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Neuronal migration disorders: from genetic diseases to developmental mechanisms

Joseph G. Gleeson and Christopher A. Walsh

Neurons that constitute the cerebral cortex must migrate hundreds of cell-body distances from their place of birth, and through several anatomical boundaries, to reach their final position within the correct cortical layer. Human neurological conditions associated with abnormal neuronal migration, together with spontaneous and engineered mouse mutants, define at least four distinct steps in cortical neuronal migration. Many of the genes that control neuronal migration have strong genetic or biochemical links to the cytoskeleton, suggesting that the field of neuronal migration might be closing in on the underlying cytoskeletal events.

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NEURONS that leave the proliferative region in the developing telencephalon must migrate hundreds of cell-body distances to arrive at their proper position within the developing cortical plate, which gives rise to the six-layered mammalian cerebral cortex. The work of His[1], Ramón y Cajal and others established that cortical neurons originate in the ventricular zone (VZ), migrate towards the pial surface and accumulate below the marginal zone to form the cortical plate. Rakic established that the migration of neurons traveling to the developing cortical plate is closely associated with radial-glial fibers[2]. Although other work[3–5] has shown there are some neurons that do not invariably follow radial glia at all times, the central role of radial glia in organizing cortical neurons is well established.

On reaching the cortex, neurons organize themselves into layers that ultimately form the adult cortex. This process of cortical lamination has been shown to be surprisingly complex. Marín-Padilla described how the earliest neurons form a precocious organization, commonly referred to as the preplate, and that the subsequent cortical plate proper deposits within this preplate[6]. The deposition of newer cortical neurons divides the preplate into an outer, marginal zone (directly beneath the pial surface and basal lamina) and a deeper layer called the subplate[6]. Angevine and Sidman pioneered the birthdating of neurons to establish that the neurons within the cortical plate are arranged in 'inside-out' order[7]. In other words, newly-arrived cortical-plate neurons bypass the subplate, as well as the older cortical plate neurons, so that the newest neurons always add to the cortical plate directly adjacent to the marginal zone. These key observations established the foundation of cortex formation and provided a framework in which to understand mutants of cortex development (Fig. 1).

Neuronal migration to the cortex lends itself to an unusually strong application of genetics, and the study of genetic disorders of cerebral cortical development in humans has played an integral role, melding itself to the study of mouse mutants. The most-familiar and certainly the best-characterized disorder of neuronal

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migration is a naturally occurring mouse mutant, reeler, which, in addition to a severe cerebellar phenotype, displays an inversion of the normal inside-out order of cortical neurons[9]. However, disordered neuronal migration has long been known to underlie several human disorders of cortex development[10], and such conditions collectively account for up to one third of cases of severe epilepsy[11], as well as specific disorders associated with severe mental retardation[12]. The identification of genes for many of these disorders now allows for integration of cortical defects in mice and in humans into a single cellular framework; this framework identifies several key stages in neuronal migration that were not clear before the study of genetic disorders.

Neuronal migration onset: periventricular heterotopia and filamin 1

Neurons are born in the ventricular zone (also known as the proliferative zone or germinal matrix) of the telencephalon. Once a postmitotic neuron is generated, it must migrate away from the ventricular zone towards the developing cortical plate. In the human disorder periventricular heterotopia (PH), a fraction of newly postmitotic neurons appears incapable of leaving the ventricular zone. In the adult, one population of differentiated neurons is present as clumps or nodules along the lining of the VZ, whereas another population migrates normally and completely to form the proper, six-layered cortex[13,14] (Fig. 2a). Thus, the abnormal neurons in PH appear to have a primary defect in neuronal migration onset.

Pedigrees in which PH is present in more than one affected person show 'X-linked dominant inheritance', with affected females displaying PH while affected males appear not to survive gestation[15]. The male–female differences in phenotype presumably reflect the fact that females inherit two X chromosomes, while males inherit a single X chromosome. Females randomly inactivate one X chromosome in each cell that leads to two populations of cells. Females with a mutation in any X-chromosome-linked gene are thus potential mosaics for two populations of neurons: one that possesses a normal allele and one that possesses a mutant allele. PH is therefore likely to be a cell-autonomous mosaic phenotype in females caused by random X-chromosome inactivation[13], presumably because neurons that express genes from the mutant X chromosome fail to migrate, and neurons that express the normal X chromosome migrate properly (Fig. 2b); however, this has not been proven. The major neurological manifestation in females with PH is epilepsy[16], which probably arises from the heterotopic collections of neurons[17]. Despite the collection of neurons that line the lateral ventricle, higher cognitive functions appear to be largely retained in affected individuals[18]. Affected males typically do not survive to term, and die sometime before the time of delivery, possibly because of a coagulation defect[19].

The gene responsible for PH was positionally cloned from Xq28 (Ref. 13) and identified as filamin 1 (*FLN1*)[19], an actin-binding/crosslinking protein, suggesting that the role of FLN1 in neuronal migration might be mediated through modulation of the actin cytoskeleton. The finding that FLN1 co-localizes with actin stress-fibers in cultured primary cortical neurons supports a role in cytoskeletal regulation[19]. *FLN1* is expressed at high levels by cells that line the lateral ventricle, which is expected, based upon the inability of these neurons to migrate in PH (Ref. 19; D. Graham, and C. Walsh, unpublished observations). In addition to its established association with, and its effects on actin, FLN1 binds to other proteins that are possibly involved in neuronal migration, including integrins[20,21], RalA (Ref. 22) and presenilin 1 (Ref. 23). It is not clear which of these potential protein interactions is crucial for the onset of neuronal migration, but based upon the prominent loss-of-function phenotype, the study of *FLN1*, particularly in a mouse model, might help identify other essential factors for the onset of neuronal migration.

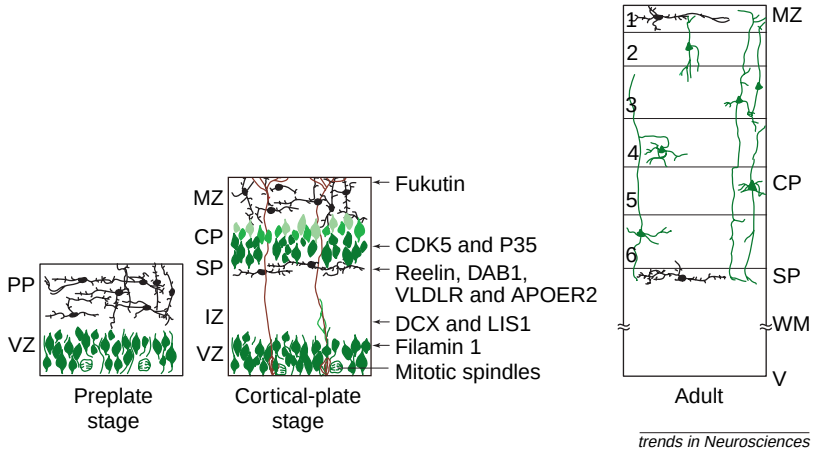

Fig. 1. ***Normal cortical development.*** *Cortical development can be divided into three stages: the preplate stage, the cortical-plate stage and the adult stage. The preplate stage is marked by the formation of the preplate (PP), a plexiform layer that forms around embryonic-day (E) 10–12 in mice and weeks 8–9 in humans. The cortical-plate stage is marked by proliferation of neuroblasts in the ventricular zone (VZ) (indicated by mitotic spindles) and migration of these cells along radial glial fibers (brown processes). Migrating neurons penetrate the preplate, split the preplate into the subplate (SP) and the marginal zone (MZ), and deposit in the cortical plate (CP). Neurons generated later must pass through the subplate and past previously deposited neurons to reach the outer limits of the developing CP (indicated by the progressive lighter shades of green in more-superficial CP neurons). The cortical-plate stage lasts from E10–E17 in mouse and weeks 10–18 in humans. The adult stage is marked by the lamination of the CP into a six-layered structure, and by loss of most of the MZ and SP cells. During the cortical-plate stage, filamin 1 (FLN1) is required for neurons to leave the VZ. Doublecortin (DCX) and LIS1 are required during the ongoing process of migration. Reelin, DAB1, VLDLR and APOER2 (also known as LRP8) are required for penetration of neurons past the subplate. CDK5 and P35 (also known as FCN2) are required for neurons to penetrate through the CP. Abbreviations: IZ, intermediate zone; V, ventricle; WM, white matter.* Modified, with permission, from Ref. 8.

The ongoing process of migration: lissencephaly, double cortex, LIS1 and doublecortin

After neurons exit the ventricular zone, they must migrate long distances towards the cortical plate. This ongoing process of migration appears to be abnormal in two other disorders in which neurons do not remain in the ventricular zone, but instead are capable of migrating partway to the cerebral cortex before arresting[24]. One such disorder in humans is 'type I' lissencephaly (literally, smooth brain, which refers to the smoothening of the contour of the cortex that results from its malformation). Lissencephaly is characterized by a more-or-less four-layered cortex. However, these four layers have no obvious relationship to the normal six layers (except perhaps that the marginal zone in lissencephaly is preserved). In lissencephaly, the majority of cortical neurons are found in the fourth layer (Fig. 3b).

Mutations of at least two genes lead to related forms of lissencephaly, *LIS1* (also known as *PAFAH1β1*) and

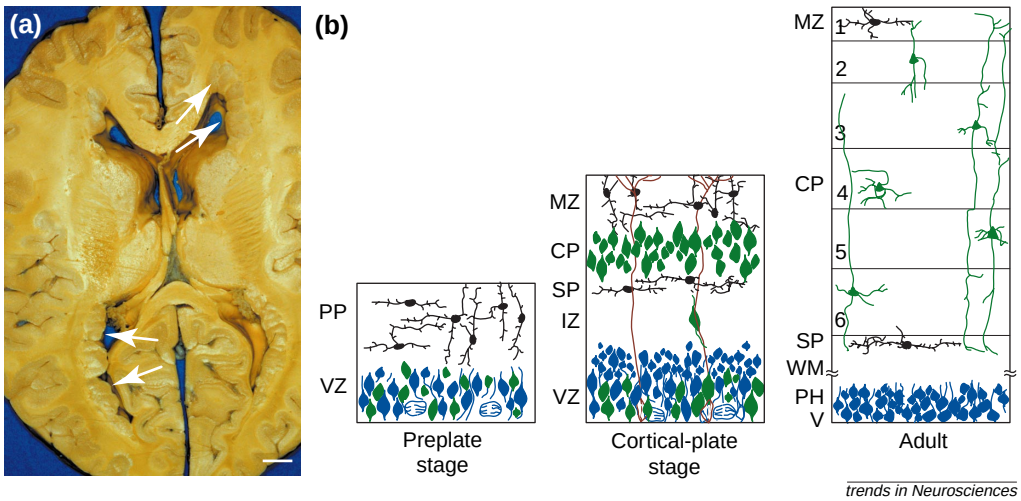

Fig. 2. ***Neuronal migration onset: periventricular heterotopia and filamin 1.*** **(a)** *Gross pathological cortical specimen from a woman with periventricular heterotopia. The key feature is neuron-containing nodules that line the lateral ventricle (arrows) and a normal-appearing outer cortex. Presumably, the neurons lining the lateral ventricle were destined for the cerebral cortex but had a defect in neuronal migration onset.* **(b)** *A model to explain the periventricular heterotopia developmental abnormality. Owing to X-chromosome inactivation, females are mosaics for two populations of cells that normally behave equivalently. In females with a mutation in* FLN1 *on one X chromosome, there is a population of neurons expressing a normal* FLN1 *gene (represented by green cells) and a population expressing a mutant* FLN1 *gene (represented by blue cells). These two populations of cells then segregate during the cortical-plate stage, with the normal cells migrating to the cortical plate and the mutant cells have a primary defect in their ability to migrate from the ventricular zone (VZ). In the adult, the result is a normal outer cortex and a collection of periventricular heterotopic (PH) neurons lining the lateral ventricle (V). The cortical plate (CP) presumably displays a normal 'inside-out' layering, but is not depicted in this model. Abbreviations: IZ, intermediate zone; MZ, marginal zone; PP, preplate; SP, subplate; V, ventricle; WM, white matter. Scale bar, 1 cm.*

doublecortin (*DCX*). The protein products of these two genes are unrelated to one another structurally, and are not known to interact physically. *LIS1* is located in 17p13 (Ref. 25), and was identified by mapping chromosomal deletions in individuals with lissencephaly[26]. Because *LIS1* is an autosomal gene, there are normally two inherited copies. Individuals with chromosome-17 lissencephaly have a mutation in one of these two copies. Therefore, individuals with lissencephaly that is caused by a *LIS1* mutation display haploinsufficiency, which suggests that a 50% decrease in LIS1 protein levels is sufficient for the lissencephaly phenotype.

A second X-chromosome-linked lissencephaly locus causes a phenotype that is nearly indistinguishable from *LIS1* mutations in males, but shows a different phenotype in heterozygous females. Several families have been described in which affected males display lissencephaly and affected females display the related disorder of neuronal migration known as double cortex (DC) or subcortical band heterotopia[27]. In DC, there is a normal six-layered outer cortex and an additional collection of neurons located in the subcortical white matter (Fig. 3d). These families fit an X-chromosome-linked pattern of inheritance, in which affected males have a more-severe phenotype than affected females, again because males have a single X chromosome and females have two X chromosomes. In males with lissencephaly that is caused by a mutation in *DCX*, all neurons express the mutant *DCX* gene, which gives rise to the lissencephaly phenotype. As appears to be true for PH, DC is also generally regarded as a cell-autonomous mosaic phenotype in females, owing to random inactivation of the X chromosome (Fig. 3e). Individuals with lissencephaly typically display severe mental retardation and intractable epilepsy, whereas individuals with DC typically display mild to moderate mental retardation and less-severe epilepsy[27,28].

Studies of a mouse model of lissencephaly, produced by an engineered *Lis1* mutation, suggest that the phenotype is quite distinct from previously described spontaneous or engineered mouse mutants with cortical neuronal migration defects[24], including reeler and its phenocopies, and either the *Cdk5* or *p35* knockouts (see below) (*p35* is also known as *Fcn35*). The cortex from the heterozygous *Lis1* mutant mouse looks relatively more normal than might be expected from the human phenotype, but neuronal birthdating studies indicate poor layer specificity, with some neurons that should be destined for the superficial layers tending to be positioned in deeper layers. Mice that are compound heterozygotes for a null *Lis1* allele and a partial loss-of-function allele die at birth with a severe cortical phenotype, whereas *Lis1* homozygous knockout mice do not survive beyond the blastula stage. Thus, in mice, two copies of *Lis1* are required for proper neuronal migration, and at least one copy is required outside the nervous system for other key events.

Although there are several putative biochemical effects of LIS1, its role in neuronal migration remains uncertain. It has been found to function as the regulatory subunit of intracellular platelet activating factor acetylhydrolase (PAF-AH)[29], which deacetylates and inactivates PAF, a potent proinflammatory phospholipid[30]. PAF itself causes neurite retraction[31,32], suggesting one mechanism of action of LIS1 in neuronal migration. Additionally, LIS1 has been found to co-localize with microtubules and regulate their dynamics[33], and the LIS1 ortholog in *Aspergillus nidulans* is required for microtubule-dependent nuclear movement[34,35], suggesting that its role in neuronal migration might involve regulation of the microtubule cytoskeleton, possibly through an effect on nucleokinesis (reviewed in Ref. 35). These seemingly disparate effects of LIS1 can be explained partially by the discovery that it is required in different amounts for different developmental stages, and thus might have more than one biochemical role.

The function of DCX in neuronal migration is also unknown, but it was also recently shown to be a microtubule-associated protein[36,37], providing a potential link between LIS1 and DCX. The sequence of DCX is entirely novel, and, except for a serine–proline-rich tail and several potential phosphorylation sites, it has no recognizable motifs[38,39]. *Dcx* appears to be expressed by all populations of neurons during periods of migration, and is tightly developmentally regulated[36,37]. It shows an overlapping localization with microtubules in cultured neurons and leads to microtubule polymerization and bundling both *in vitro* and *in vivo*[36,40], suggesting that its role in neuronal migration might also involve regulation of the microtubule cytoskeleton. It is possible that DCX and LIS1 together regulate an essential microtubule-based event in neuronal migration, but this remains to be determined.

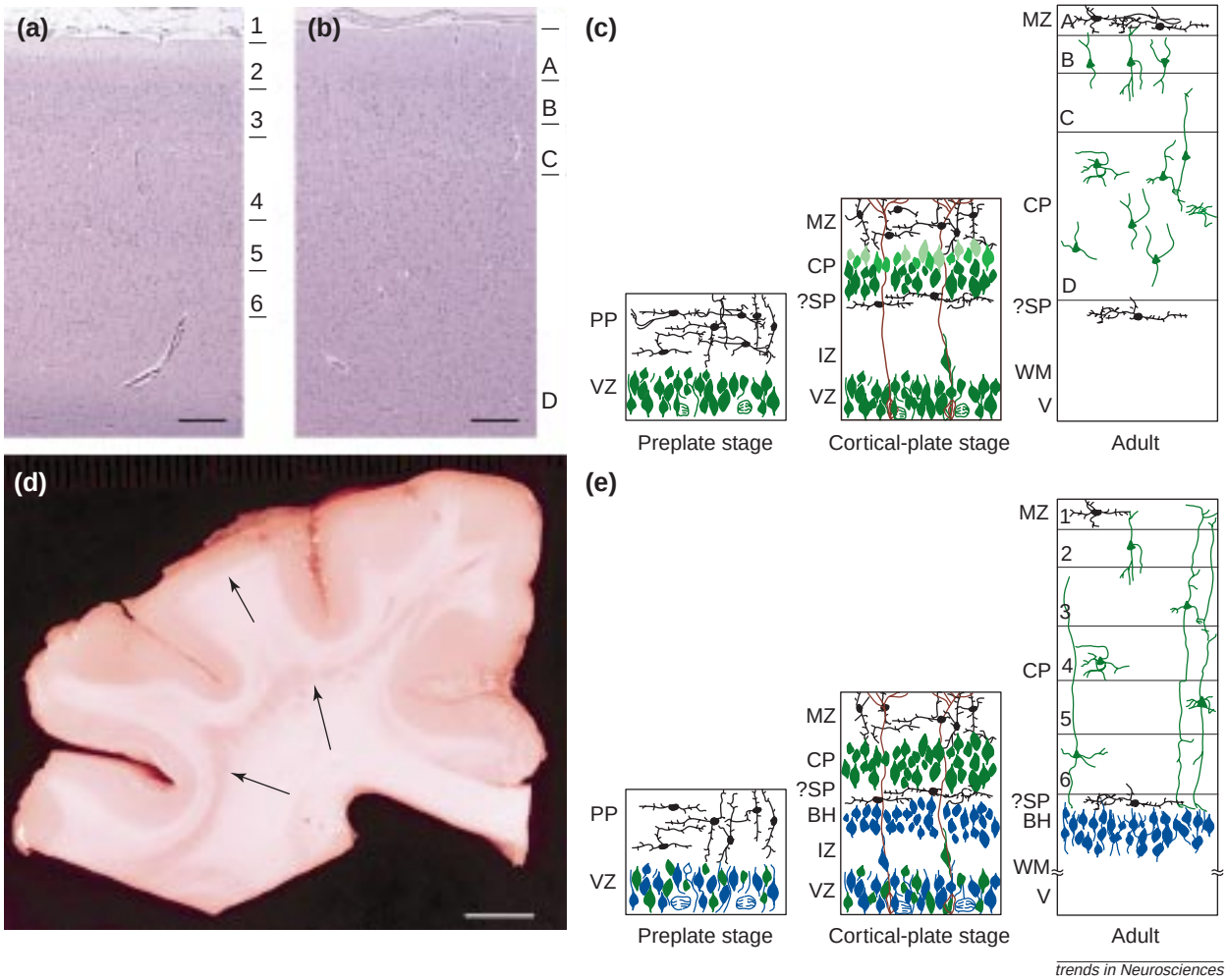

Fig. 3. ***The ongoing process of migration: lissencephaly; double cortex (DC); LIS1 and doublecortin (DCX).*** *Gross pathological cortical specimens from a normal two-year-old boy* **(a)** *and a two-year-old boy with type I lissencephaly* **(b)**. *The normal cortex is characterized by six well-defined layers (1–6), followed by white matter (layer 1 appears as two layers because of a staining artifact). In type I lissencephaly, there are four layers (A–D) but the layers do not have any correspondence to the normal layers, and are thus assigned letters instead of numbers. In type I lissencephaly, there is a cell-sparse layer (A), which probably corresponds to the normal marginal zone, followed by a cell-dense layer (B), a cell-sparse layer (C) and a cell-dense layer (D). Layer D contains the vast majority of cortical neurons, and would extend much further if it were shown completely.* **(c)** *A model to explain the type I lissencephaly developmental abnormality. In males with a mutation in the gene for doublecortin* (DCX) *or in either sex with a LIS1 mutation, all neurons express the mutant allele, leading to a generalized defect in neuronal migration. Neuronal birthdating experiments in the Lis1 knockout mouse demonstrated the proper 'inside-out' pattern [indicated by the progressive lighter shades of green in more superficial cortical-plate (CP) neurons], but in an imprecise manner. The location of the subplate in type I lissencephaly has not been described, so it is represented beneath the CP (?SP). In the adult stage, there are four layers and neuronal morphology is simplified.* **(d)** *Gross pathological cortical specimen from a woman with double cortex (DC). The key feature is a band of heterotopic neurons in the subcortical white matter (lower arrows) and a normal-appearing outer cortex (upper arrow). Presumably, the heterotopic neurons were destined for the cerebral cortex.* **(e)** *A model to explain the DC developmental abnormality. In females with a mutation in* DCX *on one X chromosome, because of X-chromosome inactivation, there is a population of neurons that expresses a normal* DCX *gene (represented by green cells) and a population that expresses a mutant* DCX *gene (represented by blue cells). These two populations of cells then segregate, with the normal cells migrating to the cortical plate and the mutant cells having a defect in the ongoing process of migration. In the adult, the result is a normal outer cortex and a band of heterotopic (BH) neurons in the subcortical white matter. The location of the subplate in DC is not known so it is represented between the CP and the heterotopic neurons (?SP). The CP presumably displays a normal 'inside-out' layering, but is not depicted in this model. Abbreviations: IZ, intermediate zone; MZ, marginal zone; PP, preplate; V, ventricle; VZ, ventricular zone; WM, white matter. Scale bars, 0.5 mm in* (a) *and* (b), *and 1 cm in* (d).

Penetration of migrating neurons through the subplate: the reeler and scrambler mice; *Reelin*, *Dab1*, *Vldlr* and *Apoer2*

As neurons complete their migration they become organized into the cortical plate in patterns that presage the adult layers. This final stage of migration has been the subject of intense investigation recently. The reeler mouse shows a rough inversion of the normal inside-out pattern of cortical migration, and an excess of neurons in the normally cell-sparse marginal zone (layer 1) (Fig. 4a,b,c; Ref. 9). The primary defect in reeler seems to be that cortical plate neurons do not insert into the preplate, but pile up underneath it[9,42]. Newly-arrived neurons do not bypass earlier neurons, which leads to an inversion of the inside-out pattern[43], possibly because the cortical neurons do not penetrate the subplate or because reeler neurons do not dissociate from radial glia properly[44] (Fig. 4d). The entire preplate is translocated to the outer margin of the cortex and is associated with the obliteration of the marginal zone into a structure known as the superplate[45]. Additionally, the reeler mouse displays a severe cerebellar developmental defect that probably accounts for the severe unsteadiness of gait. The affected gene in reeler mice was found to encode for a large extracellular protein named reelin[46] that is secreted from the horizontally oriented

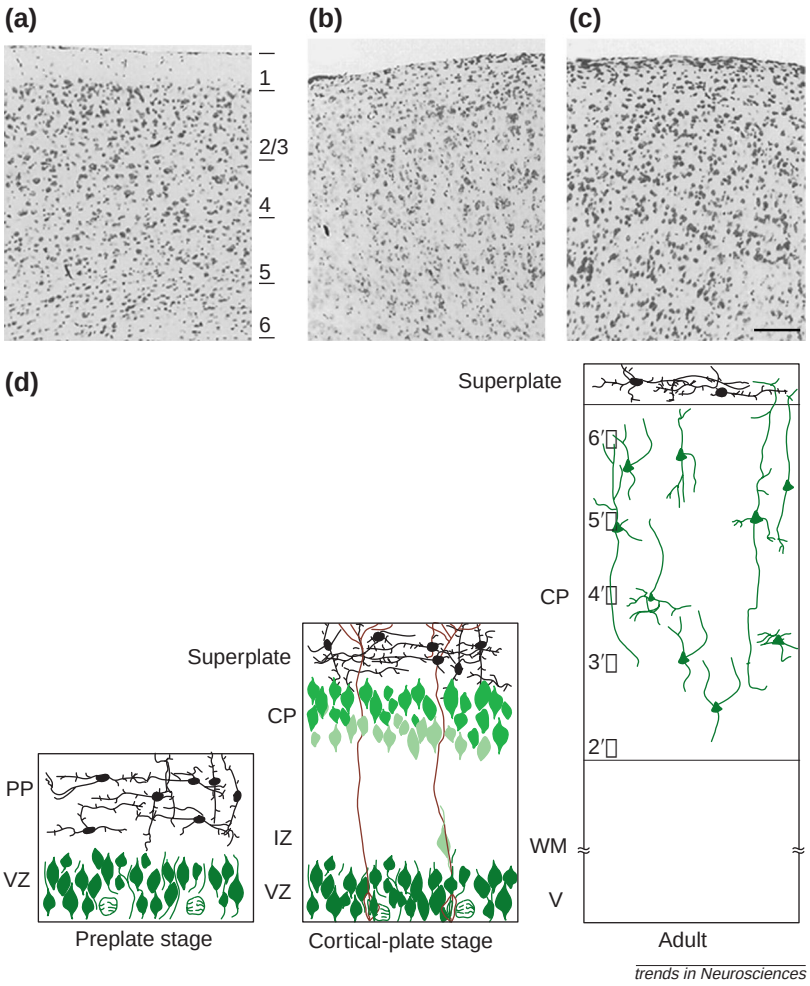

Fig. 4. ***Penetration of migrating neurons through the subplate: the reeler and scrambler mice, and reelin, DAB1, VLDLR and APOER2.*** *Histological sections stained with cresyl violet that show a normal six-layered cortex from adult mouse* **(a)** *and cortex from age-matched reeler* **(b)** *and scrambler* **(c)** *mice. Normally, the layers are distinct and there is a clear layer 1 (marginal zone, MZ). In reeler and its phenocopies, there is no clear layering and layer 1 is obliterated. Neuronal birthdating experiments demonstrated an inversion of the normal cortical layering (not depicted here). The cortex in the* Vldlr *and* Apoer2 *(also known as* Lrp8*) knockouts is each intermediate in severity.* **(d)** *Mechanism for the reeler phenotype. In reeler, incoming cortical neurons do not penetrate through the subplate. Instead, cortical neurons pile up underneath the preplate (PP), and do not migrate beyond earlier neurons [indicated by the progressive lighter shades of green in deeper cortical-plate (CP) neurons]. The MZ does not form, and instead the PP cells are present in a structure named the superplate. In the adult stage of reeler, the inverted layers are numbered 6′–2′ to represent the inverted cortical plate. Abbreviations: IZ, intermediate zone; V, ventricle; VZ, ventricular zone; WM, white matter. Scale bar, 200 μm.* (a), (b) and (c) reproduced, with permission, from Ref. 41.

Cajal–Retzius cells of the preplate and marginal zone[47]. This suggests that marginal zone signaling to cortical neurons is a crucial step in migration.

There have been several other mutations recently described in which the phenotype is remarkably similar to reeler, and the identification of the responsible genes has begun to outline a genetic pathway that regulates this crucial step in corticogenesis. The scrambler[48] and yotari[49] mice are spontaneously occurring mutants that exactly replicate the reeler phenotype[41,48], which raised the possibility that one of these genes would encode a reelin receptor. However, scrambler and yotari were both found to be caused by mutations in the mouse homolog of *Drosophila disabled*, or *Dab1*, which encodes an intracellular phosphoprotein[50–52]. DAB1 appears to act downstream of reelin, because (1) reelin is intact in the scrambler mouse[41,51], (2) DAB1 levels are elevated in the reeler mutant[53], and (3) the application of extracellular reelin leads to phosphorylation and degradation of DAB1 (Ref. 54). These studies suggest a reelin receptor might signal through DAB1 and regulate its levels, and they provided the molecular tools to identify the receptor that lies between them.

A screen for DAB1-interacting proteins identified several transmembrane glycoproteins containing the amino acids N–P–X–Y, suggesting that a protein with this motif might be the reelin receptor[55,56]. Amazingly, when two of the receptor genes with this motif [the gene for the ApoE receptor 2, *Apoer2* (also known as *Lrp8*) and the gene for the very low-density lipoprotein receptor, *Vldlr*] were simultaneously knocked out, the double mutant recapitulated the reeler–scrambler phenotype precisely[56]. Given that only the *Apoer2–Vldlr* double knockout completely recapitulated the reeler phenotype, the two structurally related receptors are thought to represent functionally redundant reelin receptors. The *Apoer2* single knockout matched the reeler phenotype more closely in the cortex, whereas the *Vldlr* knockout matched it more closely in the cerebellum, suggesting that the receptors might have some regional specificity. Finally, recent work suggests that reelin binds directly to both VLDLR and APOER2 with high affinity and this interaction can transduce the reelin signal to DAB1 (Refs 57,58), which provides strong evidence that these two proteins are the reelin receptors. Very recently, novel cadherin-like proteins have also been identified as potential reelin receptors[59], which raises the possibility that these adhesion molecules are also involved in transducing the reelin signal.

Multiple roles of Cdk5 and its activator, p35

Mutations in two other genes, which encode Cdk5 (cyclin-dependent kinase 5) and its regulator, p35 (also known as Cdk5r2), also disrupt migration, although the precise stage at which these proteins is required is a bit more complicated. Like reeler, the *Cdk5* and *p35* engineered mutations produce phenotypes in mice with an inverted cortical plate. However, these mutants are not identical to the reeler phenotype, as they leave the marginal zone intact and do not severely disrupt the accuracy with which neurons of similar type laminate (albeit in inverted fashion) (see Fig. 5). Moreover, the cortices in these two engineered mutations are not identical: in the *Cdk5* mutant, the subplate is in the middle of the cortical plate[60], whereas in the *p35* mutant it is beneath the cortical plate[61] with a proportionally larger fraction of cortical neurons present in the cortical plate. Therefore, in the *p35* mutant, the sole defect appears to be that neurons cannot migrate through the cortical plate, whereas in the *Cdk5* mutant, early cortical-plate neurons can penetrate the subplate but later cortical-plate neurons are unable to do so (Fig. 5).

The *Cdk5* gene was originally identified by homology to other members of the cyclin-dependent kinase family of serine–threonine kinases, which are largely implicated in cell-cycle regulation[62]. While *Cdk5* is expressed in many tissues, its kinase activity is surprisingly detected only in developing brain, suggesting that, similar to other such kinases, CDK5 might have a specific activator that regulates its activity. Two activators have subsequently been identified from brain: *p35* (Refs 63,64) and *p39* (Ref. 65). The similarity in the phenotype between the *Cdk5* and *p35* knockout mice suggests that CDK5 and P35 function

interdependently in neuronal migration. The role of P39 in neuronal migration is unclear because no mouse knockout has been reported.

How do the *Cdk5* and *p35* genes mediate their effect on neuronal migration? CDK5 has several putative kinase substrates and several other potential biochemical interactions, in addition to an effect on neurite outgrowth, all of which might have some role in neuronal migration. CDK5 can phosphorylate both neurofilaments[66–68] and the microtubule-associated protein, tau[69–72]. Additionally, *Cdk5* or *p35* overexpression leads to neurite outgrowth, which is inhibited by overexpression of inactivated *Cdk5* or by antisense to *p35* (Ref. 73). Although the mechanism by which CDK5 or P35 has its effects on migration is not clear, all of the effects are related to cytoskeletal changes.

Type-II lissencephalies disrupt cortical architecture by effacing the pial limiting membrane of the cortex

A number of disorders both in mice and humans appear to disrupt the architecture of the developing cerebral wall, and these conditions are also associated with severe disruptions of neuronal migration. In humans, disorders referred to collectively as 'type-II lissencephaly' produce a smooth brain (as in type-I lissencephaly) but rents are formed in the pial surface allowing the migration of neurons out of the CNS and onto the overlying subarachnoid tissue. Recessive type-II lissencephaly in association with eye and muscle abnormalities (named Fukuyama disease) is associated with mutations in the novel gene for fukutin[74]. Another non-alleleic condition, called muscle–eye–brain disease, has been mapped but the gene responsible is not yet known[75]. A third disorder, Walker–Warburg syndrome[76], is histologically similar but probably represents a third distinct locus. Several mutations in mice also cause a phenotype similar to type-II lissencephaly. Induced mutations in α6 integrin (Ref. 77) or presenilin 1 (Psen1)[78] produce defects in the pial surface with migration of neurons out of the cortex. Interestingly, the *Psen1* knockout shows defects in reelin synthesis in the marginal zone[78]; thus, some of the genes associated with type-II lissencephaly might have indirect relationships with reelin signaling, but this remains to be explored.

Potential connections between mouse and human neuronal migration defects

Is there any evidence that either the genes that cause neuronal migration defects in mice also lead to defects in human disorders of migration, or that the mouse and human genes associated with neuronal migration will interact genetically or physically? Although there are not yet any definitive connections, there are some hints that there will be convergence of mouse and human disorders of neuronal migration in the future. Human lissencephaly and the cortical defect in the *Cdk5* and *p35* engineered mutations are very similar in appearance, with an intact layer 1 and essentially normal cerebellum. However, unlike the *Cdk5* and *p35* knockouts, the *Lis1* engineered mutation does not display an inverted cortex[24]. On the other hand, it is not known whether there are human diseases caused by mutations in the reeler pathway.

Biochemically, there are no firm data to suggest a connection between the human and mouse neuronal migration genes, or between the reeler phenocopies, and *p35* and *Cdk5*, but there are some hints at a possible convergence based upon potential phosphorylation sites. The predicted protein from the expression of *FLN1* contains a potential CDK5 phosphorylation site (KSPX)[19]. Additionally, the predicted DCX protein contains a potential phosphorylation site for the non-receptor tyrosine kinase ABL (Ref. 38), which has been implicated genetically with *disabled* in *Drosophila*. Finally, there are hints that DAB1 can be phosphorylated by CDK5 *in vitro*[58]. As of yet, there are no published data to support any biochemical or genetic interactions between these proteins, however the future might bring insight into potential connections between these different cortical neuronal migration genes.

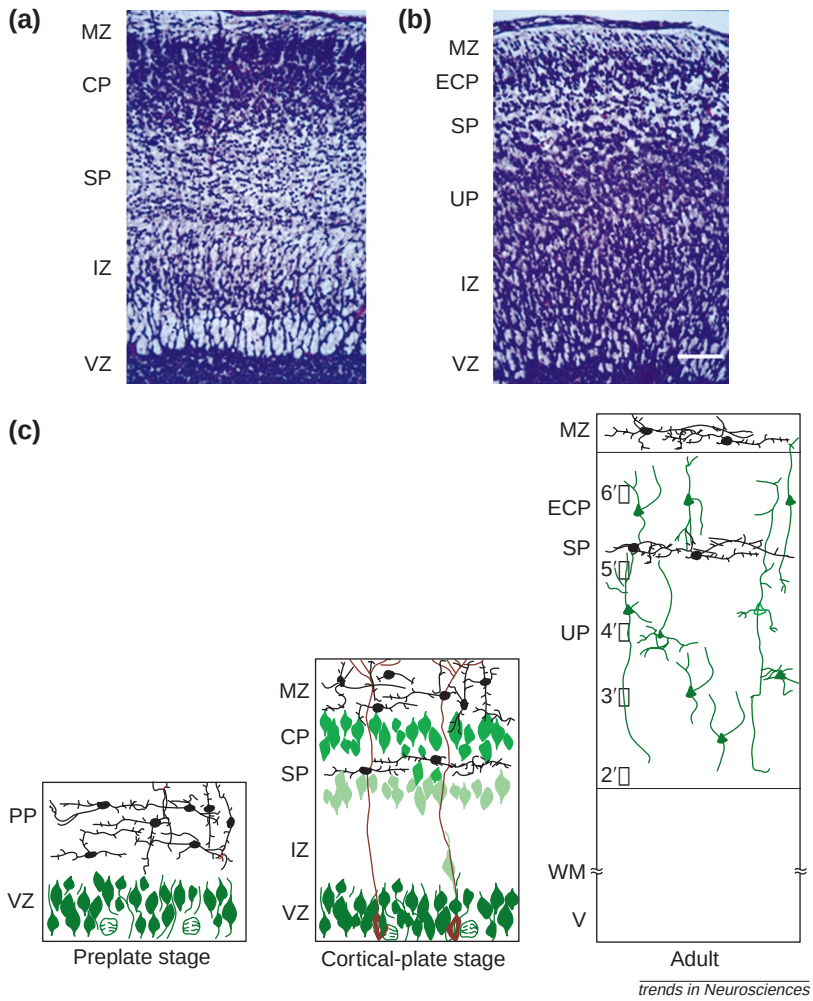

Fig. 5. ***Penetration of migrating neurons through the cortical plate: the roles of the Cdk5 and its activator, p35 in neuronal migration.*** *Histological sections stained with cresyl violet showing the entire cortical thickness from the marginal zone (MZ) to the ventricular zone (VZ) in an embryonic-day (E) 16.5 littermate* **(a)** *and a* Cdk5 *knockout mouse* **(b)**. *Normally, the MZ is most superficial, followed by the cortical plate (CP), subplate (SP), intermediate zone (IZ) and VZ. In the knockout, the MZ is followed by an early cortical plate (ECP), SP, an underplate (UP), then the IZ and VZ.* **(c)** *Presumed mechanism for the* Cdk5 *knockout phenotype. Early CP neurons can split the preplate (PP) into the MZ and the SP (dark green CP neurons in the cortical-plate stage), whereas later neurons (lighter green) cannot penetrate the SP. In adult* Cdk5 *knockout mice, the subplate is positioned between layer 6′ and layer 5′ neurons, the cortex is inverted, and there is an intact MZ; whereas in* p35- *(also know as* Fcn2*) knockout mice the subplate is positioned beneath an inverted cortical plate and there is an intact MZ (not shown). Scale bar, 200 μm.* (a) and (b) courtesy of Teruyuki Tanaka and Ashok Kulkarni.

Concluding remarks

Genetic analysis of migration mutants suggests that cortical neuronal migration can be divided into at least four discrete events and potentially more (see Fig. 1). PH and the associated mutation in *FLN1* suggest that

the onset of migration comprises a distinct event, and the strong interaction between FLN1 and actin suggests that this event might depend on actin-mediated mechanisms. Lissencephaly and double cortex appear to be caused by defects in the ongoing process of migration; the strong interactions between DCX, LIS1 and microtubules suggest that this event might be microtubule based. Mutations in the gene for reelin, and *Dab1*, *Apoer2* and *Vldlr* lead to a reeler phenocopy, with the underlying defect appearing to be an inability of cortical neurons to penetrate the preplate. The action of reelin on migrating cortical neurons is unclear, although it has been hypothesized to act as a migration stop signal to neurons[79–81], whereas the expression of other genes appears to be involved in transducing the reelin signal. Neurons in the *Cdk5* and *p35* mutants appear to have a defect in migration through the cortical plate. This phenotype is clear for the *p35* mutant, whereas the *Cdk5* mutant appears to have a phenotype that is intermediate between the *p35* mutant and reeler. Thus, neuronal migration to the cortex might be regulated by genetic mechanisms that act at the beginning of migration, during the ongoing process of migration and in several discrete steps at the completion of migration, including penetration of neurons through the subplate and through the cortical plate.

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The hypocretin/orexin ligand–receptor system: implications for sleep and sleep disorders

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The molecules originally described as the hypocretins and subsequently as the orexins were initially implicated in the control of food intake. Recent observations implicate this newly-described neurotransmitter system in the sleep disorder narcolepsy and, potentially, in the regulation of normal sleep processes. This article reviews the research that led to the isolation of the hypocretin/orexin peptides, their receptors and the activity of these molecules as we currently understand them. A model is proposed in which the cells that make these peptides might be involved in arousal state control.

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ALTHOUGH THE HYPOCRETINS were formally described early in 1998 (Ref. 1), these molecules were originally isolated in 1996 from a hypothalamic subtraction[2]. The procedure used for isolation of the clone then known as H35 has been described in detail[3] and was a modification of the directional tag PCR subtraction procedure used previously on the striatum[4]. The cloning of the gene for hypocretin from rat and mouse, the localization of the cell bodies that produce the hypocretin (Hcrt) peptides, and description of their efferent projections were first presented in 1997 (Refs 5,6). Figure 1 presents the alignment of the peptide sequences of the four mammalian species published to date, indicating the N-terminal secretory signal sequence and the two encoded peptides. Because the cell bodies that express this gene are restricted to an area of the hypothalamus centered around the perifornical nucleus (PFH) and because of a weak homology to the gut peptide secretin, these molecules have been called the 'hypocretins'. The dipeptide cleavage sites that flank the smaller of the two Hcrt peptides and the expected conversion of the C-terminal glycine

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