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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

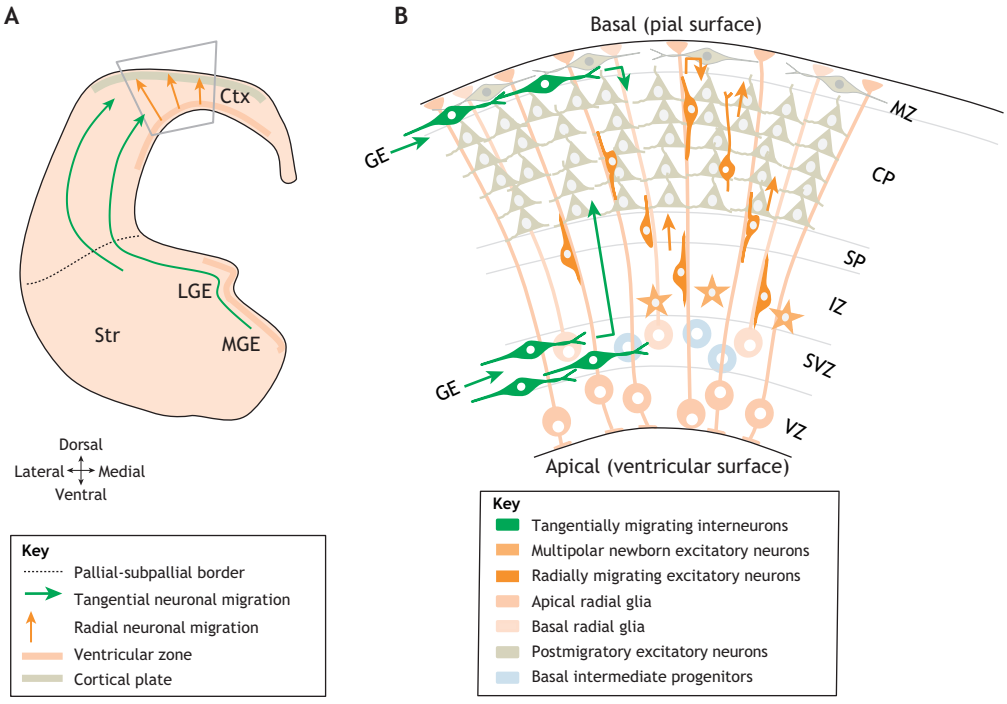

Fig. 1. Mouse cortical development. (A) Schematic of a coronal section of the early developing anterior telencephalon of the mouse at embryonic day 14 of development, showing cortical neurogenesis. The grey boxed area is enlarged in panel B. (B) Schematic of the cell composition of the developing mouse 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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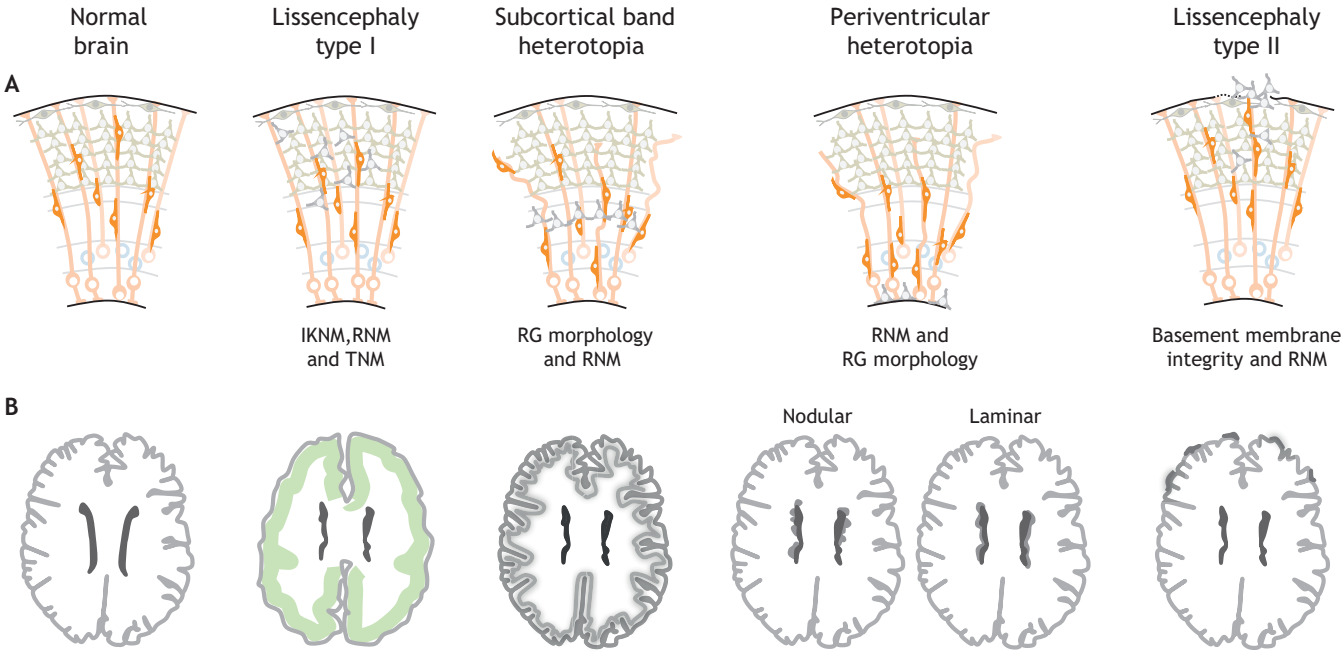

Fig. 2. Cellular and morphological defects associated with neuronal migration disorders. (A) Schematics highlighting the cellular basis of NMDs in the adult human cortex, showing the different cortical layers and neuronal migrations. Single ectopic neurons are shown in grey and the affected structures 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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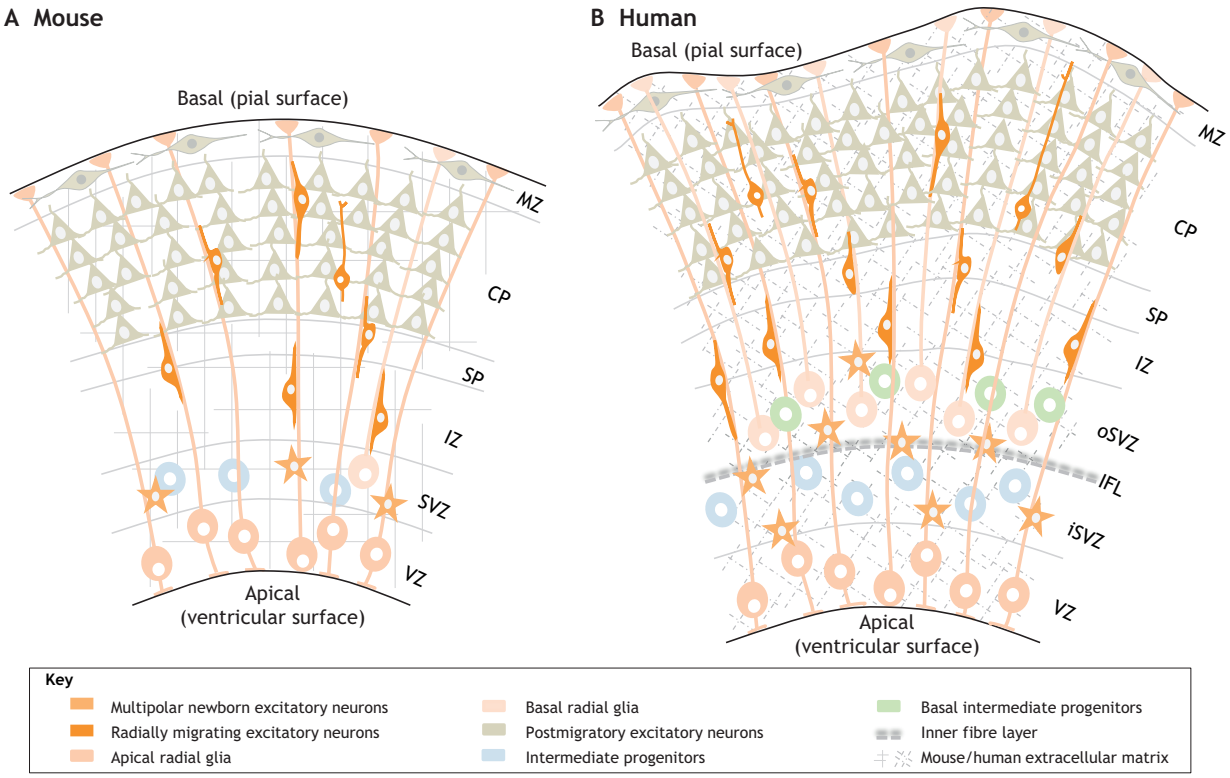

Fig. 3. Mouse and human dorsal cortical development. (A,B) Schematics illustrating the cell composition and ECM in the developing mouse (A) and human (B) dorsal cortex. Cell composition in the developing mouse lissencephalic cerebral cortex is shown (A), with aRG giving rise to IPs, or to neurons directly, 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,

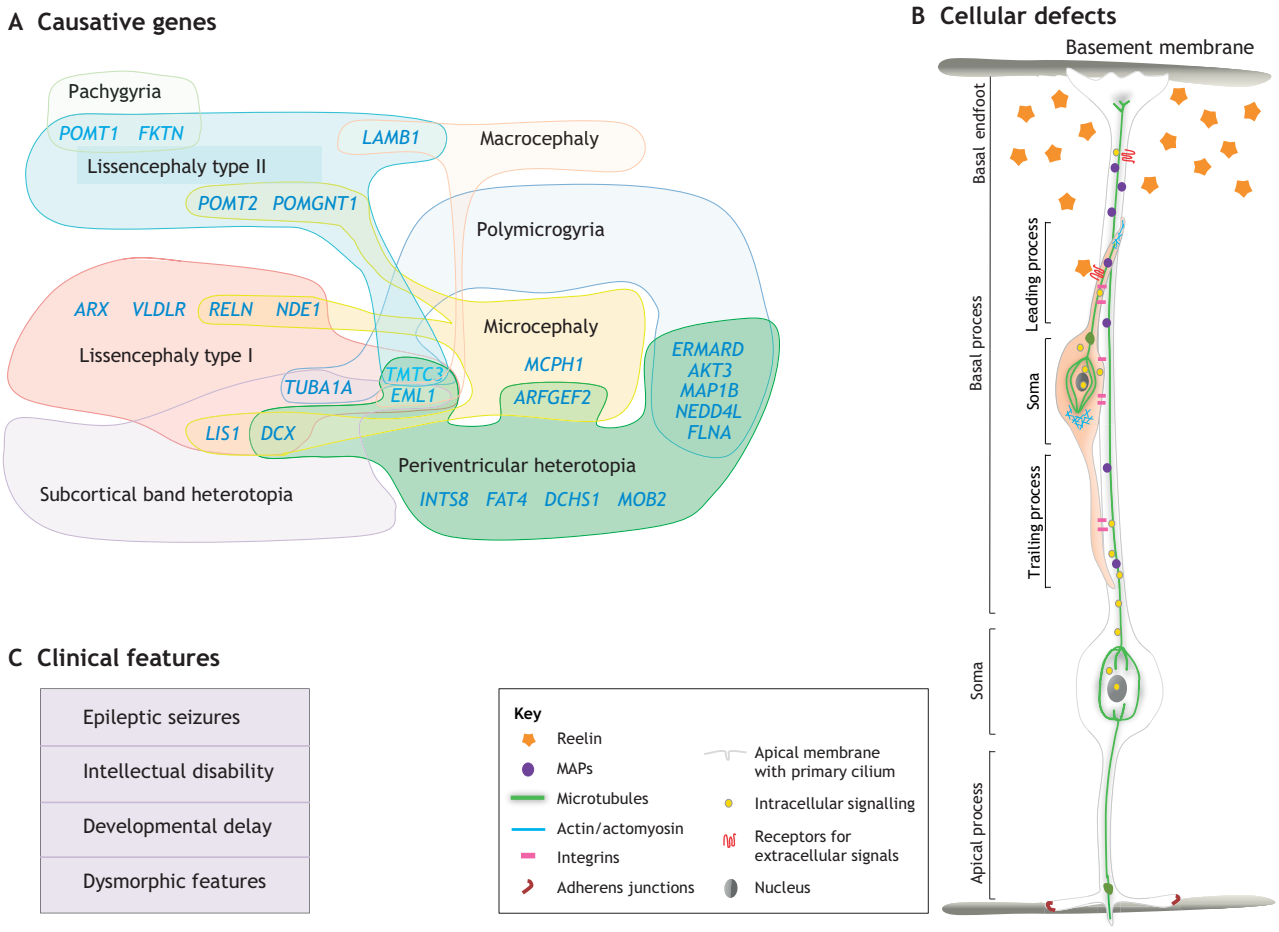

Fig. 4. Heterogeneity and complexity of neuronal migration disorders at the genetic, cellular and clinical levels. (A) NMDs (black) and some of their identified causative genes (blue) are shown; the scheme also depicts the overlap between the differently classified disorders and other malformations of 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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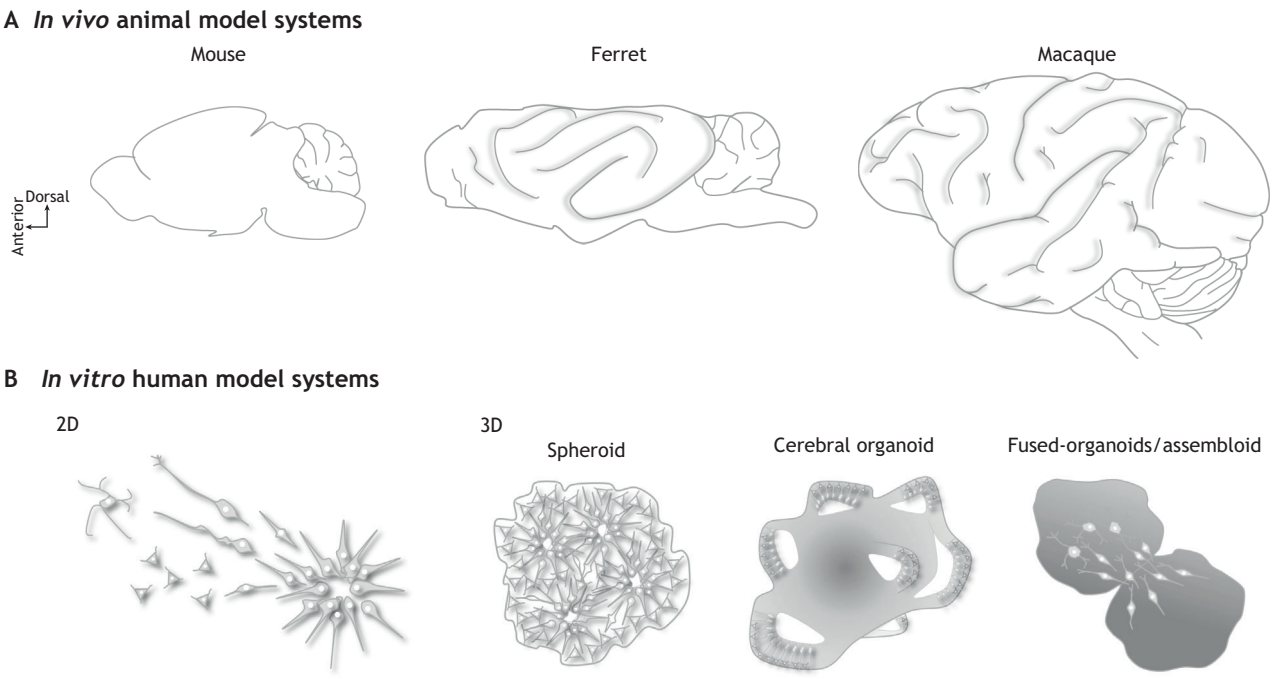

Fig. 5. *In vivo* and *in vitro* model systems of neurodevelopment and neurodevelopmental disorders. (A,B) Schematic depicting animal model systems (A) and pluripotent stem cell-based human model systems (B) of increasing complexity and similarity to the developing human brain. (A) Schematics of mouse, 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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Table S1: 3D *in vitro* human model systems and their applications for investigating neurodevelopmental disorders

Type of organoid	Modelled brain area	Technical aids	Added factors	Protocol advantages	Modelled disorder/phenotype	Identified mechanism	Reference
Undirected	Forebrain (dorsal and ventral, including forebrain organizing centers), midbrain, hindbrain, midbrain-hindbrain boundary, choroid plexus, retina; mesoderm	Matrigel embedding, spinning bioreactor	-	Generation of a variety of brain regions allows exploring human CNS diversity; recapitulation of temporal order of generation of subsequent cell types including neurons, astrocytes and oligodendrocytes. Clearing and 3D reconstruction shows interconnected ventricles (Renner et al., 2017).	**Microcephaly: *CDK5RAP2***	Premature neuronal differentiation: altered spindle orientation of radial glial cells, abundant neuronal outgrowth, smaller organoid and neuroepithelial region size due to decrease in proliferating progenitors	(Lancaster and Knoblich, 2014; Lancaster et al., 2013; Renner et al., 2017)
Undirected	Forebrain, midbrain, hindbrain	Chemically defined hydrogel material, chemically defined culture medium, static culture	-	Generation of a variety of brain regions allows to explore human CNS diversity; chemically defined medium simplifies scalability			(Lindborg et al., 2016)
Undirected	Forebrain, midbrain, hindbrain	Matrigel embedding, spinning bioreactor	-	Culture of sliced organoids shows generation of mature cortical neurons with extended long-range axons with complex branching and projections; also dendritic spines of GABAergic nature are produced	**Microcephaly + Seckel syndrome: *CPAP***	Increase in aRG with vertical cleavage plane, prematurely switching to asymmetric, neurogenic division; increased neuronal differentiation; increased number and length of cilia in aRG show defective cilium disassembly. → Role of cilium regulation in the maintenance of the stem cell pool	(Gabriel et al., 2016) (based on Lancaster and Knoblich, 2014)
Undirected	Forebrain, midbrain, hindbrain	Matrigel embedding, spinning bioreactor	-	Combination with live imaging of organoid slices and sc-RNAseq.; protocol also functions for primate iPSCs: cytoarchitecture, cell type composition, and neurogenic gene expression programs of humans and chimpanzees are remarkably similar	**Differences in cortical development between human, chimpanzee and macaque**	Lengthening of prometa-metaphase in human apical progenitors compared to chimpanzee leads to more proliferative divisions; differentially expressed genes indicate longer neurogenic period in humans. → Cortical progenitor cell clonal output regulates primate cerebral cortex size.	(Mora-Bermúdez et al., 2016) (based on Lancaster and Knoblich, 2014)

Undirected	Forebrain, midbrain, hindbrain, retina (10 clusters of cell types); mesoderm		-	Generation of a variety of brain regions allows to explore human CNS diversity; generation of mature neurons and glia, synaptic junctions, actively firing and light-sensitive retinal cells			(Quadrato et al., 2017)
Undirected	Different regional identities	Matrigel embedding, injection of organoids into Ca-Alginate microfibers	-	Ca-Alginate microfibers increase neuroectoderm induction and reproducibility of organoids	**Fetal alcohol syndrome (microcephaly)**	Reduced cell proliferation, increased cell death; premature neural differentiation with hyper production of glutamatergic neurons; reduced neurite outgrowth; increased proportion of astrocytes; altered Wnt, MAPK and Hippo signalling pathways; ectopic neurons reminiscent of cobblestone lissencephaly	(Zhu et al., 2017)
Undirected	Forebrain, midbrain, hindbrain	Matrigel embedding, orbital shaker	-	Generation of a variety of brain regions allows to explore the role of a newly identified candidate gene	**PH: biallelic mutations in *MOB2* as candidate causative gene**	Increase in the amount of primary cilia per cell upon *MOB2* knockdown → Role of cilium maintenance in neuronal migration; MOB2 is part of the Hippo pathway and phosphorylates FLNA, thereby linking both known PH disease pathways	(O'Neill et al., 2018) (based on Lancaster and Knoblich, 2014)

Slightly directed	Mostly forebrain identity	PLGA fiber microfilaments, matrigel embedding, addition of liquid matrigel to culture medium, spinning bioreactor or orbital shaker	CHIR99021 (3d pulse)	Addition of microfilament fibers results in elongated embryoid bodies and enhanced neuroectoderm formation; generating mostly forebrain tissue while maintaining self-organizing capacity; improved reproducibility and tissue architecture; generation of a basal membrane due to matrigel in the medium: RG processes reach basal membrane and form radial units	**Fetal alcohol syndrome (PH and microcephaly)**	Smaller cortical regions and reduction or complete absence of CP; PH-like heterotopic cluster of neurons; migrating neurons lacking the leading process (required for normal radial migration to the CP), resulting in disruption of locomotion and formation of heterotopic clumps	(Lancaster et al., 2017) (based on Lancaster and Knoblich, 2014)
Slightly directed	Dorsal forebrain	Matrigel embedding, orbital shaker	Dorsomorphin	Generation of larger organoids with folded surface after *PTEN* deletion due to sustained cell-cycle re-entry, expansion of progenitors and delayed neuronal differentiation	**Zika virus-mediated microcephaly**	Zika infection of neural progenitors impairs cortical growth and folding. *PTEN* deletion enhances AKT signaling, causing expansion of VZ, iSVZ, delayed neuronal differentiation and oSVZ and folding	(Li et al., 2017) (based on Lancaster and Knoblich, 2014)
Directed	Dorsal forebrain	Manual rosette selection	bFGF, DKK1 , SB431542 and BMPRIA-Fc	Rosette-based spheroids enable the study of basic concepts of development and disease in a highly reproducible system		Most similar to 8-10 PCW fetal dorsal telencephalon; recapitulation of *in vivo* cytoarchitecture, formation of excitatory neurons of different layer identities, of GABAergic interneurons, of synapses	(Mariani et al., 2012) (based on Eiraku et al., 2008)
Directed	Neocortex	40% oxygen; 2% matrigel dissolved in medium	IWR1e and SB431542; ventralised by SAG	Rolling morphogenesis and curvature with rostro-caudal polarization; cellular complexity and organization resembles early second trimester; appearance of bRG			(Kadoshima et al., 2013)

Directed	Forebrain assembloids: dorsal telencephalon- and ventral telencephalon-like regions	Manual rosette selection	Noggin, DKK1, bFGF, EGF, BDNF, GDNF, ascorbic acid, cAMP	Telencephalic organoids recapitulate transcriptional programs present in mid-fetal human cortical development	**ASD and macrocephaly**	Increased progenitor cell proliferation through decrease in cell cycle length; enhanced synaptic maturation; overproduction of GABAergic inhibitory neurons due to overexpression of transcription factor FOXG1 → Role of FOXG1 in excitation/inhibition imbalance	(Mariani et al., 2015)
Directed	Dorsal forebrain		Dorsomorphin, SB431542; bFGF and EGF; BDNF and NT3	Recapitulate later, mid-fetal stages of post conceptual weeks 19-24 and contain both neurons and non-reactive astrocytes, as well as functional synapses and electrophysiological signatures of network activity; equal proportions of deep and superficial neurons are present in 2.5 months old organoids; greater neuronal maturation in 3D than in 2D			(Paşca et al., 2015)
Directed	Neocortex	1% Matrigel dissolved in medium	IWR1e and SB431542	Species-specific timing of generation of neurons with different layer identities is preserved in organoids; combination with 2D culture for easier identification of underlying mechanisms (clonal analysis)	**Differences in cortical development between human, chimpanzee and macaque**	2D: Faster maturation of macaque neurons than chimpanzee and human; protracted progenitor proliferation in primates: more symmetric divisions in human than macaque cortical progenitors and longer cell-cycle duration in human vs. chimpanzee progenitors	(Otani et al., 2016)

Directed	Forebrain/ midbrain/ hypothalamus	Matrigel embedding; miniaturized spinning bioreactors	Forebrain: Dorsomorphin, A83–01, WNT3A, CHIR99021, SB431542; Midbrain: LDN-193189, SB431542, SHH, FGF-8, purmorphamine, CHIR99021; Hypothalamus: LDN-193189, SHH, SB431542, WNT3A, 1-Thioglycerol, , purmorphamine	Spinning bioreactors improve oxygen and nutrient diffusion to reach larger viable organoids and enable drug testing; production of neurons of all six layer identities that are produced at conserved timing; production of bRG	**Zika virus-mediated microcephaly**	Infection of neural progenitors leads to increased cell death and reduced proliferation, resulting in decreased neuronal layer volume and organoid size resembling microcephaly; enlarged ventricle-like lumen	(Qian et al., 2016)
Directed	Forebrain assembloids: fusion of dorsal telencephalon and ventral telencephalon	Matrigel embedding of dorsally and ventrally pre-patterned organoids for their fusion; orbital shaker	Ventralisation: IWP2, SAG; Dorsal forebrain identity: cyclopamine A	Generation of a dorso-ventral axis enables recapitulation of ventral-to-dorsal GABAergic interneuron migration (timelapse-imaging) and interactions between different brain regions; generation of several interneuron subtypes		Used to study interneuron migration over long distances, decipher if specific molecules act in neuronal migration in cell-autonomous or non-cell-autonomous fashion; useful because many psychiatric diseases, such as schizophrenia, are thought to involve selective deficits in specific interneuron subpopulations; organoid fusion slice culture paradigm in which confocal time-lapse imaging can be used to analyze the short-term dynamics of neuronal cell migration	(Bagley et al., 2017) (modified from Lancaster and Knoblich, 2014)

Directed	Forebrain	Matrigel embedding; shaking culture; 40 % oxygen in the beginning, addition of 2 % liquid matrigel to the medium	Small molecules LDN-193189, A83-01 and IWR-1; plus GSK3β inhibitor CHIR99021 for rescue of Wnt phenotype		**Miller-Dieker-Syndrome (lissencephaly Type I)**	Patient-derived organoids are significantly reduced in size: switch from symmetric to asymmetric cell division of aRG causes reduced expansion and premature neurogenesis; alterations in microtubule network organization in aRG and disruption of cortical niche architecture, including altered expression of cell adhesion molecules; non-cell-autonomous disturbance of the N-cadherin/β-catenin signalling axis	(Iefremova et al., 2017) (based on Kadoshima et al., 2013 that was improved in Krefft et al., 2018)
Directed	Neocortex	40% oxygen; 1% matrigel dissolved in medium	Wnt inhibitor IWR1-ε and TGF-β inhibitor SB431542	Human genetic model may be more sensitive in recapitulating lissencephaly severity as organoids contain bRG and enable genome editing; New assays: culture of organoid slides on matrigel-coated surface enables live imaging of migrating neurons; culture of organoid slices on human fetal cortical tissue enables analysis of cell-autonomous vs non-cell-autonomous mechanisms	**Miller-Dieker-Syndrome (lissencephaly Type I)**	Defective saltatory neuronal migration (reduced speed because of reduced tension during nucleokinesis; more resting timepoints; more tortuous/ tumbling migration); smaller organoid size caused by severe apoptosis of neuroepithelial stem cells accompanied by increased horizontal cell divisions due to dysregulation of mitotic spindles, leading to premature neurogenesis and overproduction of deep-layer neurons; prolonged mitosis of bRG	(Bershteyn et al., 2017) (based on Kadoshima et al., 2013)
Directed	Neocortex	Manual rosette selection; culture of 1 organoid/well in 96-well-plate	SB431542, LDN193189, PD0325901, bFGF, FGF18	Increased reproducibility with one neocortical unit/organoid makes this model useful for pharmacological applications	**Prenatal cocaine-induced impaired brain growth**	Proliferation deficit of neuroepithelial progenitors; premature neuronal differentiation; reduced CP formation	(Lee et al., 2017)

Directed	Forebrain assembloids: fusion of dorsal telencephalon- and ventral telencephalon-like spheroids	Placing of differently patterned spheroids into a common conical tube for their fusion	<u>dorsal forebrain</u>: dorsomorphin, SB431542, EGF, bFGF, BDNF, NT3 <u>ventral forebrain:</u> dorsopmorhin, SB431542, IWP-2, EGF, bFGF, SAG, retinoic acid, allopreganolene, BDNF, NT3	Fusion of dorsal cortical spheroid containing mature glutamatergic projection neurons of all layer identities with ventral telencephalic spheroid containing several types of GABAergic interneurons enables analysis of saltatory interneuron migration from ventral to dorsal part; both compartments produce astrocytes and the subpallial spheroid also produces oligodendrocytes; membrane potentials more mature in assembloids than in single spheroids; synapse formation between GABAergic interneurons and cortical neurons	**Timothy syndrome: ASD and epilepsy**	Cell-autonomous defects in saltatory migration of Timothy-syndrome derived cortical interneurons that could be restored pharmacologically by modulating the mutated L-type calcium channel	(Birey et al., 2017)
Directed	Forebrain	Organoids on a chip: grown in a 150 µm thin micro compartment between a coverslip and a semi-permeable polycarbonate membrane, embedded in matrigel	Ascorbic acid, LIF, bFGF, TGFβ1, IWR, CHIR99021, PD0325901, BIRB796, SP600125, LDN193189	Micro compartment allows tissue expansion only in the x,y dimension and long-term live imaging through the coverslip; surface wrinkles reminiscent of gyri and sulci develop as result of opposing bending and stretching forces from apical surface contraction vs. nuclear swelling in S-phase at basal position of IKNM	**Lissencephaly Type I (*LIS1* +/-)**	Less convolutions in patient organoids based on IKNM differences: softer cells and reduced opposing forces	(Karzbrun et al., 2018)