and *in vitro* models that attempt to recapitulate human-specific mechanisms of cortical development. In this Review, we discuss the advantages and limitations of these model systems and suggest that a complementary approach, using combinations of *in vivo* and *in vitro* models, will broaden our knowledge of the molecular and cellular mechanisms that underlie defective neuronal positioning in the human cerebral cortex.

KEY WORDS: Neuronal migration, Cortical malformations, Human cortical development, Model systems, Neuronal migration disorders

Introduction

Neuronal migration is a process that is essential for the development of the mammalian nervous system. In humans and rodents, a highly coordinated and regulated series of neuronal migration events is required to establish the different laminae of the cortex. When these neuronal migration processes are dysregulated, as occurs in human neuronal migration disorders (NMDs), malformations of cortical development (MCDs) can arise, which can cause a wide range of physiological and functional consequences.

The human cerebral cortex represents the largest region of the cerebrum – the most highly developed part of the human brain. It plays vital roles in processing and integrating information from all bodily senses to result in social and motor behaviours, in planning and organization, and in determining intelligence and personality (Kandel and Squire, 2000). As such, MCDs that affect the structure and functioning of this key brain region can have severe outcomes. Indeed in humans, MCDs are a recognized cause of developmental delay, intellectual disability and epilepsy, and are also associated with dysmorphic features (Guerrini and Dobyns, 2014; Jamuar and Walsh, 2015; Romero et al., 2018). MCDs have traditionally been classified according to the stage or process of cortical development that is affected (Table 1) (Barkovich et al., 2012; Pang et al., 2008). However, findings over recent years suggest that MCDs are far more heterogeneous – on a genetic, cellular and physiological level – than traditional classification schemes have indicated. Furthermore, the former boundaries between disorders of neural stem cell (NSC) proliferation, neuronal migration and cortical organisation are beginning to break down, as we deepen our understanding of their genetic and cellular aetiology.

In this Review, we first provide an overview of neuronal migration in the developing cortex and highlight the similarities and differences between the mouse and human. We then focus on NMDs, which are a subgroup of MCDs and which are often overlapping with other MCDs. We discuss the genetic, cellular and physiological heterogeneity of NMDs and briefly summarize the immense knowledge that has been gained in the past decades by analysing mouse models with specific mutations in genes that, in humans, lead to NMDs. We highlight the advantages and disadvantages of using different *in vivo* animal models and, finally, provide examples of more recently developed *in vitro* models that have been used to provide novel insights into neuronal migration and NMDs.

Neuronal migration in the developing cortex

During development, the neocortex becomes populated by two main groups of neurons – excitatory projection neurons and inhibitory interneurons. These two neuronal populations are generated in proliferative ventricular zones (VZ) and subventricular zones (SVZ) of the mammalian cortex, adjacent to the lateral ventricles of the brain (Fig. 1A). In mice, excitatory neurons are directly generated from apical radial glia (aRG; Box 1, Glossary) in the dorsal VZ or are derived from multipolar basal intermediate progenitors (bIPs; Box 1, Glossary) that have delaminated from the apical and basal surface and reside in the SVZ (Götz and Huttner, 2005; Lui et al., 2011; Taverna et al., 2014) (Fig. 1B). In humans, aRG generate heterogeneous populations of proliferative basal progenitors (BPs), including bIPs and a second population of RG that lose their apical anchoring and move their cell body into the outer SVZ (oSVZ). These basal radial glia (bRG; Box 1, Glossary) were recently described to be essential for the expansion of the cerebral cortex and for the formation of folds (gyrification; Box 1, Glossary) (Fietz and Huttner, 2011; Hansen et al., 2010; Reillo et al., 2011). At early stages of neurogenesis in mice, newborn deep-layer excitatory neurons move basally towards the marginal zone (MZ, Fig. 1B) by somal translocation (Box 1, Glossary). Once the developing cortex becomes thicker, newborn neurons shift to multipolar migration (Box 1, Glossary) until they reach the intermediate zone (IZ, Fig. 1B) (Tabata and Nakajima, 2003), in which they undergo a multipolar-to-bipolar transition (Box 1, Glossary). Neurons then begin directed radial migration (Box 1, Glossary) through the IZ and cortical plate (CP, Fig. 1B), using RG fibres as a migratory scaffold (Nadarajah et al., 2001). Glial-guided locomotion (Box 1, Glossary) is regulated by the coupled movement of cilia/centrosomes and nuclei within the neurons (Marín, 2013). Locomoting neurons migrate basally

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Box 1. Glossary

Apical radial glia. Neural stem cells, derived from neuroepithelial cells, which line the lateral ventricles in the developing dorsal and ventral cortex, thereby forming the ventricular zones. They are connected to the apical and basal surfaces via processes and are in contact with cerebrospinal fluid via their primary cilium. They self-renew and/or directly give rise to basal radial glia, basal intermediate progenitors and neurons, and, after neurogenesis, to astrocytes.

Basal radial glia. Neural stem cells that are enriched in the outer subventricular zone of the developing cortex of gyrencephalic species. They are generated from apical radial glia by delamination from the apical surface, keeping the basal process that serves as scaffold for glial-guided locomotion of newborn neurons.

Basal intermediate progenitors. Neurogenic progenitors that populate the subventricular zone, in which they amplify and generate neurons.

Cajal-Retzius cells. Reelin-producing cells in the marginal zone/layer I of the developing cerebral cortex (and in the immature hippocampus) that originate at multiple sites in the developing cortex. These cells are involved in correct development and lamination.

Embryoid body. Three-dimensional aggregates of pluripotent stem cells reminiscent of the blastocyst stage of early embryonic development in which pluripotent stem cells differentiate along all three germ lineages – endoderm, ectoderm and mesoderm.

Extracellular matrix. Composed of five classes of macromolecules – collagens, elastins, proteoglycans, hyaluronan and adhesive glycoproteins, such as laminins, reelin and tenascins – that are secreted by support cells, it serves functions in cell-cell communication, cell adhesion, and differentiation.

Ganglionic eminences. Part of the ventral telencephalon, categorized into medial, lateral and caudal ganglionic eminences. They represent the location of interneuron generation, contribute to basal ganglia formation and act in axon guidance between the thalamus and cortex.

Gyrification. The process of folding of the cortical surface, resulting in ridges (gyri) and furrows (sulci). The degree of gyrification is assessed by the gyrification index, the ratio between whole gyral contour length and the outer brain surface. Species with a folded brain surface are referred to as gyrencephalic animals.

Interkinetic nuclear migration. The characteristic movement of the nucleus of epithelial cells, including apical radial glia. The nucleus moves basally and apically within the ventricular zone in coordination with the cell cycle (with M-phase taking place at the apical surface and S-phase most basally), making the ventricular zone a pseudo-stratified tissue.

Locomotion. Glia-dependent radial neuronal migration in which neurons use radial glia as a scaffold for their migration towards the pial surface. Locomoting neurons show bipolar cell morphology, with a thick leading process and a thin trailing process, and the entire cell moves along the radial fibres that extend through the thickness of the developing cortex.

Multipolar migration. The radial glia-independent slow migration of neurons in the multipolar state, which functions through the extension and retraction of multiple processes and is not directed.

Multipolar-to-bipolar transition. The morphological transition of migrating newborn neurons that polarize and orient for radial migration in the upper subventricular zone.

Neural rosettes. In two-dimensional cultures, the radial arrangement of inside-out organized neural progenitors and neurons, with progenitors in the centre.

Pia. The outer surface of the grey matter of the brain, surrounded by the meninges (pia mater, arachnoid and dura mater).

Radial migration. Neuronal migration that proceeds from ventricular to pial surface, seen in newborn glutamatergic neurons in the developing cortex and in cerebellar Purkinje cells. Different modes of radial migration exist, such as locomotion and somal translocation.

Radial unit. A radial columnar unit of founder radial glia cells and their daughter neurons that migrate along their parental glia towards the cortical plate.

Somal translocation. A radial glia-independent mode of neuronal migration. The soma of neurons is translocated from the point of origin in the ventricular zone to the cortical plate by extending a long radially directed leading process towards the pial surface. The leading process is attached to the pial surface and progressively shortens to pull up the soma.

Terminal translocation. Final migration step of locomoting neurons that functions similarly to somal translocation. After arrival at the cortical plate and detachment from the radial glia, terminal translocation serves to reach the final position within the cortical plate.

Truncated radial glia. Human apical radial glia that lose the contact to the pial surface by acquiring a shortened, truncated morphology. They also develop a characteristic gene expression profile.

Tubulinopathies. A wide and overlapping range of brain malformations that are caused by the mutation of one of seven genes that encode different isotypes of tubulin, thus regulating the synthesis and function of microtubule and centrosome key components.

towards the pia (Box 1, Glossary), passing by earlier-born neurons; they then terminate their migration beneath the MZ once they have switched to terminal (RG-independent) translocation (Box 1, Glossary) (Sekine et al., 2011) The six layers of the cortex thus form in a birth-date-dependent and inside-out manner (Sun and Hevner, 2014) (Fig. 1B).

In contrast to excitatory neurons, inhibitory GABAergic interneurons are specified in the distant medial and caudal ganglionic eminences (GEs; Box 1, Glossary and Fig. 1A). Within the mouse GEs, an RG-containing VZ develops, as well as an SVZ that contains intermediate progenitors (IPs) and numerous subapical progenitors (SAPs) (Pilz et al., 2013). IPs and SAPs undergo 60-70% of all mitoses found in the GEs, thus expanding the interneuron population before its migration. Interneurons initially migrate tangentially in two streams over long distances into the cerebral cortex (Fig. 1A). They then switch to radial migration to integrate into the various cortical layers (Fig. 1B) (Anderson et al., 1997; Peyre et al., 2015; Silbereis et al., 2016; Wonders and Anderson, 2006).

The correct establishment of the cortical layers by neuronal migration is tightly controlled by a variety of extracellular and intracellular signals that regulate the actin and microtubule cytoskeleton, as well as their dynamics and interplay (Stouffer et al., 2015). When these precisely regulated developmental processes become dysregulated, as occurs in NMDs (Table 1), a number of key cellular and anatomical features of the cortex can become perturbed (Fig. 2), causing a range of physiological and functional consequences (Barkovich et al., 2012).

Differences between the mouse and human neocortex

A number of human-specific mechanisms of neocortical development and expansion have recently been identified (reviewed by Florio et al., 2017). Indeed, although the mouse model recapitulates a variety of common features of neurogenesis, such as the basic steps required for the generation and the migration of excitatory and inhibitory neurons, many studies have highlighted fine differences that distinguish the process of neurogenesis in mouse and human. It lies beyond the scope of this Review to go into details of these differences, but below we summarize some of the most recent findings that relate to our understanding of neuronal migration within the cortex.

There are several key aspects in which the mouse and human neocortex differ (Fig. 3), including differences in progenitor numbers, types and expansion capacity, in the composition of the extracellular matrix (ECM; Box 1, Glossary) (Pollen et al., 2015),

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Table 1. Classification scheme for malformations of cortical development (MCDs)

Affected step of development	MCDs resulting from the disturbance	Short definition of the MCD
Progenitor cell proliferation and apoptosis	Microcephaly	Abnormally small head and brain
	Macrocephaly	Abnormally big head and brain
	Hemimegalencephaly	Overgrowth of (part of) a cerebral hemisphere
	Focal cortical dysplasia	Disturbed lamination and dysmorphic neurons
Neuronal migration	Lissencephaly type I	Absence of normal convolutions/folds
	Periventricular heterotopia (PH)	Neurons accumulating at the ventricles underneath a normal cortex
	Subcortical band heterotopia/double cortex	Band of grey matter located between the lateral ventricular wall and the cortex
	Cobblestone lissencephaly/lissencephaly type II	Overmigration of neurons to localize on the surface of a brain with reduced gyri
Neuronal organisation	Polymicrogyria	Too many (usually small) folds/convolutions
	Schizencephaly	Fluid-filled cleft from ventricle(s) to pia lined by heterotopic grey matter

and in gene expression and regulation. Strikingly, the human cerebral cortex is significantly larger than the rodent cortex based on neuronal numbers; it is also highly folded, has greater complexity and has acquired higher cognitive functions (Lui et al., 2011; Rakic, 2009; Sousa et al., 2017). Two of the key factors underlying these differences are the expansion of cortical progenitors and higher neuronal production in humans (Borrell and Reillo, 2012). The increased neuronal number in humans results mainly from a larger initial pool of stem and progenitor cells at the onset of neurogenesis per unit of cortical volume, and from a prolonged neurogenic period (Charvet et al., 2011; Hansen et al., 2010; Noctor et al., 2004). Similar to the mouse, neurogenesis in humans begins with expansion of the neuroepithelium and aRG, but there are differences in human aRG morphology and proliferation (Kriegstein and Alvarez-Buylla, 2009; Nowakowski et al., 2016), such as more regenerative asymmetrical cell cycles compared with non-human primates and mice (Fish et al., 2008; Lukaszewicz et al., 2005). After the onset of neurogenesis, human aRG divide to give rise to bRG, which delaminate from the apical surface (keeping their basal process and attachment to the pial surface) and migrate basally and populate the oSVZ (Fig. 3). bRG then expand massively and make the oSVZ the predominant germinal region in the human neocortex, increasing neuronal output and cortical folding and complexity (Lui et al., 2011). The basal processes of bRG act as additional guides for migrating newborn neurons that disperse in the tangential axis to expand the surface area of the cerebral cortex (Reillo et al., 2011). Human aRG then lose their basal process and retain only the apical process (Nowakowski et al., 2016), which gives rise to truncated RG (tRG; Box 1, Glossary). Human and/or primate bRG have also been shown to form a specific niche in the oSVZ by expressing ECM proteins and growth factors (Pollen et al., 2015) (Fig. 3). A second mechanism that underlies gyrification, in which changes in intercellular adhesion influence the migration of cortical neurons, has also been identified and results in the regulation of cortical folding (del Toro et al., 2017).

In addition to the cellular-specific differences discussed above, gene duplications are a major force in human cortex evolution (Dennis and Eichler, 2016) and may contribute to species-specific features of cortex development. For example, the human-specific gene *ARHGAP11B*, a truncated duplicate of the ancestral form, promotes the generation of BPs when expressed in the mouse neocortex (Florio et al., 2015), whereas the gene *TBC1D3* (Ju et al., 2016), which is present in multiple copies in humans but not mice, promotes BP generation via aRG delamination and induces local

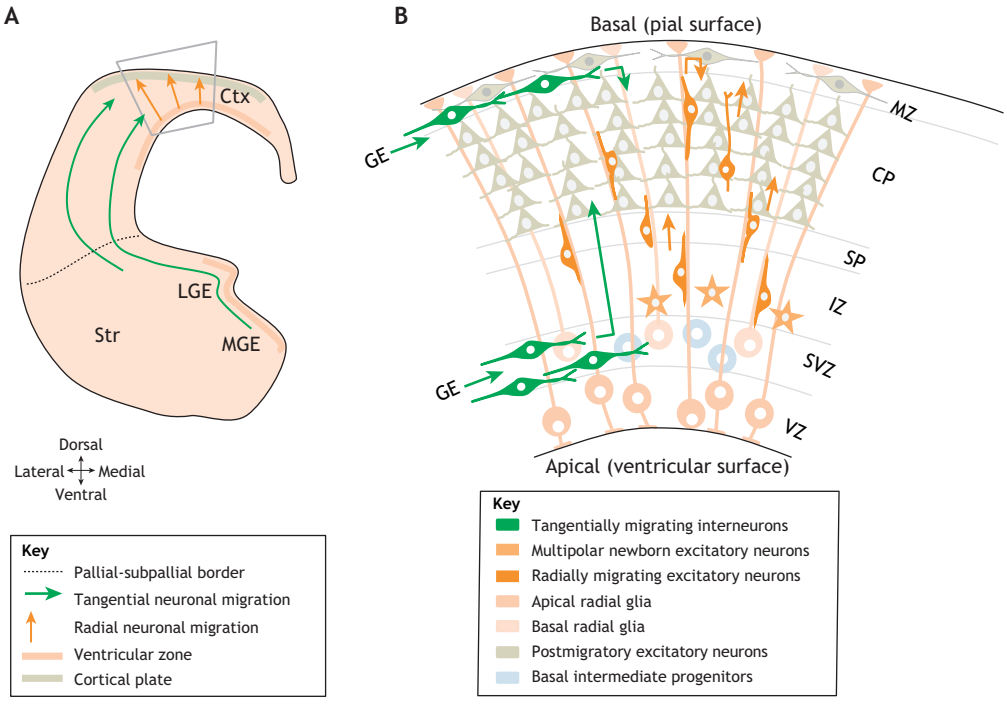

Fig. 1. Mouse cortical development. (A) Schematic of a coronal section of the early developing anterior telencephalon of the mouse at embryonic day 14 of development, showing cortical neurogenesis. The grey boxed area is enlarged in panel B. (B) Schematic of the cell composition of the developing mouse cerebral cortex showing radially migrating excitatory neurons and interneurons that enter the cortex tangentially and then switch to radial migration within the dorsal cortex. Ctx, cerebral cortex; CP, cortical plate; GE, ganglionic eminences; IZ, intermediate zone; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; MZ, marginal zone; SP, subplate; Str, Striatum; SVZ, subventricular zone, VZ, ventricular zone.

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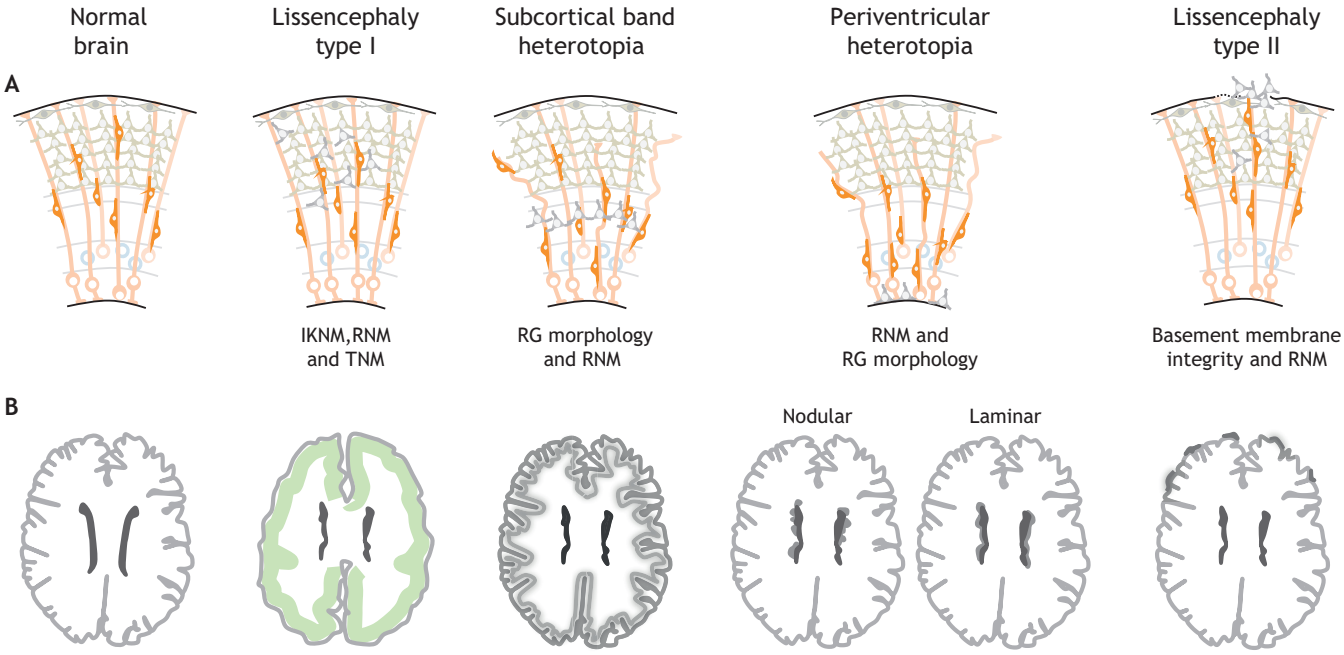

Fig. 2. Cellular and morphological defects associated with neuronal migration disorders. (A) Schematics highlighting the cellular basis of NMDs in the adult human cortex, showing the different cortical layers and neuronal migrations. Single ectopic neurons are shown in grey and the affected structures or processes are indicated. (B) Schematic showing the MRI-detectable morphological defects in the adult human brain that are caused by the cellular defects of each NMD. Ectopically located clusters of affected neurons are shown as grey shading. Lissencephaly type I is characterized by a smooth brain surface and a simplified four-layered cortex (indicated by green shading). In subcortical band heterotopia, the cortex contains an additional band of grey matter underneath the white matter. Periventricular heterotopia is characterized by clusters (nodular) or sheets (laminar) of neurons accumulating at the ventricles underneath a normal cortex. In lissencephaly type II, neurons overmigrate onto the cortical surface. Schematics are adapted from MRI images, see e.g. Bizzotto and Francis, 2015; Francis et al., 2006; Guerrini and Parrini, 2010. IKNM, interkinetic nuclear migration; RG, radial glia; RNM, radial neuronal migration; TNM, tangential neuronal migration.

cortical folding in mice upon overexpression. The NOTCH2NL genes, which arose from human-specific gene duplications of *NOTCH2*, also expand cortical progenitors and increase neuronal output when overexpressed in the developing mouse cortex (Fiddes et al., 2018; Suzuki et al., 2018). Because of this higher complexity in the regulation and heterogeneity of progenitors (which are known to guide newborn neurons during development), and in the intrinsic-extrinsic mechanisms involved in fine-tuning human neuronal migration, it has been difficult to tackle the mechanisms underlying NMDs using exclusively the mouse model. This, combined with the complexity and heterogeneity of NMDs, which we discuss below, has been an ongoing challenge for the field.

Neuronal migration disorders: genetic, cellular and physiological heterogeneity

As indicated above, recent findings suggest increased complexity in possible causes of NMDs, and of MCDs more generally, resulting in a breakdown of traditional boundaries between disorders of NSC proliferation, neuronal migration and cortical organisation (Guerrini and Dobyns, 2014). In this section, we discuss the multiple genetic, molecular, cellular and physiological levels of heterogeneity that have been recently identified in NMDs (summarised in Fig. 4).

Genetic heterogeneity of NMDs

Although environmental insults, such as *in utero* viral infection (Oliveira Melo et al., 2016), hypoxia (Golan et al., 2009), exposure to heavy metals (Kakita et al., 2001), alcohol or other drugs (Gressens et al., 1992; Mattson and Riley, 1998; Stanwood et al., 2001; Thompson et al., 2009) during pregnancy, head injury and radiation (Roper, 1998) or general genetic background (Poduri et al., 2013; Martens and van Loo, 2007) can predispose to or cause MCDs and neuropsychiatric disorders, the majority of NMDs are now thought to have a genetic basis (Table 2). Genetic variants and mutations that are associated with NMDs often function during genetically and functionally interdependent stages of cortical development. Indeed, current evidence suggests that distinct clinically defined disorders might be caused by shared risk loci (Table 2, Figs 2 and 4A) (Sullivan et al., 2012; Zhu et al., 2014), with the resulting phenotype influenced by the degree of protein dysfunction or by the levels of remaining functional protein. For example, mutations in *WDR62*, *DYNC1H1* and *TUBG1* cause a broad range of cortical malformations (Bilgüvar et al., 2010; Nicholas et al., 2010; Poirier et al., 2013; Yu et al., 2010). Tubulinopathies (Box 1, Glossary), such as those caused by mutations in *TUBA1A*, can present as lissencephaly type 1 or as polymicrogyria, (Table 1, Fig. 2 and Table 2); both of these NMDs share pathological molecular mechanisms that stem from altered microtubule function and altered interactions with microtubule-associated proteins (Cushion et al., 2013). Mutations in *LIS1* (also known as *PAFAH1B1*) can cause subcortical band heterotopia (SBH) and lissencephaly, whereas other lissencephaly causing mutations in *LIS1* and *DCX* also cause microcephaly (Table 1) (Sheen et al., 2006). In addition, *de novo* functional variants in *DCX* have been identified in patients with periventricular heterotopia (PH; Table 1) and were predicted to be causative. *MAP1B* mutations are also significantly associated with PH, with patients of this disorder additionally having deep perisylvean/insular polymicrogyria (Fig. 4A) (Heinzen et al., 2018).

It has therefore become obvious that the genetics underlying NMDs are complex and heterogeneous. As a result, genomics

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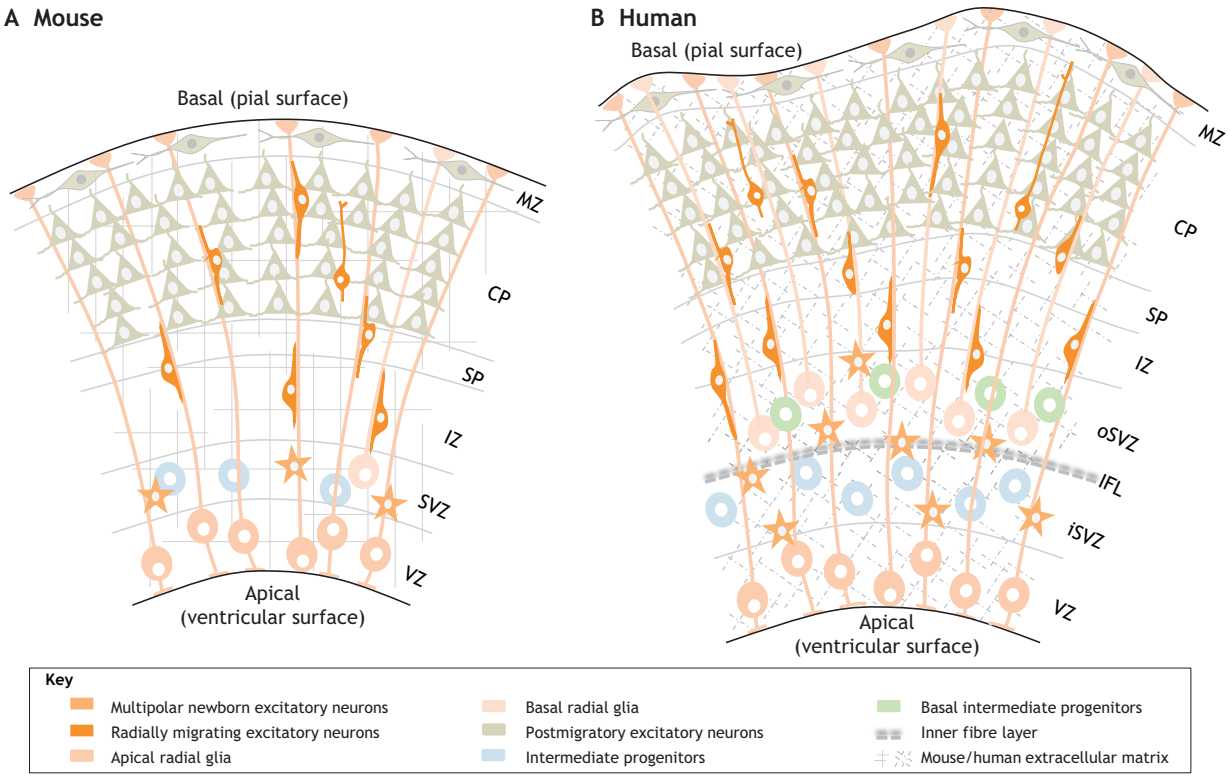

Fig. 3. Mouse and human dorsal cortical development. (A,B) Schematics illustrating the cell composition and ECM in the developing mouse (A) and human (B) dorsal cortex. Cell composition in the developing mouse lissencephalic cerebral cortex is shown (A), with aRG giving rise to IPs, or to neurons directly, that undergo multipolar-to-bipolar transition and locomote or translocate to the CP. In the developing human gyrencephalic cerebral cortex (B), the SVZ is subdivided by the inner fibre layer into the inner SVZ – corresponding to the mouse SVZ – and the oSVZ. The oSVZ is populated by outer bRG and bIPs that proliferate and generate neurons. Differences in ECM composition are indicated by different shading. CP, cortical plate; IFL, inner fibre layer; iSVZ, inner subventricular zone; IZ, intermediate zone; MZ, marginal zone; oSVZ, outer subventricular zone; SP, subplate; SVZ, subventricular zone, VZ, ventricular zone.

approaches, such as next generation sequencing (NGS) and whole exome sequencing (WES), are increasingly being used to identify the genes that contribute to MCDs. Indeed, the use of NGS to investigate families with two or more affected individuals has proven to be extraordinarily effective at identifying novel recessive mutations that contribute to neurodevelopmental and other disorders (Karaca et al., 2015; Yu et al., 2013). However, this approach is less useful in non-consanguineous families with a single affected patient, which is the case for most patients with NMDs. As a result, and because *de novo* variants are also strongly associated with neurodevelopmental conditions, WES combined with *in silico* prediction has been used to identify and to prioritize new candidate genes for various neurodevelopmental disorders, including autism spectrum disorder (ASD) (Iossifov et al., 2012) and epileptic encephalopathy (Epi4K Consortium et al., 2013). Two recent studies (Heinzen et al., 2018; O'Neill et al., 2018) have identified new candidate genes for PH by focusing on *de novo* variants and on rare inherited risk alleles. In the first study (Heinzen et al., 2018), trio-based WES of 202 patients with PH and epilepsy identified a significant enrichment of non-synonymous *de novo* variants in intolerant genes (termed 'hot-zone variants'); by combining *de novo* and very rare inherited variants, it was found that loss-of-function *MAP1B* variants are enriched in patients with this disorder, thereby identifying *MAP1B* as a new PH-associated locus. In the second study (O'Neill et al., 2018), trio-based WES was used to identify candidate genes, focussing on rare biallelic variants that contain a stop gain and/or loss or small out-of-frame insertion or deletion in at least one allele, which results in loss-of-function of the affected allele. Using this approach, the gene encoding the Hippo pathway signalling factor *MOB2* was identified as a candidate disease gene in a daughter of healthy parents that presented with epilepsy, learning difficulties and PH. Another novel, relatively fast and cost-effective approach to identifying MCD-associated genes was recently reported (Lu et al., 2018). This approach used a forward genetic screen in mice using transposon-mediated somatic mutagenesis in the developing mouse cortex to identify 33 candidate genes with potential roles in NPC proliferation, neuronal migration or differentiation (Lu et al., 2018).

Overall, these findings highlight that multiple genes can contribute to NMDs, and that the timing, severity and type of genetic (and environmental) factors that are involved in NMDs influence the type and extent of the resulting malformation.

Molecular and cellular heterogeneity

The heterogeneous genetic causes of NMDs are mirrored by the heterogeneous cellular phenotypes and functional outcomes that characterise these disorders. The genes that are implicated in NMDs encode proteins involved in various progenitor and neuronal properties and functions (Table 2). These functions include the maintenance and regulation of the morphology of the RG scaffold, the polarity and motility of neurons, the integrity of the neuroepithelium and the delamination of neurons from it, signalling between neurons and RG, basal membrane integrity, and the signalling that terminates migration in the CP (Bizzotto and Francis, 2015) (Fig. 4B).

As such, the disruption of any one of these functions can affect neuronal migration in different ways. Moreover, the proteins that are

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implicated in NMDs often function in more than one step of neuronal migration and in more than one cell type. For example, *FLNA*, which encodes the actin binding protein filamin A and has been implicated in NMDs (Fox et al., 1998; Lu et al., 2006; Parrini et al., 2006; Sheen et al., 2004b), is involved in RG proliferation and in regulating their polarized structure (Carabalona et al., 2012), in establishing neuronal polarity and in neuronal migration itself. Its mutation, therefore, could result in an array of defects. Similarly, LIS1 has been shown to be essential for both interkinetic nuclear migration (IKNM, Box 1, Glossary) of aRG and neuronal migration (Hippenmeyer et al., 2010; Moon et al., 2014). Thus, the genetic heterogeneity that underlies NMDs translates into complexity in terms of the affected molecules (Fig. 4B), cellular processes and cell types.

Physiological heterogeneity

In addition to the genetic and cellular heterogeneity of NMDs, the clinical features of patients are highly variable with regard to the absence or presence of seizures, as well as intellectual function and congenital neurological deficits (Fig. 4C). The functional outcome of PH, for example, ranges from mild, sometimes subclinical, to very severe (Barkovich and Kuzniecky, 2000; Dubeau et al., 1995), and ∼40% of NMD patients present with various types of epilepsy, whereas the rest are seizure free. Even PH patients that share mutations in the same gene, such as those with familial or sporadic *FLNA* mutations, show phenotypic heterogeneity (Parrini et al., 2006). Similarly, patients with *MAP1B* mutations can present with a range of seizures, cognitive impairments and other dysmorphic features (Heinzen et al., 2018). Furthermore, there is no clear relationship between the severity of epilepsy in PH and the extent of neuronal heterotopia (Chang et al., 2005), and epileptic activity can originate from a general imbalance of excitation versus inhibition, or it can arise locally from heterotopic clusters of neurons that can become intrinsically epileptogenic (Kothare et al., 1998) or from neurons surrounding heterotopic nodules.

Together, the genetic, cellular and functional heterogeneity of MCDs (Fig. 4) lead us to suggest that the way that NMDs have been classified to date as independent diseases is too limited. We thus propose that NMDs should be considered as an overlapping family of diseases or a spectrum of disorders.

Animal models of cortical development: their relevance, advantages and limitations

Ideally, research into the genesis of NMDs and MCDs should be performed using human tissue. However, access to human tissue – in the form of post-mortem and pathological specimens – is limited,

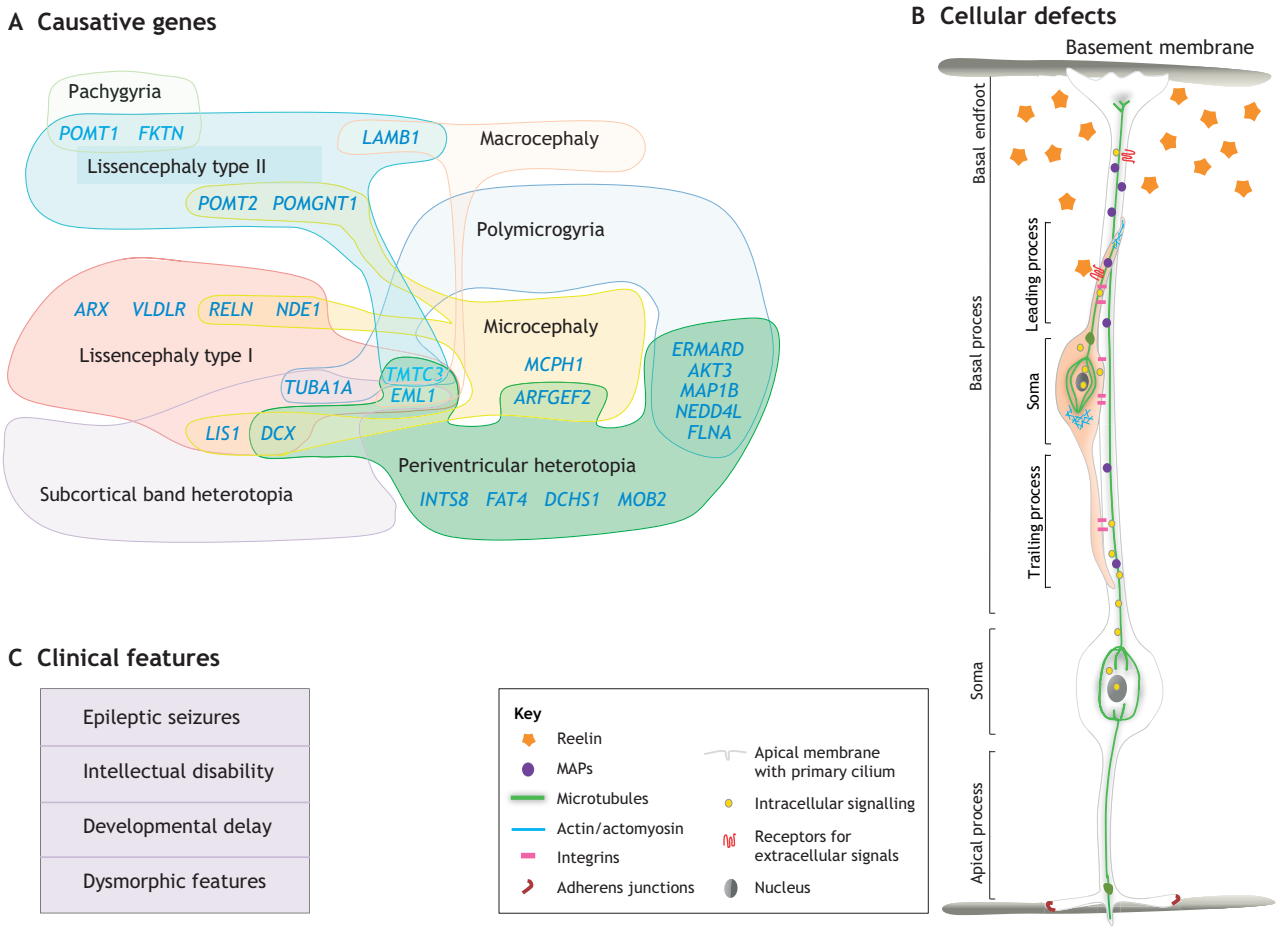

Fig. 4. Heterogeneity and complexity of neuronal migration disorders at the genetic, cellular and clinical levels. (A) NMDs (black) and some of their identified causative genes (blue) are shown; the scheme also depicts the overlap between the differently classified disorders and other malformations of cortical development not currently classified as NMDs (grey). (B) Schematic of an aRG cell (white; cellular compartments labelled on the far left), extending from apical surface to the basement membrane, and a newborn neuron (orange) using the aRG cell as a guide for its locomotion under healthy conditions. Examples of molecules and processes at the cellular level that can be compromised in NMDs in RG and radially migrating neurons are exemplified to picture the complexity of the disorders. (C) Clinical features of patients suffering from NMDs.

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Table 2. Genes known to cause neuronal migration disorders (NMDs) in humans upon disruption

Gene	Cortical malformation	Main protein function	References
Lissencephaly type I and subcortical band heterotopia			
LIS1 (*PAFAH1B1*)	Lissencephaly type I; subcortical band heterotopia; (microcephaly)	Cytoskeleton (microtubules, dynein)	des Portes et al., 1998; Faulkner et al., 2000; Reiner et al., 1993; Sheen et al., 2006
DCX	Lissencephaly type I; subcortical band heterotopia; periventricular heterotopia; (microcephaly)	Cytoskeleton (microtubule stability), dynein binding, nucleokinesis	Bahi-Buisson et al., 2013; des Portes et al., 1998; Francis et al., 1999; Gleeson et al., 1998; Horesh et al., 1999; Sicca et al., 2003
14-3-3e (*YWHAE*)	Lissencephaly type I	Cytoskeleton (microtubules), intracellular signalling	Reiner et al., 1993
TUBA1A	Lissencephaly type I; subcortical band heterotopia; polymicrogyria (with microcephaly, corpus callosum agenesis, and cerebellar hypoplasia)	Cytoskeleton (microtubule component)	Bahi-Buisson et al., 2008; Bahi-Buisson et al., 2013; Keays et al., 2007; Poirier et al., 2007
RELN	Lissencephaly type I with cerebellar hypoplasia; (microcephaly)	Secreted ECM protein; Cytoskeleton (microtubules and actin), cell adhesion	Dulabon et al., 2000; Hirota and Nakajima, 2017; Hong et al., 2000
ARX	Lissencephaly type I with corpus callosum agenesis	Transcription factor	Colombo et al., 2007; Kato et al., 2004; Kitamura et al., 2002
VLDLR	Lissencephaly type I with cerebellar hypoplasia	Reelin receptor: RELN to microtubule signalling	Schlotawa et al., 2013; Trommsdorff et al., 1999
NDE1	Extreme microcephaly with lissencephaly type I	Cytoskeleton (microtubules/ centrosome): nuclear migration, centrosome duplication, mitotic spindle assembly	Alkuraya et al., 2011
ACTG1	Lissencephaly type I	Cytoskeleton (actin component)	Verloes et al., 2015
ACTB	Lissencephaly type I	Cytoskeleton (actin component)	Verloes et al., 2015
Periventricular heterotopia			
FLNA	Periventricular nodular heterotopia; polymicrogyria	Cytoskeleton (actin binding and crosslinking protein), junction formation	Fox et al., 1998; Lu et al., 2006; Parrini et al., 2006; Sheen et al., 2004b
KIF2A	Heterotopia; subcortical band heterotopia; agyria, pachygyria; (thin corpus callosum, congenital microcephaly)	Kinesin: microtubule-associated motor	Poirier et al., 2013
TUBG1	Laminar heterotopia; agyria, pachygyria; (microcephaly, dysmorphic corpus callosum)	Cytoskeleton (microtubule component)	Poirier et al., 2013
ARFGEF2	Periventricular nodular heterotopia with microcephaly	Golgi vesicle formation and trafficking; cell-cell adhesion; interaction with FLNA; Rac/Rho signalling	Bardón-Cancho et al., 2014; Lu and Sheen, 2005; Lu et al., 2006; Sheen, 2014; Sheen et al., 2004a; Shin et al., 2005
EML1	Periventricular heterotopia; ribbon-like subcortical band heterotopia; lissencephaly type I; (macrocephaly)	Cytoskeleton (microtubules), mitotic spindle orientation, cell adhesion	Bizzotto et al., 2017; Kielar et al., 2014
FAT4	Periventricular nodular heterotopia	Protocadherin: cell-cell and apical adhesion	Badouel et al., 2015; Cappello et al., 2013
DCHS1	Periventricular nodular heterotopia	Protocadherin: cell-cell and apical adhesion	Cappello et al., 2013
ERMARD (*C6orf70*)	Periventricular nodular heterotopia with polymicrogyria and corpus callosum agenesis	ER membrane-associated RNA degradation; trafficking; cell-cell adhesion	Conti et al., 2013
NEDD4L	Periventricular nodular heterotopia; polymicrogyria	Ubiquitin ligation and protein degradation, mTOR and (PI3K) AKT pathway	Broix et al., 2016
AKT3	Periventricular heterotopia with megalencephaly; polymicrogyria	(PI3K) AKT pathway	Alcantara et al., 2017
MAP1B	Periventricular heterotopia; (polymicrogyria)	Cytoskeleton (microtubules)	Heinzen et al., 2018
MCPH1	Microcephaly with periventricular nodular heterotopia and pachygyria	DNA damage response (G2/M checkpoint)	Trimborn et al., 2004
INTS8	Periventricular nodular heterotopia	RNA processing and transcription regulation	Oegema et al., 2017
Cobblestone lissencephaly (lissencephaly type II)			
TMTC3	Cobblestone lissencephaly; periventricular heterotopia; lissencephaly type I	Protein degradation in the endoplasmic reticulum; regulation of GABAergic inhibitory synapses	Farhan et al., 2017; Jerber et al., 2016
POMT1	Cobblestone lissencephaly; pachygyria	O-glycosylase: basement membrane integrity	Beltrán-Valero de Bernabé et al., 2002; Mercuri et al., 2009
POMT2	Cobblestone lissencephaly; (microcephaly)	O-glycosylase: basement membrane integrity	Mercuri et al., 2009; van Reeuwijk et al., 2005
FKRP	Cobblestone lissencephaly	O-glycosylase: basement membrane integrity	Mercuri et al., 2009

Continued

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Table 2. Continued

Gene	Cortical malformation	Main protein function	References
FCMD (*FKTN*)	Cobblestone lissencephaly	O-glycosylase: basement membrane integrity	Mercuri et al., 2009; Yamamoto et al., 2004
POMGNT1	Cobblestone lissencephaly; (microcephaly)	O-glycosylase: basement membrane integrity	Mercuri et al., 2009; Vuillaumier-Barrot et al., 2011
LARGE (*LARGE1*)	Cobblestone lissencephaly	O-glycosylase: basement membrane integrity	Longman et al., 2003; Vuillaumier-Barrot et al., 2011
LAMB1	Cobblestone lissencephaly; (macrocephaly)	ECM component: basement membrane integrity	Radmanesh et al., 2013
GPR56 (*ADGRG1*)	Bilateral fronto-parietal polymicrogyria and/or cobblestone lissencephaly; (white matter abnormalities, cerebellar dysplasia)	G-protein coupled receptor: basement membrane integrity	Bahi-Buisson et al., 2010; Li et al., 2008
COL4A1	Cobblestone lissencephaly	ECM component: basement membrane integrity/linkage of RG to the pial basement membrane	Labelle-Dumais et al., 2011

Accompanying MCDs are listed in brackets; owing to partial overlap in causative genes, polymicrogyria was taken into this list of NMDs.

particularly in the case of rare diseases. We therefore require suitable model systems to improve our understanding of human cortical development and disorders. To date, the mouse has been the principal model organism used to investigate the basis of cortical development and has been essential for revealing some of the molecular, cellular and functional mechanisms that underlie the formation of the most common types of NMDs. Extensive detailed reviews regarding the use of mouse models in this context are available (Guerrini and Dobyns, 2014; Jamuar and Walsh, 2015; Romero et al., 2018); however, a key challenge to modelling NMDs in mouse models is to take into account species-specific differences in cortical development. Below, we discuss examples of the advantages and limitations of the mouse model and highlight additional *in vivo* model systems that more closely resemble some features of the human brain (e.g. bRGs and folds) and, therefore, could be used to help refine our findings.

Mouse models of NMDs

Mouse models have been widely used to uncover essential mechanisms that underlie neurogenesis and neuronal migration. Moreover, modelling MCDs (including NMDs) *in vivo* using specific knockout (KO) models of the genes that have mutated in patients with MCDs has highlighted basic essential mechanisms that cause microcephaly, lissencephaly type I, neuronal heterotopia and other MCDs (Guerrini and Parrini, 2010; Stouffer et al., 2015). However, although several mouse models recapitulate the morphological phenotypes of human NMDs, the genetic mutations involved are not always those that are associated with human disorders. For example, impaired neuronal migration phenotypes – similar to those seen in humans with SBH – have been achieved by conditionally inactivating genes in mice that function in the apical adherens junction, including *Ctnna1* (Schmid et al., 2014), *Rapgef2*, *Rapgef6* (Maeta et al., 2016), *Mllt4* (*Afdn*) and *Cdh2* (Gil-Sanz et al., 2014). The conditional inactivation of *RhoA*, which encodes the small GTPase, in the developing mouse brain also leads to SBH, thereby revealing that the integrity of both the actin and microtubule cytoskeleton in RG is important for generating a functional glial scaffold for radial migration (Cappello et al., 2012). PH can also be generated in mice by mutating genes that are involved in signalling via FLNA, for example, by conditionally inactivating *Mekk4* (*Map3k4*; Sarkisian et al., 2006) or acutely knocking down *Rcan1* (Li et al., 2015). In addition, a missense mutation in the mouse *Napa* gene (which encodes alphaSnap) has revealed a role for vesicle trafficking in PH, similar to that mediated by *ARFGEF2* in humans (Chae et al., 2004).

An example of an excellent mouse model that does recapitulate the patient phenotype with the same genetics is the KO of the microtubule-associated *Eml1*: patient brains have ribbon-like neuronal heterotopia and the mouse brain also shows SBH (Bizzotto et al., 2017; Kielar et al., 2014). Such mouse models have been very useful for understanding the general aetiology of neuronal heterotopia.

Mouse models for the neuronal overmigration seen in cobblestone lissencephaly have also been described (Bizzotto and Francis, 2015). These models highlight the importance of an intact basement membrane and of Cajal-Retzius cells (Box 1, Glossary). Mutations that produce neuronal overmigration phenotypes in mice include loss-of-function mutations in *Ps1* (*Psen1*; Hartmann et al., 1999) and in basement membrane receptors, such as the alpha 6 integrins (Georges-Labouesse et al., 1998) and *Ilk* (Niewmierzycka et al., 2005).

Rodent models of NMDs have also been generated by manipulating a mouse homologue of a human NMD-associated gene, but this approach does not always produce the expected human phenotype. These models include the mouse *Dcx* KO model, which does not recapitulate the human phenotype of isocortical malformation. By contrast, acute knockdown (KD) of *Dcx* in the rat does mimic the human phenotype, displaying aberrant electrophysiology (Nosten-Bertrand et al., 2008; Ramos, 2005). Likewise, the *Flna* KO mouse does not develop PH, whereas RNAi-mediated KD of *Flna* in the rat leads to ectopic neurons (Carabalona et al., 2012). *Fat4* KO in mice does not cause cortical heterotopia, as found in patients with *FAT4* mutations (Badouel et al., 2015), but instead leads to overproliferation and to reduced neuronal differentiation when acutely knocked down (Cappello et al., 2013). Finally, whereas *TUBA1A* mutations can cause severe lissencephaly, microcephaly, SBH and abnormal gyrification in human patients (Aiken et al., 2017), a mouse mutant of *Tuba1a* develops with hippocampal, but no cortical, defects (Liu, 2011).

The knowledge we have gained by studying the development of the cortex in these models is, without doubt, highly valuable. However, it is somewhat fragmentary: we can extrapolate basic ideas of why progenitor cells fail to expand or differentiate in the correct manner and why neurons fail to reach their final destination, but we are facing a simplified system that may mask or not adequately display key human-specific mechanisms. In addition, it is becoming clear that common phenotypes are sometimes driven by different genetic human and/or mouse mutations, and that multiple or missing phenotypes are often observed in the mouse brain, which suggests an additional level of regulation in humans.

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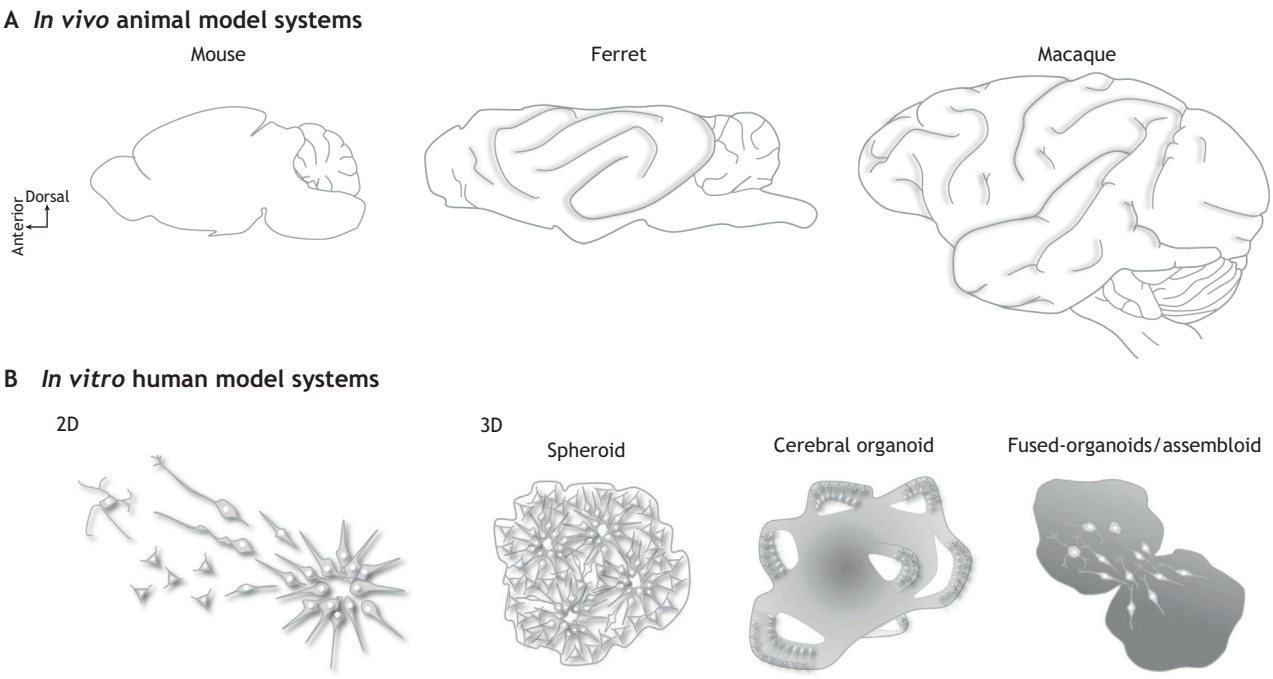

Fig. 5. *In vivo* and *in vitro* model systems of neurodevelopment and neurodevelopmental disorders. (A,B) Schematic depicting animal model systems (A) and pluripotent stem cell-based human model systems (B) of increasing complexity and similarity to the developing human brain. (A) Schematics of mouse, ferret and non-human primate brains as *in vivo* model systems. Axes are indicated. (B) 2D human models include NPCs and neurons, and 3D models include rosette-based spheroids, brain region or whole-brain organoids, and assembloids with different brain regions fused together, which are particularly promising for the study of both radial and tangential neuronal migration. Note that *in vitro*-derived cells/aggregates can also be transplanted into the mouse brain (not shown), in which neurons can integrate, mature, become vascularized and generate functional circuits with the host cells.

Non-rodent models of cortical development

To overcome the challenges of modelling cortical development and NMDs in mice, researchers have turned to new *in vivo* models, including the ferret and non-human primates (Fig. 5A). Several lines of evidence suggest that the ferret neocortex represents a more 'human-like' model system than that of the mouse. The ferret neocortex is, for example, folded and equipped with an oSVZ that contains abundant bRG (Fietz et al., 2010) and discrete domains of gene expression (de Juan Romero et al., 2015); for example, the differential expression of adhesion molecules in future gyri and sulci (del Toro et al., 2017). Furthermore, the dynamics of neuronal migration are more complex in the ferret brain than in the rodent brain: concomitant with the start of cortical folding, neurons can acquire tortuous migratory routes, using processes of multiple neighbouring RG to disperse laterally and generate a complex cortical architecture (Gertz and Kriegstein, 2015). Importantly, similar to the mouse, gene expression can be acutely manipulated in the developing ferret brain by *in utero* electroporation (Kawasaki et al., 2013; Smith et al., 2018). As such, the ferret is emerging as a powerful model for studying cortical development (Johnson et al., 2018; Smith et al., 2018).

The brains of non-human primates are much more similar to the human cortex in terms of their size, neuronal numbers and gyrification. In the developing cortex of gyrencephalic monkeys, such as the macaque, an inner and outer SVZ can be clearly distinguished (Dehay et al., 2015; Smart et al., 2002), and the oSVZ contains an abundant population of bRG. The diversity of precursor cell types is also much higher in primates than in rodent germinal zones, with heterogeneity of bRG evident in primates (Betizeau et al., 2013; Dehay and Kennedy, 2007; Dehay et al., 2015; Lukaszewicz et al., 2005). Despite the high degree of similarity to the human cortex, the cortex of the monkey is still decisively smaller. This highlights that additional mechanisms underlying differences in brain development likely exist and remain to be elucidated; this observation also highlights the shortcomings of using non-human primates as models of human cortical development. A further limitation in the use of non-human primates is their long gestation and developmental time and the difficulty of genetic manipulation compared with rodents, although there are some successful examples of the generation of transgenic animals with germline transmission [e.g. in marmosets (Sasaki et al., 2009) and macaques (Liu et al., 2016)]. Taken together, *in vivo* studies of cortex development in mouse, ferret and primates (Fig. 5A) have been, and will continue to be, essential to understand neuronal migration and NMDs, but we clearly need an accessible human-specific system to validate our *in vivo* findings in a human context.

In vitro models of cortical development

Given the aforementioned limitations of *in vivo* models for studying human cortex development and malformation, a number of *in vitro* models of cortical development have been created. Many of these use mouse and human stem cells as a starting point, and thus represent highly accessible systems that can be manipulated in various ways. Indeed, both embryonic stem cells (ESCs) (Evans and Kaufman, 1981; Thomson et al., 1998) and induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006) can be differentiated in culture to generate neurons via neural progenitor cells (NPCs) (Zhang et al., 2001). The ability of such pluripotent stem cells (PSCs) to spontaneously acquire neural identity (Ying et al., 2003) and self-organize and differentiate *in vitro* is remarkable, and is most likely underscored by the fact that few external factors are needed to induce neuroectoderm formation (Holtfreter, 1944). Genome editing (Ran et al., 2013) and acute

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manipulation by transfection and transduction are also relatively simple in human PSCs, further strengthening their utility. In addition, human PSCs can be differentiated *in vitro* to create 2D or more complex 3D model systems (Fig. 5B), and the resulting cells and tissues can also be transplanted into animal models. The advantages that 3D systems offer for modelling and studying NMDs relative to other approaches, as discussed in more detail below, include the possibility of exploring molecular, cellular and functional properties of human progenitors and neurons in an accessible, three-dimensional structure that, in a rudimental manner, resembles the early steps of human brain development.

2D models

In vitro protocols for generating 2D neuronal models are based on the capacity of polarized neuroepithelial cells to self-organize into neural rosettes (Box 1, Glossary) around a pseudo-lumen (Elkabetz et al., 2008). The default fate of NPCs is forebrain cells (Levine and Brivanlou, 2007) that sequentially generate layer-specific neurons in a stereotypical temporal order; these neurons then mature to build networks of neurons that form synapses and that are able to actively fire (Espuny-Camacho et al., 2013; Gaspard et al., 2008; Pankratz et al., 2007; Paşca et al., 2011; Shi et al., 2012). A key advantage of such 2D neuronal *in vitro* systems is that they recapitulate the human species-specific molecular clock of development and maturation (Suzuki and Vanderhaeghen, 2015), enabling cellular morphology and proliferation, as well as migration and differentiation, to be easily studied to uncover disease mechanisms (Iefremova et al., 2017; Paşca et al., 2014). However, their disadvantages are based on the limitations of an *in vitro* system, in that spatial organisation is restricted and cellular behaviour is highly dependent on culture conditions.

3D models

3D suspension cultures have recently been developed to generate neural model systems that more closely resemble *in vivo* tissue. NPCs can self-organize to form 3D aggregates – termed organoids or spheroids – that produce various CNS lineages (Eiraku et al., 2011; Nakano et al., 2012; Reynolds and Weiss, 1992; Turner et al., 2016). Based on this inherent genetically encoded ability, several protocols have been developed to generate brain region-specific spheroids and cerebral organoids that can be used to investigate cortex development and MCDs (summarised in Table S1). All of these approaches result in VZs containing aRG that are organized around a ventricle-like lumen. Basally to this lumen lies an SVZ-like zone that contains bIPs and bRG, a CP-like zone with neurons of different layer identities, and a marginal-like zone with Cajal-Retzius neurons. These 3D tissues are classified according to the amount of external patterning they undergo, which is dependent on them undergoing either directed or undirected approaches (as discussed below), or according to the complexity of the generated tissue. As the approaches used to generate 3D neuronal tissues have recently been reviewed (Di Lullo and Kriegstein, 2017; Paşca, 2018), we focus here on the differences between protocols and illustrate the type of question they can be used to investigate.

In protocols of 'directed' spheroid generation, 3D aggregates known as embryoid bodies (Box 1, Glossary) are patterned (Chambers et al., 2009; Watanabe et al., 2005) to acquire an ectodermal fate and are then instructed to develop towards a certain brain region using exogenously supplied morphogens to mimic endogenous patterning events (Mariani et al., 2012; Bagley et al., 2017; Eiraku et al., 2008; Li et al., 2017; Qian et al., 2016) (Fig. 5B). Cortical spheroids recapitulate mid-foetal stages and contain bRG, neurons and glia, as well as functional synapses and electrophysiological signatures of network activity (Paşca et al., 2015). They even mature to resemble postnatal stages of cortex development, containing mature astrocytes that are very similar to human primary astrocytes (Sloan et al., 2017). Special protocols that combine the action of small molecules, culture in high oxygen and miniaturized bioreactors have been designed to direct differentiation towards forebrain, midbrain, or hypothalamus, adenohypophysis and cerebellum identity, and to facilitate drug screening (Bershteyn et al., 2017; Iefremova et al., 2017; Jo et al., 2016; Kadoshima et al., 2013; Krefft et al., 2018; Muguruma et al., 2015; Qian et al., 2016; Rigamonti et al., 2016; Sakaguchi et al., 2015; Suga et al., 2011). These directed spheroid approaches are more reproducible than unpatterned approaches (see below) and reach a higher degree of maturation than do cultures in 2D. Spheroids and/or organoids can also undergo long-term *in situ* live imaging, using the 'organoids-on-a-chip' method (Karzbrun et al., 2018), in which cerebral organoids are grown in micro-fabricated compartments, allowing tissue expansion only in the *x*,*y*-plane and imaging through the coverslip (Table S1).

By contrast, 'undirected' approaches can be used to develop spheroids and/or organoids without the addition of external patterning factors (Lancaster and Knoblich, 2014; Lancaster et al., 2013; Lindborg et al., 2016; Renner et al., 2017). They rely on the intrinsic capacity of cells to differentiate along a lineage and self-organise. The resulting cerebral organoids contain germinal zones with cells of all brain region identities, including dorsal and ventral forebrain and forebrain organising centres, midbrain, hindbrain, midbrain-hindbrain boundary, choroid plexus, and retina (Table S1). These cell types are generated via the same transcriptional programmes and developmental trajectories as those that occur in the human foetal brain (Camp et al., 2015; Quadrato et al., 2017). A key advantage of undirected protocols is the high complexity of the interacting areas – reminiscent of different brain regions – that they generate. They therefore enable a more comprehensive study of brain development and disease, enabling the identification of cell types that are affected by certain disease genes.

Regardless of how they are generated, both brain region-specific spheroids and cerebral organoids can be analysed using an array of unbiased approaches, including single cell RNA sequencing, single cell live imaging and fluorescence-activated cell sorting. Their disadvantages include the high variability in the efficiency of neural induction, in the brain regions generated, and between organoids and batches (Camp et al., 2015; Jabaudon and Lancaster, 2018; Quadrato et al., 2017). Organoid size is also currently limited by the diffusion of oxygen and nutrients because of the absence of vascularisation. The addition of microfilaments and scaffolding can nevertheless improve neural induction efficiency, the production of regions with dorsal cortical identity and the generation of radial units (Box 1, Glossary; Krefft et al., 2018; Lancaster et al., 2017; Zhu et al., 2017).

It is also possible to fuse together formerly patterned spheroids to acquire different regional identities and to create tissues – termed fused-organoids or assembloids – of high, defined complexity *in vitro*. For example, pallial and subpallial spheroids have been fused to create mature glutamatergic projection neurons of all layer identities, as well as several different GABAergic interneuron types that subsequently migrate towards a dorsal cortex-like region (Birey et al., 2017). Both pallial and subpallial 'brain regions' give rise to astrocytes, and the subpallial spheroids also produce oligodendrocytes. In additional examples, assembloids of ventral and dorsal telencephalon were created to study the radial migration of glutamatergic neurons and the saltatory tangential migration of

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interneurons, as well as interneuron integration into dorsal cortical laminae with the establishment of electrophysiologically active microcircuits (Bagley et al., 2017; Birey et al., 2017; Xiang et al., 2017). Such assembloids can be used to study altered neural circuit formation in patient-derived cells and to distinguish cell-autonomous mechanisms from non-cell-autonomous ones by combining patient- and control-derived spheroids.

Transplantation of *in vitro* generated tissues and cells

In vitro-generated neural cell preparations can also be transplanted into mice in order to obtain an additional level of complexity. When *in vitro*-generated human NPCs or neurons are transplanted into the mouse brain (Fig. 5A,B), they migrate into the host tissue, undergo further morphological and electrophysiological maturation, show long-term survival and functionally integrate into host neural circuits (Kriks et al., 2011; Reddington et al., 2014; Zhu et al., 2016). The intrinsic, human-specific molecular clock of development and maturation is thus recapitulated *in vitro* and retained upon transplantation into the mouse or rat cortex (Espuny-Camacho et al., 2013; Suzuki and Vanderhaeghen, 2015). Using such transplantation experiments, disease mechanisms can be elucidated by studying the development, migration, integration and physiological function of neurons *in vivo* (Espuny-Camacho et al., 2017). In an example of this approach, iPSC-derived glial progenitor cells, created from cells obtained from a schizophrenia patient, were transplanted into the mouse brain to generate a 'human iPSC glial mouse chimera' (Windrem et al., 2017). This chimeric model revealed a role for oligodendrocytes and astrocytes in the aetiology of schizophrenia. Behavioural experiments even showed patient-like changes in the host mice following the transplantation of patient-derived cells, such as increased anxiety and anhedonia, and disturbed social interaction and sleep-wake rhythm.

As mentioned above, a key limitation of organoids is their lack of vascularization. A recent study attempted to overcome this by mixing iPSC-derived tissue-specific progenitors with endothelial cells and mesenchymal stem cells and transplanting the resulting organ buds into mouse hosts (Takebe et al., 2015). The subsequent vascularisation of the transplanted organ buds increased their self-organisation capacity and enabled the transplanted tissues to successfully mature, marking a step towards the generation of functional complex organs. A method for implanting human cerebral organoids into the adult mouse brain has also been described (Mansour et al., 2018). The transplanted organoids integrate into the host brain and became vascularized, with grafted neurons establishing functional synaptic connections with host neurons and responding to physiological stimuli.

Thus, by combining human cell-based *in vitro* models with mouse *in vivo* models, it is possible to generate physiological environments that can facilitate research into the human-specific mechanisms of cortical development and also enable improved disease modelling. As mentioned in previous sections, however, a transitional and combinatorial approach is needed in order to validate basic mechanisms identified in *in vivo* animal models.

Modelling human neurodevelopmental disorders *in vitro*

In recent years, the *in vitro* systems discussed above have been applied to model various NMDs and MCDs, as well as other neurological disorders in which aberrant neuronal migration might be implicated. 2D iPSC models, for example, have been used to shed light on the mechanisms that underlie certain human neurological disorders, such as schizophrenia (Brennand et al., 2011), bipolar disorder (Madison et al., 2015; Mertens et al., 2015), and Rett syndrome (Marchetto et al., 2010), and have helped researchers to analyse important cell processes, such as gene expression, cell morphology and motility, neuronal excitability, and synapse formation (Flaherty and Brennand, 2017; Wen et al., 2016). Both 2D and 3D models have been applied to investigate species-specific differences in cortical development, specifically between humans and non-human primates (Mora-Bermúdez et al., 2016; Otani et al., 2016). In addition, 3D models can be used to elucidate the effects of genomic variants between humans and their hominid ancestors (Cohen, 2018; Hajdinjak et al., 2018), and to address the regulation of cortical folding (Karzbrun et al., 2018; Li et al., 2017). They have also served to model human neuropsychiatric disorders and MCDs, e.g. microcephaly (Cugola et al., 2016; Gabriel et al., 2016, 2017; Lancaster et al., 2013; Li et al., 2017; Ming et al., 2016) (summary Table S1). Importantly, many of these studies have provided novel insights into how aberrant neuronal migration might contribute to human disease.

Cerebral spheroids, organoids and assembloids have been applied by several groups to investigate the pathophysiology of ASD (recently reviewed by Ilieva et al., 2018). For example, the upregulation of forkhead box G1 (*FOXG1*) expression, accelerated cell cycle progression and decrease in cell cycle length, enhanced synaptic maturation and overproduction of inhibitory GABAergic neurons were all identified in patient-derived forebrain spheroids (Mariani et al., 2015). Timothy syndrome, a rare disorder in which ASD and epilepsy can be observed, has also been modelled using forebrain assembloids (Birey et al., 2017). This study identified cell-autonomous defects in the saltatory migration of cortical interneurons that were derived from Timothy syndrome patients, which could be restored pharmacologically (Birey et al., 2017). Taken together, these two novel studies suggest that neuronal migration and perhaps the (consequent) imbalance of excitatory and inhibitory neurons are at the basis of ASD (Table S1).

Classic NMDs have also been modelled using 3D human *in vitro* approaches (Table S1). Many of these studies have focused on lissencephaly, as the lissencephalic mouse brain cannot serve as a model system owing to its intrinsic physiological lack of folds. Of note, three recent studies, using different complementary organoid technologies, have identified several novel factors that contribute to this disease. In the first study (Bershteyn et al., 2017), cerebral organoids were generated using lissencephaly patient-derived iPSCs, with the patient carrying a heterozygous 17p13.3 deletion that results in Miller-Dieker syndrome (MDS), the most severe form of lissencephaly, which features epilepsy and intellectual disability. The organoids that are generated from the patient-derived iPSCs recapitulate specific cellular phenotypes that have been previously identified in mouse models of this syndrome, e.g. spindle and migration defects. However, the organoids also reveal some additional human-specific features, including severe apoptosis and the increased horizontal division of aRG, resulting in more neurogenic aRG divisions, overproduction of deep-layer neurons and smaller organoid size. The nuclei of aRG are less elongated, consistent with a reduced tension during nucleokinesis, and bRG show a cell-type-specific mitotic defect, which causes delayed cell division. A second study used forebrain-specific organoids to elucidate the mechanisms that underlie MDS (Iefremova et al., 2017). In support of the findings reported from mouse models of this syndrome, and in line with the findings reported by Bershteyn et al. (2017), this study found that aRG show reduced expansion (but no increase in apoptosis), resulting from a transition to more asymmetrical divisions and leading to premature neurogenesis. In addition, the microtubule network of aRG in patient organoids is altered and truncated in appearance, with reduced extension towards

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the basal membrane. Altered expression of cell adhesion molecules added to the disruption of cortical niche architecture, which led to a non-cell-autonomous disturbance of the N-cadherin/β-catenin signalling axis. The third study, using an 'organoids-on-a-chip' approach (Karzbrun et al., 2018), found that, in contrast to control organoids, CRISPR/Cas9-generated *LIS1* (+/−) mutant organoids show fewer convolutions, leading to a decreased gyrification index that is consistent with the lissencephaly patient phenotype. Using atomic force microscopy, the researchers found mutant cells to be softer and to swell less than control S-phase cells, indicating defective interkinetic nuclear migration.

Foetal alcohol syndrome (FAS) has also been modelled *in vitro* by treating cerebral organoids with ethanol (Lancaster et al., 2017; Zhu et al., 2017). This results in smaller cortical-like regions and cortical plate disruption, with ectopic clusters of neurons present at the organoid surface or in the VZ (reminiscent of cobblestone lissencephaly and PH). Importantly, disrupted leading processes – which are needed for neuronal locomotion – were identified as the cause of these aberrant features (Lancaster et al., 2017). These studies also found that reduced NPC proliferation, increased cell death and premature neural differentiation, with a concomitant increase in glutamatergic neurons, might underlie the excitation/inhibition imbalance that causes, for example, the hyperactivity symptoms observed in FAS patients.

Finally, cerebral organoids can also be used to identify a cellular role for candidate causative genes that have been identified in patients with MCDs. In a recent study, cerebral organoids were used to confirm the phenotype seen in a mouse KD model of *MOB2*, which is associated with PH (O'Neill et al., 2018). As in the KD mouse model, defects in cilia number were observed in cerebral organoids, which highlights the importance of proper MOB2 levels for cilia maintenance and neuronal positioning in human neurons. Taken together, these studies exemplify how cerebral organoids can serve both to reveal human-specific roles of known disease-associated genes, adding human-specific aspects to the knowledge gained from *in vivo* models, and to decipher new candidate causative genes and their human-specific mechanisms of action.

Conclusions and future perspectives

As we have highlighted here, recent studies of *in vivo* and *in vitro* models of cortical development have provided important insights into the role played by neuronal migration, both in the context of normal development and in the case of human neuronal disorders. Of particular importance for future research in this field are recent advances in cerebral organoid technology that aim to improve the reproducibility and patterning of organoids. This has been achieved using various biomaterials (Lancaster et al., 2017; Zhu et al., 2017) in combination with instructive factors that mimic the morphogen gradients that pattern axis formation *in vivo*, and via improved modelling of the basement membrane for the establishment of functional radial units (Kadoshima et al., 2013; Krefft et al., 2018; Lancaster et al., 2017). Until recently, cerebral organoids were suggested to lack microglia, which are of non-ectodermal origin (Paşca, 2018). However, it is has been reported (Ormel et al., 2018) that mesoderm-derived progenitor cells that are innately present in cerebral organoids (Quadrato et al., 2017) can differentiate to mature microglia that transcriptionally resemble adult human microglia and acquire typical ramified morphology and microglial functions. Cerebral organoids are thus more 'complete' than had previously been thought, and represent a valuable and highly accessible tool with which to study neuron-glia interactions in normal and diseased human brain development.

Nevertheless, organoids are not without limitations, and they clearly represent a simplification of *in vivo* neural tissue, being relatively immature, small and heterogeneous (Camp et al., 2015; Jabaudon and Lancaster, 2018; Paşca, 2018; Quadrato et al., 2017). This creates uncertainty about the specificity of the established neuronal connections, especially as input and output organs are missing. In addition, the proportion of astrocytes in cerebral organoids is lower than in primary tissue and endothelial cells are missing, as well as white matter regions, meninges and a circulation. In addition to modelling developmental axes and morphogen gradients in organoids, the recapitulation of the ECM and perineuronal nets needs to be tackled in the future. These are human-specific and of importance during a crucial window of development, as well as for adult neuronal plasticity (Galtrey and Fawcett, 2007). Despite these limitations, cerebral organoids have successfully been used to uncover human-specific aspects of cortical development and have helped to elucidate mechanisms that underlie NMDs – especially when combined with *in vivo* models. Exploiting all of the described model systems will be important for advancing our knowledge of neuronal migration, NMDs and MCDs, and of neuronal development and disorders more broadly.

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Competing interests

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Supplementary information

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References

Aiken, J., Buscaglia, G., Bates, E. A. and Moore, J. K. (2017). The α-tubulin gene TUBA1A in brain development: a key ingredient in the neuronal isotype blend. *J. Dev. Biol.* **5**, 8.

Alcantara, D., Timms, A. E., Gripp, K., Baker, L., Park, K., Collins, S., Cheng, C., Stewart, F., Mehta, S. G., Saggar, A. et al. (2017). Mutations of AKT3 are associated with a wide spectrum of developmental disorders including extreme megalencephaly. *Brain* **140**, 2610-2622.

Alkuraya, F. S., Cai, X., Emery, C., Mochida, G. H., Al-Dosari, M. S., Felie, J. M., Hill, R. S., Barry, B. J., Partlow, J. N., Gascon, G. G. et al. (2011). Human mutations in NDE1 cause extreme microcephaly with lissencephaly. *Am. J. Hum. Genet.* **88**, 536-547.

Anderson, S. A., Eisenstat, D. D., Shi, L. and Rubenstein, J. L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* **278**, 474-476.

Badouel, C., Zander, M. A., Liscio, N., Bagherie-Lachidan, M., Sopko, R., Coyaud, E., Raught, B., Miller, F. D. and McNeill, H. (2015). Fat1 interacts with Fat4 to regulate neural tube closure, neural progenitor proliferation and apical constriction during mouse brain development. *Development* **142**, 2781-2791.

Bagley, J. A., Reumann, D., Bian, S., Lévi-Strauss, J. and Knoblich, J. A. (2017). Fused cerebral organoids model interactions between brain regions. *Nat. Methods* **14**, 743-751.

Bahi-Buisson, N., Poirier, K., Boddaert, N., Saillour, Y., Castelnau, L., Philip, N., Buyse, G., Villard, L., Joriot, S., Marret, S. et al. (2008). Refinement of cortical dysgeneses spectrum associated with TUBA1A mutations. *J. Med. Genet.* **45**, 647-653.

Bahi-Buisson, N., Poirier, K., Boddaert, N., Fallet-Bianco, C., Specchio, N., Bertini, E., Caglayan, O., Lascelles, K., Elie, C., Rambaud, J. et al. (2010). GPR56-related bilateral frontoparietal polymicrogyria: further evidence for an overlap with the cobblestone complex. *Brain* **133**, 3194-3209.

Bahi-Buisson, N., Souville, I., Fourniol, F. J., Toussaint, A., Moores, C. A., Houdusse, A., Yves Lemaitre, J., Poirier, K., Khalaf-Nazzal, R., Hully, M. et al. (2013). New insights into genotype-phenotype correlations for the doublecortin-related lissencephaly spectrum. *Brain* **136**, 223-244.

Bardón-Cancho, E. J., Muñoz-Jiménez, L., Vázquez-López, M., Ruíz-Martín, Y., García-Morín, M. and Barredo-Valderrama, E. (2014). Periventricular nodular heterotopia and dystonia due to an ARFGEF2 mutation. *Pediatr. Neurol.* **51**, 461-464.

Barkovich, A. J. and Kuzniecky, R. I. (2000). Gray matter heterotopia. *Neurology* **55**, 1603-1608.

Barkovich, A. J., Guerrini, R., Kuzniecky, R. I., Jackson, G. D. and Dobyns, W. B. (2012). A developmental and genetic classification for malformations of cortical development: update 2012. *Brain* **135**, 1348-1369.

Beltrán-Valero de Bernabé, D., Currier, S., Steinbrecher, A., Celli, J., van Beusekom, E., van der Zwaag, B., Kayserili, H., Merlini, L., Chitayat, D., Dobyns, W. B. et al. (2002). Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. *Am. J. Hum. Genet.* **71**, 1033-1043.

Bershteyn, M., Nowakowski, T. J., Pollen, A. A., Di Lullo, E., Nene, A., Wynshaw-Boris, A. and Kriegstein, A. R. (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.

Betizeau, M., Cortay, V., Patti, D., Pfister, S., Gautier, E., Bellemin-Ménard, A., Afanassieff, M., Huissoud, C., Douglas, R. J., Kennedy, H. et al. (2013). Precursor diversity and complexity of lineage relationships in the outer subventricular zone of the primate. *Neuron* **80**, 442-457.

Bilgüvar, K., Öztürk, A. K., Louvi, A., Kwan, K. Y., Choi, M., Tatli, B., Yalnizoğlu, D., Tüysüz, B., Çağlayan, A. O., Gökben, S. et al. (2010). Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. *Nature* **467**, 207-210.

Birey, F., Andersen, J., Makinson, C. D., Islam, S., Wei, W., Huber, N., Fan, H. C., Metzler, K. R. C., Panagiotakos, G., Thom, N. et al. (2017). Assembly of functionally integrated human forebrain spheroids. *Nature* **545**, 54-59.

Bizzotto, S. and Francis, F. (2015). Morphological and functional aspects of progenitors perturbed in cortical malformations. *Front. Cell. Neurosci.* **9**, 30.

Bizzotto, S., Uzquiano, A., Dingli, F., Ershov, D., Houllier, A., Arras, G., Richards, M., Loew, D., Minc, N., Croquelois, A. et al. (2017). Eml1 loss impairs apical progenitor spindle length and soma shape in the developing cerebral cortex. *Sci. Rep.* **7**, 17308.

Borrell, V. and Reillo, I. (2012). Emerging roles of neural stem cells in cerebral cortex development and evolution. *Dev. Neurobiol.* **72**, 955-971.

Brennand, K. J., Simone, A., Jou, J., Gelboin-Burkhart, C., Tran, N., Sangar, S., Li, Y., Mu, Y., Chen, G. Yu, D. et al. (2011). Modelling schizophrenia using human induced pluripotent stem cells. *Nature* **473**, 221-225.

Broix, L., Jagline, H., Ivanova, E. L., Schmucker, S., Drouot, N., Clayton-Smith, J., Pagnamenta, A. T., Metcalfe, K. A., Isidor, B., Louvier, U. W. et al. (2016). Mutations in the HECT domain of NEDD4L lead to AKT-mTOR pathway deregulation and cause periventricular nodular heterotopia. *Nat. Genet.* **48**, 1349-1358.

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.

Cappello, S., Böhringer, C. R. J., Bergami, M., Conzelmann, K.-K., Ghanem, A., Tomassy, G. S., Arlotta, P., Mainardi, M., Allegra, M., Caleo, M. et al. (2012). A radial glia-specific role of RhoA in double cortex formation. *Neuron* **73**, 911-924.

Cappello, S., Gray, M. J., Badouel, C., Lange, S., Einsiedler, M., Srour, M., Chitayat, D., Hamdan, F. F., Jenkins, Z. A., Morgan, T. et al. (2013). Mutations in genes encoding the cadherin receptor-ligand pair DCHS1 and FAT4 disrupt cerebral cortical development. *Nat. Genet.* **45**, 1300-1308.

Carabalona, A., Beguin, S., Pallesi-pocachard, E., Buhler, E., Pellegrino, C., Arnaud, K., Hubert, P., Oualha, M., Siffroi, J. P., Khantane, S. et al. (2012). A glial origin for periventricular nodular heterotopia caused by impaired expression of Filamin-A. *Hum. Mol. Genet.* **21**, 1004-1017.

Chae, T. H., Kim, S., Marz, K. E., Hanson, P. I. and Walsh, C. A. (2004). The hyh mutation uncovers roles for αSnap in apical protein localization and control of neural cell fate. *Nat. Genet.* **36**, 264-270.

Chambers, S. M., Fasano, C. A., Papapetrou, E. P., Tomishima, M., Sadelain, M. and Studer, L. (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* **27**, 275-280.

Chang, B. S., Ly, J., Appignani, B., Bodell, A., Apse, K. A., Ravenscroft, R. S., Sheen, V. L., Doherty, M. J., Hackney, D. B., O'Connor, M. et al. (2005). Reading impairment in the neuronal migration disorder of periventricular nodular heterotopia. *Neurology* **64**, 799-803.

Charvet, C. J., Striedter, G. F. and Finlay, B. L. (2011). Evo-devo and brain scaling: candidate developmental mechanisms for variation and constancy in vertebrate brain evolution. *Brain. Behav. Evol.* **78**, 248-257.

Cohen, J. (2018). Neanderthal brain organoids come to life. *Science* **360**, 1284-1284.

Colombo, E., Collombat, P., Colasante, G., Bianchi, M., Long, J., Mansouri, A., Rubenstein, J. L. R. and Broccoli, V. (2007). Inactivation of Arx, the murine ortholog of the X-linked lissencephaly with ambiguous genitalia gene, leads to severe disorganization of the ventral telencephalon with impaired neuronal migration and differentiation. *J. Neurosci.* **27**, 4786-4798.

Conti, V., Carabalona, A., Pallesi-Pocachard, E., Parrini, E., Leventer, R. J., Buhler, E., McGillivray, G., Michel, F. J., Striano, P., Mei, D. et al. (2013). Periventricular heterotopia in 6q terminal deletion syndrome: Role of the C6orf70 gene. *Brain* **136**, 3378-3394.

Cugola, F. R., Fernandes, I. R., Russo, F. B., Freitas, B. C., Dias, J. L. M., Guimarães, K. P., Benazzato, C., Almeida, N., Pignatari, G. C., Romero, S. et al. (2016). The Brazilian Zika virus strain causes birth defects in experimental models. *Nature* **534**, 267-271.

Cushion, T. D., Dobyns, W. B., Mullins, J. G. L., Stoodley, N., Chung, S.-K., Fry, A. E., Hehr, U., Gunny, R., Aylsworth, A. S., Prabhakar, P. et al. (2013). Overlapping cortical malformations and mutations in TUBB2{B} and TUBA1{A}. *Brain* **136**, 536-548.

de Juan Romero, C., Bruder, C., Tomasello, U., Sanz-Anquela, J. M. and Borrell, V. (2015). Discrete domains of gene expression in germinal layers distinguish the development of gyrencephaly. *EMBO J.* **34**, 1859-1874.

Dehay, C. and Kennedy, H. (2007). Cell-cycle control and cortical development. *Nat. Rev. Neurosci.* **8**, 438-450.

Dehay, C., Kennedy, H. and Kosik, K. S. (2015). The outer subventricular zone and primate-specific cortical complexification. *Neuron* **85**, 683-694.

del Toro, D., Ruff, T., Cederfjäll, E., Villalba, A., Seyit-Bremer, G., Borrell, V. and Klein, R. (2017). Regulation of cerebral cortex folding by controlling neuronal migration via FLRT adhesion molecules. *Cell* **169**, 621-635.e16.

Dennis, M. Y. and Eichler, E. E. (2016). Human adaptation and evolution by segmental duplication. *Curr. Opin. Genet. Dev.* **41**, 44-52.

des Portes, V., Pinard, J. M., Billuart, P., Vinet, M. C., Koulakoff, A., Carrié, A., Gelot, A., Dupuis, E., Motte, J., Berwald-Netter, Y. et al. (1998). A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. *Cell* **92**, 51-61.

Di Lullo, E. and Kriegstein, A. R. (2017). The use of brain organoids to investigate neural development and disease. *Nat. Rev. Neurosci.* **18**, 573-584.

Dubeau, F., Tampieri, D., Lee, N., Andermann, E., Carpenter, S., Leblanc, R., Olivier, A., Radtke, R., Villemure, J. G. and Andermann, F. (1995). Periventricular and subcortical nodular heterotopia. A study of 33 patients. *Brain* **118**, 1273-1287.

Dulabon, L., Olson, E. C., Taglienti, M. G., Eisenhuth, S., McGrath, B., Walsh, C. A., Kreidberg, J. A. and Anton, E. S. (2000). Reelin binds alpha3beta1 integrin and inhibits neuronal migration. *Neuron* **27**, 33-44.

Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K. and 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.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T. and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472**, 51-56.

Elkabetz, Y., Panagiotakos, G., Al Shamy, G., Socci, N. D. and Tabar, V. (2008). Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. **22**, 152-165.

Epi4K Consortium, **Epilepsy Phenome/Genome Project**, **Allen, A. S., Berkovic, S. F., Cossette, P., Delanty, N., Dlugos, D., Eichler, E. E., Epstein, M. P., Glauser, T. et al.** (2013). De novo mutations in epileptic encephalopathies. *Nature* **501**, 217-221.

Espuny-Camacho, I., Michelsen, K. A., Gall, D., Linaro, D., Hasche, A., Bonnefont, J., Bali, C., Orduz, D., Bilheu, A., Herpoel, A. et al. (2013). Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. *Neuron* **77**, 440-456.

Espuny-Camacho, I., Arranz, A. M., Fiers, M., Snellinx, A., Ando, K., Munck, S., Bonnefont, J., Lambot, L., Corthout, N., Omodho, L. et al. (2017). Hallmarks of Alzheimer's disease in stem-cell-derived human neurons transplanted into mouse brain. *Neuron* **93**, 1066-1081.e8.

Evans, M. J. and Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**, 154-156.

Farhan, S. M. K., Nixon, K. C. J., Everest, M., Edwards, T. N., Long, S., Segal, D., Knip, M. J., Arts, H. H., Chakrabarti, R., Wang, J. et al. (2017). Identification of a novel synaptic protein, TMTC3, involved in periventricular nodular heterotopia with intellectual disability and epilepsy. *Hum. Mol. Genet.* **26**, 4278-4289.

Faulkner, N. E., Dujardin, D. L., Tai, C.-Y., Vaughan, K. T., O'Connell, C. B., Wang, Y. and Vallee, R. B. (2000). A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function. *Nat. Cell Biol.* **2**, 784-791.

Fiddes, I. T., Lodewijk, G. A., Mooring, M., Bosworth, C. M., Ewing, A. D., Mantalas, G. L., Novak, A. M., van den Bout, A., Bishara, A., Rosenkrantz, J. L. et al. (2018). Human-specific NOTCH2NL genes affect notch signaling and cortical neurogenesis. *Cell* **173**, 1356-1369.e22.

Fietz, S. A. and Huttner, W. B. (2011). Cortical progenitor expansion, self-renewal and neurogenesis-a polarized perspective. *Curr. Opin. Neurobiol.* **21**, 23-35.

Fietz, S. A., Kelava, I., Vogt, J., Wilsch-Bräuninger, M., Stenzel, D., Fish, J. L., Corbeil, D., Riehn, A., Distler, W., Nitsch, R. et al. (2010). OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nat. Neurosci.* **13**, 690-699.

Fish, J. L., Dehay, C., Kennedy, H. and Huttner, W. B. (2008). Making bigger brains-the evolution of neural-progenitor-cell division. *J. Cell Sci.* **121**, 2783-2793.

Flaherty, E. K. and Brennand, K. J. (2017). Using hiPSCs to model neuropsychiatric copy number variations (CNVs) has potential to reveal underlying disease mechanisms. *Brain Res.* **1655**, 283-293.
Florio, M., Albert, M., Taverna, E., Namba, T., Brandl, H., Lewitus, E., Haffner, C., Sykes, A., Wong, F. K., Peters, J. et al. (2015). Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. *Science* **347**, 1465-1470.
Florio, M., Borrell, V. and Huttner, W. B. (2017). Human-specific genomic signatures of neocortical expansion. *Curr. Opin. Neurobiol.* **42**, 33-44.
Fox, J. W., Lamperti, E. D., Ekşioğlu, Y. Z., Hong, S. E., Feng, Y., Graham, D. A., Scheffer, I. E., Dobyns, W. B., Hirsch, B. A., Radtke, R. A. et al. (1998). Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* **21**, 1315-1325.
Francis, F., Koulakoff, A., Boucher, D., Chafey, P., Schaar, B., Vinet, M.-C., Friocourt, G., McDonnell, N., Reiner, O., Kahn, A. et al. (1999). Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron* **23**, 247-256.
Francis, F., Meyer, G., Fallet-Bianco, C., Moreno, S., Kappeler, C., Socorro, A. C., Tuy, F. P. D., Beldjord, C. and Chelly, J. (2006). Human disorders of cortical development: from past to present. *Eur. J. Neurosci.* **23**, 877-893.
Gabriel, E., Wason, A., Ramani, A., Gooi, L. M., Keller, P., Pozniakovsky, A., Poser, I., Noack, F., Telugu, N. S., Calegari, F. et al. (2016). CPAP promotes timely cilium disassembly to maintain neural progenitor pool. *EMBO J.* **35**, 803-819.
Gabriel, E., Ramani, A., Karow, U., Gottardo, M., Natarajan, K., Gooi, L. M., Goranci-Buzhala, G., Krut, O., Peters, F., Nikolic, M. et al. (2017). Recent Zika virus isolates induce premature differentiation of neural progenitors in human brain organoids. *Cell Stem Cell* **20**, 397-406.e5.
Galtrey, C. M. and Fawcett, J. W. (2007). The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. *Brain Res. Rev.* **54**, 1-18.
Gaspard, N., Bouschet, T., Hourez, R., Dimidschstein, J., Naeije, G., van den Ameele, J., Espuny-Camacho, I., Herpoel, A., Passante, L., Schiffmann, S. N. et al. (2008). An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature* **455**, 351-357.
Georges-Labouesse, E., Mark, M., Messaddeq, N. and Gansmüller, A. (1998). Essential role of α6 integrins in cortical and retinal lamination. *Curr. Biol.* **8**, 983-986.
Gertz, C. C. and Kriegstein, A. R. (2015). Neuronal migration dynamics in the developing Ferret cortex. *J. Neurosci.* **35**, 14307-14315.
Gil-Sanz, C., Landeira, B., Ramos, C., Costa, M. R. and Muller, U. (2014). Proliferative defects and formation of a double cortex in mice lacking Mltt4 and Cdh2 in the dorsal telencephalon. *J. Neurosci.* **34**, 10475-10487.
Gleeson, J. G., Allen, K. M., Fox, J. W., Lamperti, E. D., Berkovic, S., Scheffer, I., Cooper, E. C., Dobyns, W. B., Minnerath, S. R., Ross, M. E. et al. (1998). Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell* **92**, 63-72.
Golan, M. H., Mane, R., Molczadzki, G., Zuckerman, M., Kaplan-Louson, V., Huleihel, M. and Perez-Polo, J. R. (2009). Impaired migration signaling in the hippocampus following prenatal hypoxia. *Neuropharmacology* **57**, 511-522.
Götz, M. and Huttner, W. B. (2005). The cell biology of neurogenesis. *Nat. Rev. Mol. Cell Biol.* **6**, 777-788.
Gressens, P., Kosofsky, B. E. and Evrard, P. (1992). Cocaine-induced disturbances of corticogenesis in the developing murine brain. *Neurosci. Lett.* **140**, 113-116.
Guerrini, R. and Dobyns, W. B. (2014). Malformations of cortical development: clinical features and genetic causes. *Lancet Neurol.* **13**, 710-726.
Guerrini, R. and Parrini, E. (2010). Neuronal migration disorders. *Neurobiol. Dis.* **38**, 154-166.
Hajdinjak, M., Fu, Q., Hübner, A., Petr, M., Mafessoni, F., Grote, S., Skoglund, P., Narasimham, V., Rougier, H., Crevecoeur, I. et al. (2018). Reconstructing the genetic history of late Neanderthals. *Nature* **555**, 652-656.
Hansen, D. V., Lui, J. H., Parker, P. R. and Kriegstein, A. R. (2010). Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* **464**, 554-561.
Hartmann, D., De Strooper, B. and Saftig, P. (1999). Presenilin-1 deficiency leads to loss of Cajal-Retzius neurons and cortical dysplasia similar to human type 2 lissencephaly. *Curr. Biol.* **9**, 719-727.
Heinzen, E. L., O'Neill, A. C., Zhu, X., Allen, A. S., Bahlo, M., Chelly, J., Chen, M. H., Dobyns, W. B., Freytag, S., Guerrini, R. et al. (2018). De novo and inherited private variants in MAP1B in periventricular nodular heterotopia. *PLoS Genet.* **14**, e1007281.
Hippenmeyer, S., Youn, Y. H., Moon, H. M., Miyamichi, K., Zong, H., Wynshaw-Boris, A. and Luo, L. (2010). Genetic mosaic dissection of Lis1 and Ndel1 in neuronal migration. *Neuron* **68**, 695-709.
Hirota, Y. and Nakajima, K. (2017). Control of neuronal migration and aggregation by reelin signaling in the developing cerebral cortex. *Front. Cell Dev. Biol.* **5**, 40.
Holtfreter, J. (1944). Neural differentiation of ectoderm through exposure to saline solution. *J. Exp. Zool.* **95**, 307-343.
Hong, S. E., Shugart, Y. Y., Huang, D. T., Shahwan, S. A., Grant, P. E., Hourihane, J. O. B., Martin, N. D. T. and Walsh, C. A. (2000). Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat. Genet.* **26**, 93-96.
Horesh, D., Sapir, T., Francis, F., Wolf, S. G., Caspi, M., Elbaum, M., Chelly, J. and Reiner, O. (1999). Doublecortin, a stabilizer of microtubules. *Hum. Mol. Genet.* **8**, 1599-1610.
Iefremova, V., Manikakis, G., Krefft, O., Jabali, A., Weynans, K., Wilkens, R., Marsoner, F., Brändl, B., Müller, F.-J., Koch, P. et al. (2017). An organoid-based model of cortical development identifies non-cell-autonomous defects in Wnt signaling contributing to Miller-Dieker syndrome. *Cell Rep.* **19**, 50-59.
Ilieva, M., Fex Svenningsen, Å., Thorsen, M. and Michel, T. M. (2018). Psychiatry in a dish: stem cells and brain organoids modeling autism spectrum disorders. *Biol. Psychiatry* **83**, 558-568.
Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y., Narzisi, G., Leotta, A. et al. (2012). De novo gene disruptions in children on the autistic spectrum. *Neuron* **74**, 285-299.
Jabaudon, D. and Lancaster, M. (2018). Exploring landscapes of brain morphogenesis with organoids. *Development* **145**, dev172049.
Jamuar, S. S. and Walsh, C. A. (2015). Genomic variants and variations in malformations of cortical development. *Pediatr. Clin. North Am.* **62**, 571-585.
Jerber, J., Zaki, M. S., Al-Aama, J. Y., Rosti, R. O., Ben-Omran, T., Dikoglu, E., Silhavy, J. L., Caglar, C., Musaev, D., Albrecht, B. et al. (2016). Biallelic mutations in TMTC3, encoding a transmembrane and TPR-containing protein, lead to cobblestone lissencephaly. *Am. J. Hum. Genet.* **99**, 1181-1189.
Jo, J., Xiao, Y., Sun, A. X., Cukuroglu, E., Tran, H.-D., Göke, J., Tan, Z. Y., Saw, T. Y., Tan, C.-P., Lokman, H. et al. (2016). Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. *Cell Stem Cell* **19**, 248-257.
Johnson, M. B., Sun, X., Kodani, A., Borges-Monroy, R., Girskis, K. M., Ryu, S. C., Wang, P. P., Patel, K., Gonzalez, D. M., Woo, Y. M. et al. (2018). Aspm knockout ferret reveals an evolutionary mechanism governing cerebral cortical size. *Nature* **556**, 370-375.
Ju, X.-C., Hou, Q.-Q., Sheng, A.-L., Wu, K.-Y., Zhou, Y., Jin, Y., Wen, T., Yang, Z., Wang, X. and Luo, Z.-G. (2016). The hominoid-specific gene TBC1D3 promotes generation of basal neural progenitors and induces cortical folding in mice. *eLife* **5**, e18197.
Kadoshima, T., Sakaguchi, H., Nakano, T., Soen, M., Ando, S., Eiraku, M. and 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. USA* **110**, 20284-20289.
Kakita, A., Wakabayashi, K., Su, M., Piao, Y.-S. and Takahashi, H. (2001). Experimentally induced leptomeningeal glioneuronal heterotopia and underlying cortical dysplasia of the lateral limbic area in rats treated transplacentally with methylmercury. *J. Neuropathol. Exp. Neurol.* **60**, 768-777.
Kandel, E. R. and Squire, L. R. (2000). Neuroscience: breaking down scientific barriers to the study of brain and mind. *Science* **290**, 1113-1120.
Karaca, E., Harel, T., Pehlivan, D., Jhangiani, S. N., Gambin, T., Coban Akdemir, Z., Gonzaga-Jauregui, C., Erdin, S., Bayram, Y., Campbell, I. M. et al. (2015). Genes that affect brain structure and function identified by rare variant analyses of Mendelian neurologic disease. *Neuron* **88**, 499-513.
Karzbrun, E., Kshirsagar, A., Cohen, S. R., Hanna, J. H. and Reiner, O. (2018). Human brain organoids on a chip reveal the physics of folding. *Nat. Phys.* **14**, 515-522.
Kato, M., Das, S., Petras, K., Kitamura, K., Morohashi, K., Abuelo, D. N., Barr, M., Bonneau, D., Brady, A. F., Carpenter, N. J. et al. (2004). Mutations of ARX are associated with striking pleiotropy and consistent genotype-phenotype correlation. *Hum. Mutat.* **23**, 147-159.
Kawasaki, H., Toda, T. and Tanno, K. (2013). In vivo genetic manipulation of cortical progenitors in gyrencephalic carnivores using in utero electroporation. *Biol. Open* **2**, 95-100.
Keays, D. A., Tian, G., Poirier, K., Huang, G.-J., Siebold, C., Cleak, J., Oliver, P. L., Fray, M., Harvey, R. J., Molnár, Z. et al. (2007). Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell* **128**, 45-57.
Kielar, M., Tuy, F. P. D., Bizzotto, S., Lebrand, C., De Juan Romero, C., Poirier, K., Oegema, R., Mancini, G. M., Bahi-Buisson, N., Olaso, R. et al. (2014). Mutations in Eml1 lead to ectopic progenitors and neuronal heterotopia in mouse and human. *Nat. Neurosci.* **17**, 923-933.
Kitamura, K., Yanazawa, M., Sugiyama, N., Miura, H., Iizuka-Kogo, A., Kusaka, M., Omichi, K., Suzuki, R., Kato-Fukui, Y., Kamiirisa, K. et al. (2002). Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat. Genet.* **32**, 359-369.
Kothare, S. V., VanLandingham, K., Armon, C., Luther, J. S., Friedman, A. and Radtke, R. A. (1998). Seizure onset from periventricular nodular heterotopias: depth-electrode study. *Neurology* **51**, 1723-1727.
Krefft, O., Jabali, A., Iefremova, V., Koch, P. and Ladewig, J. (2018). Generation of standardized and reproducible forebrain-type cerebral organoids from human induced pluripotent stem cells. *J. Vis. Exp.* **131**, e56768.

Kriegstein, A. and Alvarez-Buylla, A. (2009). The glial nature of embryonic and adult neural stem cells. *Annu. Rev. Neurosci.* **32**, 149-184.
Kriks, S., Shim, J.-W., Piao, J., Ganat, Y. M., Wakeman, D. R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A. et al. (2011). Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* **480**, 547-551.
Labelle-Dumais, C., Dilworth, D. J., Harrington, E. P., de Leau, M., Lyons, D., Kabaeva, Z., Manzini, M. C., Dobyns, W. B., Walsh, C. A., Michele, D. E. et al. (2011). COL4A1 mutations cause ocular dysgenesis, neuronal localization defects, and myopathy in mice and Walker-Warburg syndrome in humans. *PLoS Genet.* **7**, e1002062.
Lancaster, M. A. and Knoblich, J. A. (2014). Generation of cerebral organoids from human pluripotent stem cells. *Nat. Protoc.* **9**, 2329-2340.
Lancaster, M. A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L. S., Hurles, M. E., Homfray, T., Penninger, J. M., Jackson, A. P. and Knoblich, J. A. (2013). Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373-379.
Lancaster, M. A., Corsini, N. S., Wolfinger, S., Gustafson, E. H., Phillips, A. W., Burkard, T. R., Otani, T., Livesey, F. J. and Knoblich, J. A. (2017). Guided self-organization and cortical plate formation in human brain organoids. *Nat. Biotechnol.* **35**, 659-666.
Lee, C.-T., Chen, J., Kindberg, A. A., Bendriem, R. M., Spivak, C. E., Williams, M. P., Richie, C. T., Handreck, A., Mallon, B. S., Lupica, C. R. et al. (2017). CYP3A5 mediates effects of cocaine on human neocorticogenesis: studies using an in vitro 3D self-organized hPSC model with a single cortex-like unit. *Neuropsychopharmacology* **42**, 774-784.
Levine, A. J. and Brivanlou, A. H. (2007). Proposal of a model of mammalian neural induction. *Dev. Biol.* **308**, 247-256.
Li, S., Jin, Z., Koirala, S., Bu, L., Xu, L., Hynes, R. O., Walsh, C. A., Corfas, G. and Piao, X. (2008). GPR56 regulates pial basement membrane integrity and cortical lamination. *J. Neurosci.* **28**, 5817-5826.
Li, Y., Wang, J., Zhou, Y., Li, D. and Xiong, Z.-Q. (2015). Rcan1 deficiency impairs neuronal migration and causes periventricular heterotopia. *J. Neurosci.* **35**, 610-620.
Li, Y., Muffat, J., Omer, A., Bosch, I., Lancaster, M. A., Sur, M., Gehrke, L., Knoblich, J. A. and Jaenisch, R. (2017). Induction of expansion and folding in human cerebral organoids. *Cell Stem Cell* **20**, 385-396.e3.
Lindborg, B. A., Brekke, J. H., Vegoe, A. L., Ulrich, C. B., Haider, K. T., Subramaniam, S., Venhuizen, S. L., Eide, C. R., Orchard, P. J., Chen, W. et al. (2016). Rapid induction of cerebral organoids from human induced pluripotent stem cells using a chemically defined hydrogel and defined cell culture medium. *Stem Cells Transl. Med.* **5**, 970-979.
Liu, J. S. (2011). Molecular genetics of neuronal migration disorders. *Curr. Neurol. Neurosci. Rep.* **11**, 171-178.
Liu, Z., Li, X., Zhang, J.-T., Cai, Y.-J., Cheng, T.-L., Cheng, C., Wang, Y., Zhang, C.-C., Nie, Y.-H., Chen, Z.-F. et al. (2016). Autism-like behaviours and germline transmission in transgenic monkeys overexpressing MeCP2. *Nature* **530**, 98-102.
Longman, C., Brockington, M., Torelli, S., Jimenez-Mallebrera, C., Kennedy, C., Khalil, N., Feng, L., Saran, R. K., Voit, T., Merlini, L. et al. (2003). Mutations in the human LARGE gene cause MDC1D, a novel form of congenital muscular dystrophy with severe mental retardation and abnormal glycosylation of alpha-dystroglycan. *Hum. Mol. Genet.* **12**, 2853-2861.
Lu, J. and Sheen, V. (2005). Periventricular heterotopia. *Epilepsy Behav.* **7**, 143-149.
Lu, J., Tiao, G., Folkerth, R., Hecht, J., Walsh, C. and Sheen, V. (2006). Overlapping expression of ARFGEF2 and filamin A in the neuroependymal lining of the lateral ventricles: Insights into the cause of periventricular heterotopia. *J. Comp. Neurol.* **494**, 476-484.
Lu, I.-L., Chen, C., Tung, C.-Y., Chen, H.-H., Pan, J.-P., Chang, C.-H., Cheng, J.-S., Chen, Y.-A., Wang, C.-H., Huang, C.-W. et al. (2018). Identification of genes associated with cortical malformation using a transposon-mediated somatic mutagenesis screen in mice. *Nat. Commun.* **9**, 2498.
Lui, J. H., Hansen, D. V. and Kriegstein, A. R. (2011). Development and evolution of the human neocortex. *Cell* **146**, 18-36.
Lukaszewicz, A., Savatier, P., Cortay, V., Giroud, P., Huissoud, C., Berland, M., Kennedy, H. and Dehay, C. (2005). G1 phase regulation, area-specific cell cycle control, and cytoarchitectonics in the primate cortex. *Neuron* **47**, 353-364.
Madison, J. M., Zhou, F., Nigam, A., Hussain, A., Barker, D. D., Nehme, R., van der Ven, K., Hsu, J., Wolf, P., Fleishman, M. et al. (2015). Characterization of bipolar disorder patient-specific induced pluripotent stem cells from a family reveals neurodevelopmental and mRNA expression abnormalities. *Mol. Psychiatry* **20**, 703-717.
Maeta, K., Edamatsu, H., Nishihara, K., Ikutomo, J., Bilasy, S. E. and Kataoka, T. (2016). Crucial role of Rapgef2 and Rapgef6, a family of guanine nucleotide exchange factors for Rap1 small GTPase, in formation of apical surface adherens junctions and neural progenitor development in the mouse cerebral cortex. *eNeuro* **3**, ENEURO.0142-16.2016..
Mansour, A. A. F., Gonçalves, J. T., Bloyd, C. W., Li, H., Fernandes, S., Quang, D., Johnston, S., Parylak, S. L., Jin, X. and Gage, F. H. (2018). An in vivo model of functional and vascularized human brain organoids. *Nat. Biotechnol.* **36**, 432-441.
Marchetto, M. C. N., Carromeu, C., Acab, A., Yu, D., Yeo, G. W., Mu, Y., Chen, G., Gage, F. H. and Muotri, A. R. (2010). A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* **143**, 527-539.
Mariani, J., Simonini, M. V., Palejev, D., Tomasini, L., Coppola, G., Szekely, A. M., Horvath, T. L. and Vaccarino, F. M. (2012). Modeling human cortical development in vitro using induced pluripotent stem cells. *Proc. Natl. Acad. Sci. USA* **109**, 12770-12775.
Mariani, J., Coppola, G., Zhang, P., Abyzov, A., Provini, L., Tomasini, L., Amenduni, M., Szekely, A., Palejev, D., Wilson, M. et al. (2015). FOXG1-dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders. *Cell* **162**, 375-390.
Marín, O. (2013). Cellular and molecular mechanisms controlling the migration of neocortical interneurons. *Eur. J. Neurosci.* **38**, 2019-2029.
Martens, G. J. M. and van Loo, K. M. J. (2007). Genetic and environmental factors in complex neurodevelopmental disorders. *Curr. Genomics* **8**, 429-444.
Mattson, S. N. and Riley, E. P. (1998). A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol. *Alcohol. Clin. Exp. Res.* **22**, 279-294.
Mercuri, E., Messina, S., Bruno, C., Mora, M., Pegoraro, E., Comi, G. P., D'Amico, A., Aiello, C., Biancheri, R., Berardinelli, A. et al. (2009). Congenital muscular dystrophies with defective glycosylation of dystroglycan: a population study. *Neurology* **72**, 1802-1809.
Mertens, J., Wang, Q.-W., Kim, Y., Yu, D. X., Pham, S., Yang, B., Zheng, Y., Diffenderfer, K. E., Zhang, J., Soltani, S. et al. (2015). Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature* **527**, 95-99.
Ming, G., Tang, H. and Song, H. (2016). Advances in Zika virus research: stem cell models, challenges, and opportunities. *Cell Stem Cell* **19**, 690-702.
Moon, H. M., Youn, Y. H., Pemble, H., Yingling, J., Wittmann, T. and Wynshaw-Boris, A. (2014). LIS1 controls mitosis and mitotic spindle organization via the LIS1-NDEL1-dynein complex. *Hum. Mol. Genet.* **23**, 449-466.
Mora-Bermúdez, F., Badsha, F., Kanton, S., Camp, J. G., Vernot, B., Köhler, K., Voigt, B., Okita, K., Maricic, T., He, Z. et al. (2016). Differences and similarities between human and chimpanzee neural progenitors during cerebral cortex development. *eLife* **5**, e18683.
Muguruma, K., Nishiyama, A., Kawakami, H., Hashimoto, K. and Sasai, Y. (2015). Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep.* **10**, 537-550.
Nadarajah, B., Brunstrom, J. E., Grutzendler, J., Wong, R. O. L. and Pearlman, A. L. (2001). Two modes of radial migration in early development of the cerebral cortex. *Nat. Neurosci.* **4**, 143-150.
Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M. and Sasai, Y. (2012). Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* **10**, 771-785.
Nicholas, A. K., Khurshid, M., Désir, J., Carvalho, O. P., Cox, J. J., Thornton, G., Kausar, R., Ansar, M., Ahmad, W., Verloes, A. et al. (2010). WDR62 is associated with the spindle pole and is mutated in human microcephaly. *Nat. Genet.* **42**, 1010-1014.
Niewmierzycka, A., Mills, J., St-Arnaud, R., Dedhar, S. and Reichardt, L. F. (2005). Integrin-linked kinase deletion from mouse cortex results in cortical lamination defects resembling cobblestone lissencephaly. *J. Neurosci.* **25**, 7022-7031.
Noctor, S. C., Martínez-Cerdeño, V., Ivic, L. and Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* **7**, 136-144.
Nosten-Bertrand, M., Kappeler, C., Dinocourt, C., Denis, C., Germain, J., Phan Dinh Tuy, F., Verstraeten, S., Alvarez, C., Métin, C., Chelly, J. et al. (2008). Epilepsy in Dcx knockout mice associated with discrete lamination defects and enhanced excitability in the hippocampus. *PLoS ONE* **3**, e2473.
Nowakowski, T. J., Pollen, A. A., Sandoval-Espinosa, C. and Kriegstein, A. R. (2016). Transformation of the radial glia scaffold demarcates two stages of human cerebral cortex development. *Neuron* **91**, 1219-1227.
Oegema, R., Baillat, D., Schot, R., van Unen, L. M., Brooks, A., Kia, S. K., Hoogeboom, A. J. M., Xia, Z., Li, W., Cesaroni, M. et al. (2017). Human mutations in integrator complex subunits link transcriptome integrity to brain development. *PLoS Genet.* **13**, e1006809.
Oliveira Melo, A. S., Malinger, G., Ximenes, R., Szejnfeld, P. O., Alves Sampaio, S. and Bispo de Filippis, A. M. (2016). Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet. Gynecol.* **47**, 6-7.
O'Neill, A. C., Kyrousi, C., Einsiedler, M., Burtscher, I., Drukker, M., Markie, D. M., Kirk, E. P., Götz, M., Robertson, S. P. and Cappello, S. (2018). Mob2 insufficiency disrupts neuronal migration in the developing cortex. *Front. Cell. Neurosci.* **12**, 57.
Ormel, P. R., Vieira de Sá, R., van Bodegraven, E. J., Karst, H., Harschnitz, O., Sneeboer, M. A. M., Johansen, L. E., van Dijk, R. E., Scheefhals, N., Berdenis

van Berlekom, A. et al. (2018). Microglia innately develop within cerebral organoids. *Nat. Commun.* **9**, 4167.
Otani, T., Marchetto, M. C., Gage, F. H., Simons, B. D. and Livesey, F. J. (2016). 2D and 3D stem cell models of primate cortical development identify species-specific differences in progenitor behavior contributing to brain size. *Cell Stem Cell* **18**, 467-480.
Pang, T., Atefy, R. and Sheen, V. (2008). Malformations of cortical development. *Neurologist* **14**, 181-191.
Pankratz, M. T., Li, X.-J., LaVaute, T. M., Lyons, E. A., Chen, X. and Zhang, S.-C. (2007). Directed neural differentiation of human embryonic stem cells via an obligated primitive anterior stage. *Stem Cells* **25**, 1511-1520.
Parrini, E., Ramazzotti, A., Dobyns, W. B., Mei, D., Moro, F., Veggiotti, P., Marini, C., Brilstra, E. H., Dalla Bernardina, B., Goodwin, L. et al. (2006). Periventricular heterotopia: phenotypic heterogeneity and correlation with Filamin A mutations. *Brain* **129**, 1892-1906.
Paşca, S. P. (2018). The rise of three-dimensional human brain cultures. *Nature* **553**, 437.
Paşca, S. P., Portmann, T., Voineagu, I., Yazawa, M., Shcheglovitov, A., Paşca, A. M., Cord, B., Palmer, T. D., Chikahisa, S., Nishino, S. et al. (2011). Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nat. Med.* **17**, 1657-1662.
Paşca, S. P., Panagiotakos, G. and Dolmetsch, R. E. (2014). Generating human neurons in vitro and using them to understand neuropsychiatric disease. *Annu. Rev. Neurosci.* **37**, 479-501.
Paşca, A. M., Sloan, S. A., Clarke, L. E., Tian, Y., Makinson, C. D., Huber, N., Kim, C. H., Park, J.-Y., O'Rourke, N. A., Nguyen, K. D. et al. (2015). Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat. Methods* **12**, 671-678.
Peyre, E., Silva, C. G. and Nguyen, L. (2015). Crosstalk between intracellular and extracellular signals regulating interneuron production, migration and integration into the cortex. *Front. Cell. Neurosci.* **9**, 129.
Pilz, G.-A., Shitamukai, A., Reillo, I., Pacary, E., Schwausch, J., Stahl, R., Ninkovic, J., Snippert, H. J., Clevers, H., Godinho, L. et al. (2013). Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. *Nat. Commun.* **4**, 2125.
Poduri, A., Evrony, G. D., Cai, X. and Walsh, C. A. (2013). Somatic mutation, genomic variation, and neurological disease. *Science* **341**, 1237758-1237758.
Poirier, K., Keays, D. A., Francis, F., Saillour, Y., Bahi, N., Manouvrier, S., Fallet-Bianco, C., Pasquier, L., Toutain, A., Tuy, F. P. D. et al. (2007). Large spectrum of lissencephaly and pachygyria phenotypes resulting from de novo missense mutations in tubulin alpha 1A (*TUBA1A*). *Hum. Mutat.* **28**, 1055-1064.
Poirier, K., Lebrun, N., Broix, L., Tian, G., Saillour, Y., Boscheron, C., Parrini, E., Valence, S., Pierre, B. S., Oger, M. et al. (2013). Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat. Genet.* **45**, 639-647.
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.
Qian, X., Nguyen, H. N., Song, M. M., Hadiono, C., Ogden, S. C., Hammack, C., Yao, B., Hamersky, G. R., Jacob, F., Zhong, C. et al. (2016). Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell*.
Quadrato, G., Nguyen, T., Macosko, E. Z., Sherwood, J. L., Min Yang, S., Berger, D. R., Maria, N., Scholvin, J., Goldman, M., Kinney, J. P. et al. (2017). Cell diversity and network dynamics in photosensitive human brain organoids. *Nature* **545**, 48-53.
Radmanesh, F., Caglayan, A. O., Silhavy, J. L., Yilmaz, C., Cantagrel, V., Omar, T., Rosti, B., Kaymakcalan, H., Gabriel, S., Li, M. et al. (2013). Mutations in LAMB1 cause cobblestone brain malformation without muscular or ocular abnormalities. *Am. J. Hum. Genet.* **92**, 468-474.
Rakic, P. (2009). Evolution of the neocortex: a perspective from developmental biology. *Nat. Rev. Neurosci.* **10**, 724-735.
Ramos, R. L. (2005). Heterotopia formation in rat but not mouse neocortex after RNA interference knockdown of DCX. *Cereb. Cortex* **16**, 1323-1331.
Ran, F. A., Hsu, P. P. D., Wright, J., Agarwala, V., Scott, D. A. and Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.* **8**, 2281-2308.
Reddington, A. E., Rosser, A. E. and Dunnett, S. B. (2014). Differentiation of pluripotent stem cells into striatal projection neurons: a pure MSN fate may not be sufficient. *Front. Cell. Neurosci.* **8**, 398.
Reillo, I., de Juan Romero, C., García-Cabezas, M. Á. and Borrell, V. (2011). A role for intermediate radial Glia in the tangential expansion of the mammalian cerebral cortex. *Cereb. Cortex* **21**, 1674-1694.
Reiner, O., Carrozzo, R., Shen, Y., Wehnert, M., Faustinella, F., Dobyns, W. B., Caskey, C. T. and Ledbetter, D. H. (1993). Isolation of a Miller-Dicker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature* **364**, 717-721.
Renner, M., Lancaster, M. A., Bian, S., Choi, H., Ku, T., Peer, A., Chung, K. and Knoblich, J. A. (2017). Self-organized developmental patterning and differentiation in cerebral organoids. *EMBO J.* **36**, 1316-1329.
Reynolds, B. A. and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707-1710.
Rigamonti, A., Repetti, G. G., Sun, C., Price, F. D., Reny, D. C., Rapino, F., Weisinger, K., Benkler, C., Peterson, Q. P., Davidow, L. S. et al. (2016). Large-scale production of mature neurons from human pluripotent stem cells in a three-dimensional suspension culture system. *Stem Cell Rep.* **6**, 993-1008.
Romero, D. M., Bahi-Buisson, N. and Francis, F. (2018). Genetics and mechanisms leading to human cortical malformations. *Semin. Cell Dev. Biol.* **76**, 33-75.
Roper, S. N. (1998). In utero irradiation of rats as a model of human cerebrocortical dysgenesis: a review. *Epilepsy Res.* **32**, 63-74.
Sakaguchi, H., Kadoshima, T., Soen, M., Narii, N., Ishida, Y., Ohgushi, M., Takahashi, J., Eiraku, M. and Sasai, Y. (2015). Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. *Nat. Commun.* **6**, 8896.
Sarkisian, M. R., Bartley, C. M., Chi, H., Nakamura, F., Hashimoto-Torii, K., Torii, M., Flavell, R. A. and Rakic, P. (2006). MEKK4 signaling regulates filamin expression and neuronal migration. *Neuron* **52**, 789-801.
Sasaki, E., Suemizu, H., Shimada, A., Hanazawa, K., Oiwa, R., Kamioka, M., Tomioka, I., Sotomaru, Y., Hirakawa, R., Eto, T. et al. (2009). Generation of transgenic non-human primates with germline transmission. *Nature* **459**, 523-527.
Schlotawa, L., Hotz, A., Zeschnigk, C., Hartmann, B., Gärtner, J. and Morris-Rosendahl, D. (2013). Cerebellar ataxia, mental retardation and dysequilibrium syndrome 1 (CAMRQ1) caused by an unusual constellation of VLDLR mutation. *J. Neurol.* **260**, 1678-1680.
Schmid, M.-T. T., Weinandy, F., Wilsch-Bräuninger, M., Huttner, W. B., Cappello, S., Götz, M., Wilsch-Brauninger, M., Huttner, W. B., Cappello, S. and Gotz, M. (2014). The role of alpha-E-catenin in cerebral cortex development: radial glia specific effect on neuronal migration. *Front. Cell Neurosci.* **8**, 215.
Sekine, K., Honda, T., Kawauchi, T., Kubo, K.-I. and Nakajima, K. (2011). The outermost region of the developing cortical plate is crucial for both the switch of the radial migration mode and the Dab1-dependent "inside-out" lamination in the neocortex. *J. Neurosci.* **31**, 9426-9439.
Sheen, V. L. (2014). Filamin A mediated Big2 dependent endocytosis. *Tissue Barriers* **2**, 1-6.
Sheen, V. L., Ganesh, V. S., Topcu, M., Sebire, G., Bodell, A., Hill, R. S., Grant, P. E., Shugart, Y. Y., Imitola, J., Khoury, S. J. et al. (2004a). Mutations in ARFGEF2 implicate vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex. *Nat. Genet.* **36**, 69-76.
Sheen, V. L., Basel-Vanagaite, L., Goodman, J. R., Scheffer, I. E., Bodell, A., Ganesh, V. S., Ravenscroft, R., Hill, R. S., Cherry, T. J., Shugart, Y. Y. et al. (2004b). Etiological heterogeneity of familial periventricular heterotopia and hydrocephalus. *Brain Dev.* **26**, 326-334.
Sheen, V. L., Ferland, R. J., Harney, M., Hill, R. S., Neal, J., Banham, A. H., Brown, P., Chenn, A., Corbo, J., Hecht, J. et al. (2006). Impaired proliferation and migration in human Miller-Dieker neural precursors. *Ann. Neurol.* **60**, 137-144.
Shi, Y., Kirwan, P., Smith, J., Robinson, H. P. and Livesey, F. J. (2012). Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. *Nat. Neurosci.* **15**, 477-486, S1.
Shin, H.-W., Shinotsuka, C. and Nakayama, K. (2005). Expression of BIG2 and analysis of its function in mammalian cells. *Methods Enzymol.* **404**, 206-215.
Sicca, F., Kelemen, A., Genton, P., Das, S., Mei, D., Moro, F., Dobyns, W. B. and Guerrini, R. (2003). Mosaic mutations of the LIS1 gene cause subcortical band heterotopia. *Neurology* **61**, 1042-1046.
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.
Sloan, S. A., Darmanis, S., Huber, N., Khan, T. A., Birey, F., Caneda, C., Reimer, R., Quake, S. R., Barres, B. A. and Paşca, S. P. (2017). Human astrocyte maturation captured in 3D cerebral cortical spheroids derived from pluripotent stem cells. *Neuron* **95**, 779-790.e6.
Smart, I. H. M., Dehay, C., Giroud, P., Berland, M. and 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.
Smith, R. S., Kenny, C. J., Ganesh, V., Jang, A., Borges-Monroy, R., Partlow, J. N., Hill, R. S., Shin, T., Chen, A. Y., Doan, R. N. et al. (2018). Sodium channel SCN3A (NaV1.3) regulation of human cerebral cortical folding and oral motor development. *Neuron* **99**, 905-913.e7.
Sousa, A. M. M., Meyer, K. A., Santpere, G., Gulden, F. O. and Sestan, N. (2017). Evolution of the human nervous system function, structure, and development. *Cell* **170**, 226-247.
Stanwood, G. D., Washington, R. A. and Levitt, P. (2001). Identification of a sensitive period of prenatal cocaine exposure that alters the development of the anterior cingulate cortex. *Cereb. Cortex* **11**, 430-440.
Stouffer, M. A., Golden, J. A. and Francis, F. (2015). Neuronal migration disorders: focus on the cytoskeleton and epilepsy. *Neurobiol. Dis.* **92**, 18-45.

Suga, H., Kadoshima, T., Minaguchi, M., Ohgushi, M., Soen, M., Nakano, T., Takata, N., Wataya, T., Muguruma, K., Miyoshi, H. et al. (2011). Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* **480**, 57-62.
Sullivan, P. F., Daly, M. J. and O'Donovan, M. (2012). Genetic architectures of psychiatric disorders: the emerging picture and its implications. *Nat. Rev. Genet.* **13**, 537-551.
Sun, T. and Hevner, R. F. (2014). Growth and folding of the mammalian cerebral cortex: from molecules to malformations. *Nat. Rev. Neurosci.* **15**, 217-232.
Suzuki, I. K. and Vanderhaeghen, P. (2015). Is this a brain which I see before me? Modeling human neural development with pluripotent stem cells. *Development* **142**, 3138-3150.
Suzuki, I. K., Gacquer, D., Van Heurck, R., Kumar, D., Wojno, M., Bilheu, A., Herpoel, A., Lambert, N., Cheron, J., Polleux, F. et al. (2018). Human-specific NOTCH2NL genes expand cortical neurogenesis through Delta/Notch regulation. *Cell* **173**, 1370-1384.e16.
Tabata, H. and Nakajima, K. (2003). Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. *J. Neurosci.* **23**, 9996-10001.
Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.
Takebe, T., Enomura, M., Yoshizawa, E., Kimura, M., Koike, H., Ueno, Y., Matsuzaki, T., Yamazaki, T., Toyohara, T., Osafune, K. et al. (2015). Vascularized and complex organ buds from diverse tissues via mesenchymal cell-driven condensation. *Cell Stem Cell* **16**, 556-565.
Taverna, E., Götz, M. and Huttner, W. B. (2014). *The Cell Biology of Neurogenesis: Toward an Understanding of the Development and Evolution of the Neocortex*. *Annu. Rev. Cell Dev. Biol.* **30**, 465-502.
Thompson, B. L., Levitt, P. and Stanwood, G. D. (2009). Prenatal exposure to drugs: effects on brain development and implications for policy and education. *Nat. Rev. Neurosci.* **10**, 303-312.
Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S. and Jones, J. M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145-1147.
Trimborn, M., Bell, S. M., Felix, C., Rashid, Y., Jafri, H., Griffiths, P. D., Neumann, L. M., Krebs, A., Reis, A., Sperling, K. et al. (2004). Mutations in microcephalin cause aberrant regulation of chromosome condensation. *Am. J. Hum. Genet.* **75**, 261-266.
Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R. E., Richardson, J. A. and Herz, J. (1999). Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* **97**, 689-701.
Turner, D. A., Baillie-Johnson, P. and Martinez Arias, A. (2016). Organoids and the genetically encoded self-assembly of embryonic stem cells. *BioEssays* **38**, 181-191.
van Reeuwijk, J., Janssen, M., van den Elzen, C., Beltran-Valero de Bernabé, D., Sabatelli, P., Merlini, L., Boon, M., Scheffer, H., Brockington, M., Muntoni, F. et al. (2005). POMT2 mutations cause alpha-dystroglycan hypoglycosylation and Walker-Warburg syndrome. *J. Med. Genet.* **42**, 907-912.
Verloes, A., Di Donato, N., Masliah-Planchon, J., Jongmans, M., Abdul-Raman, O. A., Albrecht, B., Allanson, J., Brunner, H., Bertola, D., Chassaing, N. et al. (2015). Baraitser–Winter cerebrofrontofacial syndrome: delineation of the spectrum in 42 cases. *Eur. J. Hum. Genet.* **23**, 292-301.
Vuillaumier-Barrot, S., Bouchet-Seraphin, C., Chelbi, M., Eude-Caye, A., Charluteau, E., Besson, C., Quentin, S., Devisme, L., Le Bizec, C., Landrieu, P. et al. (2011). Intragenic rearrangements in LARGE and POMGNT1 genes in severe dystroglycanopathies. *Neuromuscul. Disord.* **21**, 782-790.
Watanabe, K., Kamiya, D., Nishiyama, A., Katayama, T., Nozaki, S., Kawasaki, H., Watanabe, Y., Mizuseki, K. and Sasai, Y. (2005). Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat. Neurosci.* **8**, 288-296.
Wen, Z., Christian, K. M., Song, H. and Ming, G. (2016). Modeling psychiatric disorders with patient-derived iPSCs. *Curr. Opin. Neurobiol.* **36**, 118-127.
Windrem, M. S., Osipovitch, M., Liu, Z., Bates, J., Chandler-Militello, D., Zou, L., Munir, J., Schanz, S., McCoy, K., Miller, R. H. et al. (2017). Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia. *Cell Stem Cell* **21**, 195-208.e6.
Wonders, C. P. and Anderson, S. A. (2006). The origin and specification of cortical interneurons. *Nat. Rev. Neurosci.* **7**, 687-696.
Xiang, Y., Tanaka, Y., Patterson, B., Kang, Y.-J., Govindaiah, G., Roselaar, N., Cakir, B., Kim, K.-Y., Lombroso, A. P., Hwang, S.-M. et al. (2017). Fusion of regionally specified hPSC-derived organoids models human brain development and interneuron migration. *Cell Stem Cell* **21**, 383-398.e7.
Yamamoto, T., Kato, Y., Kawaguchi, M., Shibata, N. and Kobayashi, M. (2004). Expression and localization of fukutin, POMGnT1, and POMT1 in the central nervous system: consideration for functions of fukutin. *Med. Electron Microsc.* **37**, 200-207.
Ying, Q.-L., Stavridis, M., Griffiths, D., Li, M. and Smith, A. (2003). Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nat. Biotechnol.* **21**, 183-186.
Yu, T. W., Mochida, G. H., Tischfield, D. J., Sgaier, S. K., Flores-Sarnat, L., Sergi, C. M., Topçu, M., McDonald, M. T., Barry, B. J., Felie, J. M. et al. (2010). Mutations in WDR62, encoding a centrosome-associated protein, cause microcephaly with simplified Gyri and abnormal cortical architecture. *Nat. Genet.* **42**, 1015-1020.
Yu, T. W., Chahrour, M. H., Coulter, M. E., Jiralerspong, S., Okamura-Ikeda, K., Ataman, B., Schmitz-Abe, K., Harmin, D. A., Adli, M., Malik, A. N. et al. (2013). Using whole-exome sequencing to identify inherited causes of autism. *Neuron* **77**, 259-273.
Zhang, S.-C., Wernig, M., Duncan, I. D., Brüstle, O. and Thomson, J. A. (2001). In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat. Biotechnol.* **19**, 1129-1133.
Zhu, X., Need, A. C., Petrovski, S. and Goldstein, D. B. (2014). One gene, many neuropsychiatric disorders: lessons from Mendelian diseases. *Nat. Neurosci.* **17**, 773-781.
Zhu, X., Ai, Z., Hu, X. and Li, T. (2016). Efficient generation of corticofugal projection neurons from human embryonic stem cells. *Sci. Rep.* **6**, 28572.
Zhu, Y., Wang, L., Yin, F., Yu, Y., Wang, Y., Shepard, M. J., Zhuang, Z. and Qin, J. (2017). Probing impaired neurogenesis in human brain organoids exposed to alcohol. *Integr. Biol. (Camb).* **9**, 968-978.