RESEARCH ARTICLE

The role of the Eph/ephrin family during cortical development and cerebral malformations

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Abstract

Neuronal numbers and the associated size of the cerebral cortex, surface folding and laminar organisation are determined by precise developmental mechanisms that are orchestrated by several intrinsic and extrinsic molecules. Abnormalities during development can cause manifold microscopic and macroscopic cortical malformations, mostly accompanied by clinical consequences such as mental disorders, intellectual disabilities, or epileptic seizures. Most cortical malformations and associated neurological disorders result from genetic defects, however the cellular mechanisms remain complex and poorly understood. Eph receptor tyrosine kinases and their ligands, the ephrins, are abundantly expressed in the developing brain where they regulate several developmental processes that are crucial for correct brain formation. Ephrin family members represent membrane-bound proteins that are key players in complex short-range cell-cell communication. In addition, mechanisms for long-range interactions have been described recently. Several ephrins have already been shown to control cell cycle dynamics of cortical stem cells during corticogenesis and the positioning of postmitotic neurons. In addition, mutations in genes encoding for members of the Eph/ephrin family are implicated in mental disorders, although the underlying mechanisms remain to be elucidated. A deeper understanding of Eph/ephrin interactions during cerebral cortex development will be beneficial to shed light on developmental disabilities. Here, we discuss the function of Eph/ephrin system during the different processes of corticogenesis and the impact on cerebral malformations.

Keywords: ephrins, corticogenesis, malformation

Introduction

The formation of the cerebral cortex, the seat of higher cognitive functions in the mammalian brain, is a highly sophisticated process requiring the precise interplay of several developmental steps. Perturbation of neural development can result in manifold cortical malformations, often associated with psychological disorders and mental disabilities. The human cerebral cortex is generated during the first two trimesters of gestation. Within this period, neuronal stem cells residing in the epithelium of the neural tube generate diverse subtypes of progenitor, neuronal and glial cells. These stem cells display a polarized morphology with a basal process anchored to the pial surface and an apical process that is in contact with the cerebrospinal fluid. During mitotic cell division, they perform characteristic interkinetic nuclear migration synchronized with cell cycle progression. The S-phase takes place in the basal part of the ventricular zone, whereas G2 nuclei translocate apically towards the ventricular surface, where the M-phase occurs (1). However, the relevance of this nuclear translocation along the vertical axis is not clear and until now no human cortical malformations are associated with defects in these nuclear movements. For this reason, we will not discuss it further.

Before the first neurons are generated, neuronal stem cells divide symmetrically to expand the pool of progenitor cells (2, 3). At the onset of neurogenesis, stem cells divide asymmetrically to generate post-mitotic neurons or intermediate, transient amplifying progenitor cells. These intermediate progenitors translocate their cell bodies more basally, forming the subventricular zone and dividing symmetrically to indirectly generate the majority of neurons (1, 4, 5). The transient amplifying progenitors are already present at early stages of neurogenesis and are suggested to contribute to the neuronal production of all cortical layers (1, 4, 6).

The precise regulation of the progenitor pool is crucial for the correct development of the cerebral cortex. Overproduction of stem cells can lead to megalencephaly, whereas the loss of neuronal stem cells caused by precocious differentiation or increased apoptosis results in microencephaly (7). The cerebral cortex is formed in a temporally regulated inside-out fashion. Neurons destined for deep layers are generated first, whereas those born later migrate through the already existing deeper layers to form the superficial ones (8). Thereby, the radial processes of the progenitor cells serve as a scaffold guiding the migrating post-mitotic neurons to their target layers. Impaired neuronal migration is implicated in cortical malformations like lissencephaly, polymicrogyria, or hetero-topia (9). Interestingly, defects in cellular adhesion result in equal phenotypes (9), as adhesion is critical for neuronal migration.

Eph (Erythropoietin-producing hepato-cellular) receptor tyrosine kinases, and their membrane-bound ligands, the ephrins (Eph receptor interacting proteins), are critically involved in the regulation of developmental processes underlying the formation of the cerebral cortex including proliferation,

apoptosis, cellular adhesion, division orientation, and cell fate determination. Here we discuss the Eph/ephrin-dependent regulation of developmental processes and potential implications in cortical malformations and neurological disorders.

Structure, interactions and signalling of the Eph/ephrin family

Eph receptor tyrosine kinases and their ephrin ligands are widely expressed in the developing brain (10-13) and numerous studies have demonstrated their function in various developmental processes. The 15 Eph receptor tyrosine kinases and 9 ephrin ligands are classified into two groups, according to their structural similarities and binding affinities (Figure 1; 14). Class-A ephrins are tethered to the membrane by a glycosylphosphatidyl-inositol anchor, whereas class-B ephrins are trans-membrane proteins with a cytoplasmic domain and a PDZ binding motif (15, 16). Eph receptor tyrosine kinases are monomeric receptors that homodimerize and phosphorylate each other upon ligand binding. Furthermore, the receptors contain a C-terminal PDZ-domain that potentially modulates intracellular signalling (15, 17, 18). EphA receptors bind promiscuously to A-ligands and EphB receptors to B-ephrins (Figure 2). However, class-crossing interactions as well as exclusive binding affinities have also been described (14, 19, 20). For instance, EphA4 shows class-crossing interactions with ephrinB2 and ephrinB3, whereas EphB2 binds ephrinA5. In turn, EphA1 and EphB4 exclusively interact with ephrinA1 (14, 19, 20) and EphrinB2 (21), respectively. Furthermore, ephrins display different affinities to distinct receptors. For example, ephrinA5 has a stronger affinity to EphA4 and EphA5 than to EphA3 (22).

A special feature of the Eph/ephrin family is the potential of bidirectional signalling (Figure 1). *Forward signalling* describes the induction of intracellular signalling in the receptor-bearing cell upon ligand binding inducing receptor auto-phosphorylation and subsequent activation of downstream targets. Although ephrins do not exhibit a catalytic activity, they can trigger a *reverse signalling* in ligand-expressing cells after binding to cognate receptors (17, 23-25). For class B-ephrins, the *reverse signalling* results in the phosphorylation of their cytoplasmic domain, which triggers signal transduction. Although class A-ephrins do not possess an intracellular domain, there is some evidence for the initiation of *reverse signalling* through interactions with adapter proteins (26, 27).

Another unique feature of Eph receptors among tyrosine kinases is the capability to form higher-order clusters (28, 29). Such protein assemblies can include more than one Eph receptor, and the size as well as the composition of the cluster determines the cellular response. Various factors regulate cluster size and several Eph receptors have different capacities to cluster (30). Thus, the nature and specificity of a biological response arises from the particular clustering of a receptor.

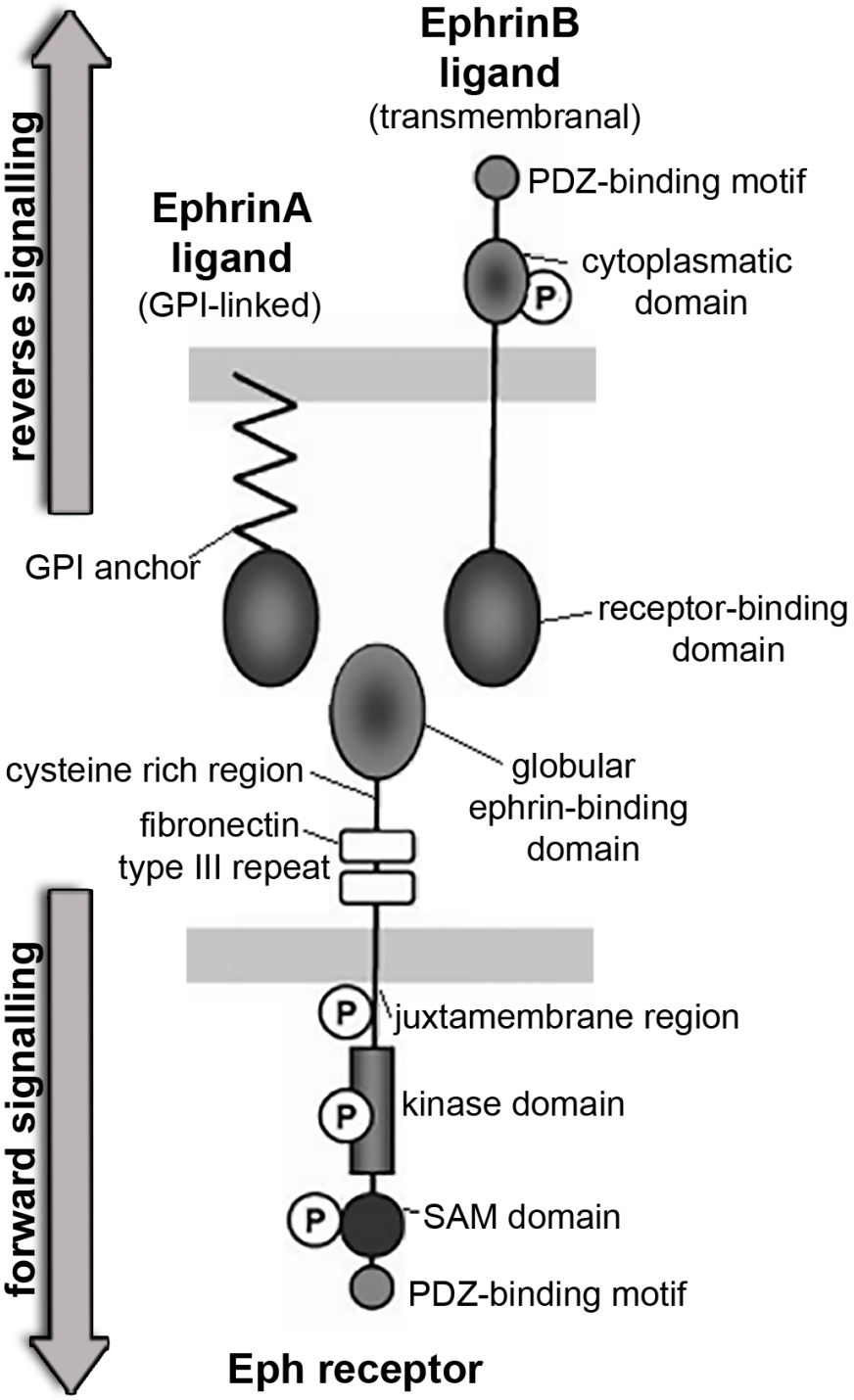

Figure 1: Schematic structure of Eph-receptors and their membrane-bound ephrin ligands. Class A-ephrins are tethered to the membrane by a glycosylphosphatidyl-inositol anchor, whereas class B-ephrins are trans-membrane proteins with a cytoplasmic domain and a PDZ binding motif. Upon ligand binding, *forward* signalling is induced in the receptor-bearing cell. Although ephrins do not exhibit a catalytic activity, they can trigger a *reverse signalling* after receptor binding.

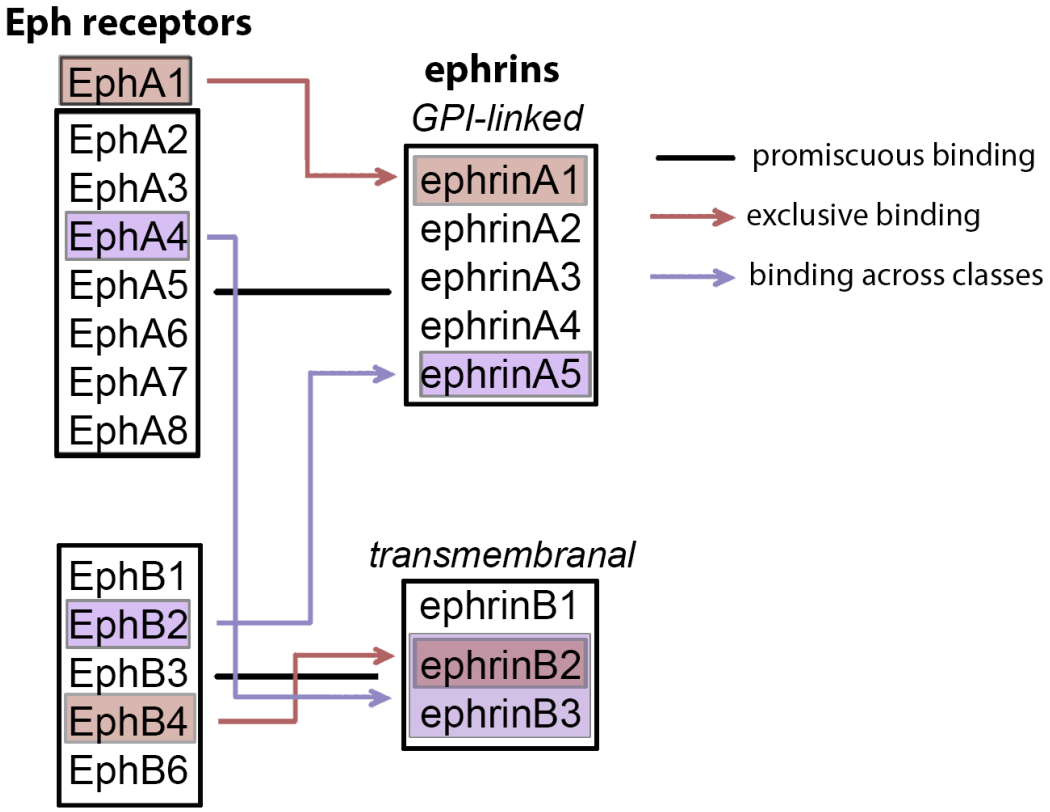

Figure 2: Illustration of structural similarities and binding affinities of Eph receptors and their ephrin ligands. EphA receptors bind promiscuously to A-ligands and EphB receptors to B-ephrins (black line). However class-crossing interactions (brown arrows) and exclusive binding affinities (violet arrows) have been described.

Receptor-ligand interactions between different cells are named *trans*-interactions. When cells express both, receptors as well as corresponding ligands, *cis*-interactions of neighboured receptors and ligands are possible, which often diminishes forward signalling responses, resulting from trans-interactions (31). Thus, co-expression of ligands and Eph receptors can modulate cell-cell signalling and further increases the spectrum of physiological responses. For *trans*-binding of ephrins and Eph receptors, direct cell-cell contacts are required for short-range communication (32) that usually involves paracrine interactions between neighbouring cells. However, this established model of short-range interaction has been extended to long-range communication by recent work. For instance, the developing thalamus was suggested to exert inter-regional control over the proliferation of cortical progenitor cells through axonal fibres growing into the cerebral cortex during development, thereby modulating cell cycle kinetics of apical progenitor cells through interactions with the radial processes (6). Besides this cell contact-dependent mechanism, class A-

ephrins can also be cleaved and released by proteases activating EphA receptors as diffusible molecules (33). This emphasizes that soluble forms of ephrins can also act as long-range cues (34, 35). In addition, it has been described that guidance proteins from the cerebrospinal fluid influence the cell cycle kinetics of neuronal progenitor cells that are lining the cerebral lumen (36, 37). Recent studies reported the existence of several ephrin members in the embryonic cavities (38), which provides the exciting possibility for an extrinsic regulation of neuronal stem cells by the cerebrospinal fluid. More recently it has been published that ephrins were also detected in extracellular vesicles that are released by different cell types in the brain (39). The authors have shown that EphB2 present in exosomes can induce a *reverse signalling* in ephrinB1-expressing cells, whereby the initiated signalling cascade results in axonal retraction. These findings demonstrate that Eph/ephrin signalling exhibits a wide range of interactions in addition to the classical binding, opening new perspectives in regard to their implication in developmental mechanisms.

The Eph/ephrin family is involved in the control of brain size through the regulation of cortical proliferation

The size of the cerebral cortex depends on the multiplication of neuronal stem cells in the neuroepithelium that generate diverse progenitor, neuronal and glial subtypes (40). Several mechanisms control the size and composition of the cortical stem cell pool, orchestrating the production of appropriate numbers of neurons and intermediate precursor cells. The modulation of the balance between proliferation, differentiation, adhesion and apoptosis is crucial to generate a functional cortex and defects are associated with severe brain malformations accompanied by mental disorders. Several ephrins are expressed in the neurogenic niches and they have been identified to influence various aspects of cortical proliferation.

Paracrine activation of EphA4 by ephrinB1 binding promotes cortical stem cell proliferation and the expansion of the progenitor cell pool (41). In turn, ephrinB1 *reverse signalling* induced by EphB1 binding maintains the cortical progenitor cell state (Figure 3; 42). Genetic depletion of ephrinB1 promotes premature cell cycle exit accompanied by a concomitant loss of cortical progenitor cells (42). It has been suggested that ephrinB1 serves as a molecular switch during development, controlling the balance between progenitor cell maintenance and neuronal differentiation (43). Other proteins that interact with ephrinB1 potentially mediate this function. For instance, the zinc-finger and homeodomain protein 2 (ZHX2) is expressed in cortical progenitor cells and acts as transcriptional repressor whose activity is enhanced by co-expression of the ephrinB1 intracellular domain. ZHX2 binds directly to the cytoplasmatic domain of ephrinB1, which prevents the differentiation of neuronal progenitor cells (44). More recently it was shown that extrinsic ephrinA5 ligands expressed by thalamic fibres activate EphA4 receptors of apical progenitors, thereby controlling their

proliferation and cell fate decision (Figure 3). Deletion of *EFNA5* results in an increased production of basal precursors at the expense of apical progenitors (6). Moreover, EphA receptors have been identified as positive regulators of mitogen-activated protein kinase pathway that controls neuronal differentiation in the developing nervous system (45). Further, it has been published recently that ephrinA2 seems crucial for the production of excitatory neurons (Figure 3; 46).

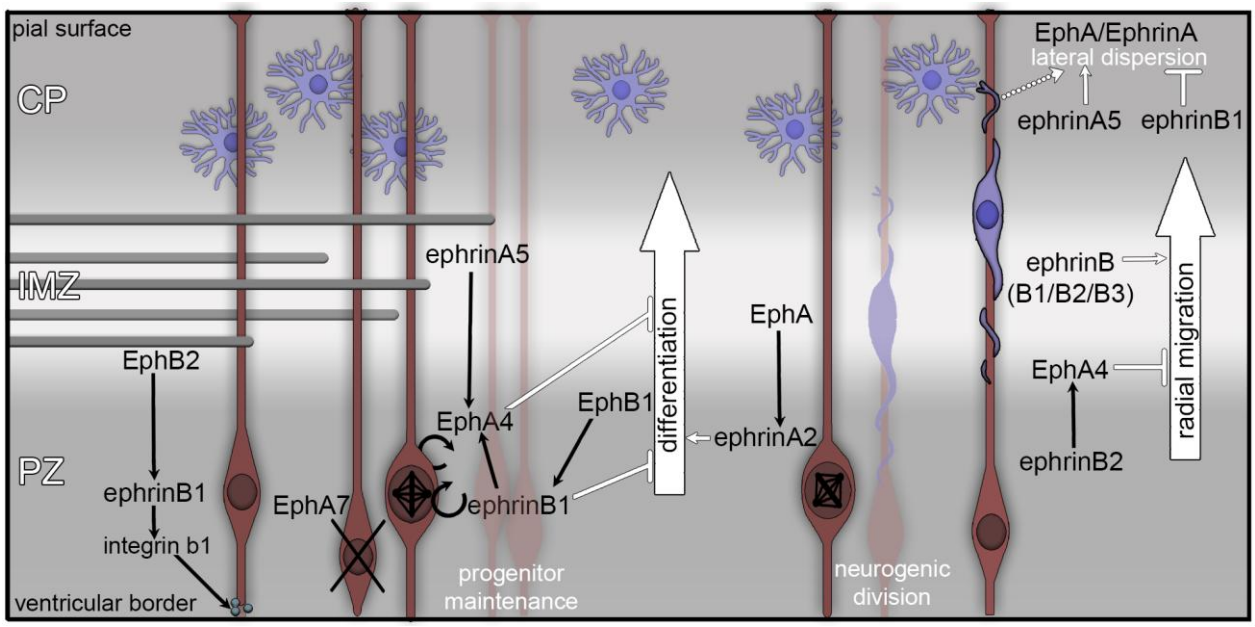

Figure 3: Eph/ephrin family members regulate essential cellular processes during cortical development. EphB2-induced *reverse* signalling of ephrinB1 promotes integrin-based apical adhesion. Activation of EphA7 by ephrinA5 causes cell death of cortical stem cells and regulates the size of the progenitor pool. Paracrine activation of EphA4 by ephrinB1 or due to binding to ephrinA5 on thalamic axons promotes symmetric divisions and cortical progenitor maintenance. EphB1-induced ephrinB1 *reverse* signalling maintains the cortical progenitor cell state. EphrinA2 *reverse* signalling promotes neurogenic divisions and neuronal maturation. EphrinB2-induced EphA4 activation inhibits migrating neurons. Furthermore EphA/ephrinA signalling controls lateral dispersion of postmitotic neurons. EphrinA5 is required to generate cortical columns by restricting lateral dispersion, whereas ephrinB1 promotes the lateral distribution of post-mitotic cells.

Apical progenitor cells give rise to intermediate progenitor cells, a more restricted type of transient amplifying precursor that generates the majority of excitatory neurons in the developing cortex. In mice, these intermediate progenitors were suggested to perform just one terminal neurogenic division to produce two post-mitotic neurons (47). In contrast, in primates and humans there are many types of transient amplifying precursor characterized by a remarkably increased

potential to proliferate and to produce more diverse progeny (48). These transient amplifying precursor cells are thought to promote the tangential expansion during cortical evolution (49, 50) and are correlated with the appearance and degree of gyrification (50-52). Therefore, generating correct numbers of transient amplifying precursors is crucial for correct cerebral cortex development and defects result in cortical malformations such as lissencephaly, polymicrogyria, or autism spectrum disorders in which the patients exhibit an overall higher degree of gyrification (53-55).

Just a few genes are known to induce cortical expansion and to increase folding (52, 56). There is emerging evidence that Eph/ephrin family members are implicated in the regulation of the transient amplifying progenitor pool. EphA4/ephrinA5-interactions influence the generation of intermediate progenitor cells during neurogenesis (6). Moreover, the transcription factor PAX6 is expressed in apical progenitor cells and critical for the generation of intermediate progenitors (57). Interestingly, it has already been shown that *EFNA5*-deficient mice, which show altered numbers of intermediate progenitor cells (6), display decreased *PAX6* expression levels (11). Furthermore, EphA3 and EphA5 are expressed in the primate SVZ, suggesting that they play a role in transient amplifying precursors in higher mammals (58). Together, these studies suggest that the Eph/ephrin system is implicated in the generation of transient amplifying precursor cells and potentially plays a role during cerebral cortex expansion and gyrification.

The Eph/ephrin system affects the survival and apoptosis of neuronal progenitor cells

The size of the cortical progenitor pool also depends on the regulation of programmed cell death. Inhibiting Caspase-dependent cortical apoptosis results in developmental abnormalities. *CASP3* and *CASP9*-deficient embryos exhibit reduced apoptosis of neuroepithelial stem cells, which is accompanied by an abnormal expansion of the progenitor pool, severe cortical malformations and excessive hyperplasia (59, 60). Ephrin family members are involved in the control of cortical apoptosis with consequential impact on cortical expansion. For example, EphA4 promotes Caspase-dependent cell death in the ventricular wall with relevance for adult neurogenesis. In turn, depletion of EphA4 or infusion of soluble ephrinB3 into the lateral ventricle inhibits apoptosis (61). Moreover, expression of EphA7 and ephrinB2 is mutually exclusive in the cells of the ventricular wall, and EphA7-induced ephrinB2 *reverse signalling* causes apoptosis and thereby negatively regulates neural progenitor proliferation and neurogenesis in adult mice (62). In addition, EphB3/ephrinB3-interactions in the adult SVZ are transiently inhibited after traumatic brain injury, probably to enhance the expansion and survival of neuronal precursors (63). During development, overexpression of EphA8 in the dorsal midline of the diencephalon and mesencephalon causes apoptosis of neuroepithelial cells (64). Another study has shown that the activation of EphA7 by ectopic overexpression of ephrinA5 induces

death of cortical stem cells (Figure 3) accompanied by reduced numbers of cortical progenitor cells and dramatic decrease of brain size. In turn, depletion of EphA7 results in reduced cell death and exencephalic overgrowth of the forebrain (65). However, analyses of *EFNA5*-deficient embryos reveal no differences in the numbers of apoptotic cells in the ventricular zone and therefore do not support a direct pro-apoptotic effect of ephrinA5 on cortical progenitor cells (6). Overexpression of ephrinA5 might induce apoptosis, whereas physiological levels of the ligand may not reach the threshold required to induce cell death. Thus, Eph/ephrin-bidirectional signalling seems to generally promote physiological apoptosis, whereas overstimulation of the system results in massive cell death (64). The spatially restricted mutual expression patterns of ephrin family members was already suggested to play a role during tissue boundary formation and may prevent excessive growth through induction of apoptosis as shown for the overstimulation of the Eph/ephrin-system in neuroepithelial cells, thereby affecting brain size and tissue morphogenesis.

Functional implications of ephrins in progenitor cell division

Recent studies suggest that ephrin family members are implicated in the modulation of cell division orientation. Patients with a deletion of a gene cluster of ephrin family members, including *EFNA1*, *EFNA3* and *EFNA4*, exhibit microencephaly and developmental delay accompanied by mental retardation (66). Microencephaly is associated to defects in progenitor cell division; however, the exact cellular mechanisms remain to be investigated.

During early corticogenesis, the neuronal stem cells divide symmetrically with a planar division angle that expands the stem cell pool. However, at the onset of neurogenesis, cells divide asymmetrically with more deviated angles to produce various daughter cells with different fates (4, 5), presumably as a result of asymmetric inheritance of cell fate determining factors (67, 68). The orientation of cell division is precisely controlled by several intrinsic and extrinsic signals and spindle orientation defects are associated with cortical malformations like microencephaly as well as macroencephaly. Moreover, alterations in relative numbers of symmetric and asymmetric cell divisions result in changes of the cortical surface area. For instance, the delayed onset of asymmetric divisions results in an increased cortical surface area (69).

There is a link between mechanical cues from the environment and the alignment of the mitotic spindle (70). The distribution of adhesive contacts during interphase precisely predicts the future orientation of the mitotic spindle (71). As the Eph/ephrin system is known to modulate cellular adhesion, it is feasible that they do not just regulate apical attachment, but may also exert mechanical tension on dividing progenitor cells to influence fate determination. In ascidian embryos extracellular FGF and ephrin signals polarize the mitotic mother cell via

interactions with the adjacent ectoderm, thereby promoting asymmetric cell division and inducing distinct cell fates in the resultant daughter pair (72). Moreover, it has been shown that ephrinA5 is implicated in the regulation of asymmetric divisions in the neural tube and notochord of *Ciona intestinalis* by functionally polarizing the mother cell and thereby promoting the generation of neuronal daughters (73). Defects in the ephrin signalling results in symmetric divisions and generated cells adapt the notochord fate.

In the developing cortex, ephrinB1 and ephrinB2 regulate the activity of the Par-protein complex (74-76) that is located in the apical end feet of neuronal stem cells (1). This complex plays a key role for apico-basal polarity, mitosis and promotes proliferative divisions as well as the generation of basal precursors (77). EphrinB1 *reverse* signalling promotes the transition from proliferative symmetric division towards neurogenic asymmetric divisions (42). In contrast, it has been shown that ephrinA5 promotes symmetric, proliferative divisions and the maintenance of apical progenitors during early neurogenesis (6). Loss of ephrinA5 function results in asymmetric divisions accompanied by increased numbers of intermediate progenitors and post-mitotic neurons. More recently it has been published that ephrinA2 *reverse* signalling promotes neurogenic divisions and neuronal differentiation (Figure 3; 46). Together, these studies strongly support a role for ephrins in the regulation of division orientation and fate determination in the developing neuroepithelium, which would provide an explanation for the microcephaly observed in patients with deletions in the *EPH/EFN* gene cluster (66).

In addition, ephrins may also influence cortical progenitor divisions through the modulation of the canonical Wnt pathway. Wnt is essential for the maintenance of cortical progenitor cells and self-renewal divisions (78), and defects in canonical Wnt signalling result in disturbed laminar organisation of the cortex (79). EphB receptors are Wnt-target genes in the intestinal epithelium (80) and it has been shown that ephrinA3 suppresses Wnt-signalling in retinal stem cells through the activation of EphA4, thereby inhibiting proliferation and increasing differentiation (81). Additionally, in the developing cortex canonical Wnt-signalling was reported to negatively regulate *EFNB3*-expression (82).

Ephrin family members are also implicated in cytokinesis, the final phase of cell division, during which the daughter cells become separated. The completion of cytokinesis is controlled by factors of the environment. The function of citron kinase is crucial for the cytokinesis (83) and mutations in the coding gene affect brain development (84) and cause primary microcephaly in humans (85). It has been shown that Eph-mediated tyrosine phosphorylation of citron kinase regulates abscission and EphB2 forward signalling causes midbody forming failures, thereby preventing the completion of cytokinesis (86). Hence, the Eph/ephrin family seems relevant for various subcellular regulatory processes underlying progenitor division.

Eph/ephrins and cellular adhesion

Regulation of cellular adhesion is crucial for the appropriate formation of the cerebral cortex and defects in adhesion are associated with cortical malformations. Cortical stem cells are highly polarized cells that are basally anchored to the pial surface and apically to the ventricular border. Adhesion is intimately linked to proliferation, the precise generation of the neuronal numbers and cortical precursors as well as to neuronal migration. Thus, the disruption of either basal or apical attachment can cause proliferation and migration disorders (9).

Cortical stem cells are tightly attached to adjacent neighbours and receive pro-mitotic signals from surrounding cells and the cerebrospinal fluid (36, 41). Attachment to the ventricular surface is associated with symmetric division and loss of apical adhesion during asymmetric division causes changes of cellular fate (87). Delamination of neuronal precursors from the apical surface could be responsible for the emergence of intermediate progenitor cells during evolution (88). Apical attachment defects result in precocious delamination, accompanied by concomitant differentiation and loss of cortical stem cells (87, 89). However, cells that are committed to differentiate reduce adhesiveness and disengage from the neuroepithelium.

A key component of the adherent junction complex is N-Cadherin and loss of function results in precocious delamination and accumulation of ectopic neurons in the ventricular zone (87, 89). It has already been described, that ephrinA5 recruits N-Cadherin to the adherent junctions during lens development and thereby promotes the formation of cell-cell contacts (90). Eph receptor stimulation with pre-clustered ephrins induces aggregation of endothelial and embryonic cortical cells in culture and Eph/ephrin-interactions can regulate the function of other cell adhesion molecules (91-93). It has been further shown that the activation of Eph receptors potentially increases the adhesiveness to the extracellular matrix via integrins (94). Moreover, ephrinA5 regulates integrin function and thereby modulates cellular adhesion (95). Paracrine interactions between ephrinB1 and EphB2 on adjacent cells promote apical integrin-based adhesion (Figure 3) and deletion of *EFNB1* results in abnormal neuroepithelia and exencephaly (88).

The basal process is anchored to the basal membrane adjacent to the pial surface and serves as a scaffold for radially migrating neurons. Thus, incorrect basal attachment can cause migratory defects such as heterotopia or lissencephaly. The basal membrane represents an anchor for the basal end feet and a physical barrier, respectively. Basal attachment of the radial processes regulates cortical proliferation via integrin-signalling (96, 97) and integrin-deficiency causes defects in the cortical organisation (9). It has been published that ephrin family members can directly or indirectly modulate integrin signalling (98, 99). However, an implication in the regulation of basal attachment has not yet been shown. Another adhesion protein that is secreted in the basal membrane is laminin and deficiency of this glycoprotein is

associated with cobblestone lissencephaly (100). Stimulation of EphA2 forward signalling increases the secretion of laminin in proximal tubular cell lines (101) and B-ephrins modulate laminin-mediated interactions of thymocytes (102). However, it remains to be determined whether ephrins regulate laminin-mediated adhesion in the developing cortex.

Another important developmental step in which ephrins are intimately involved through regulation of adhesion is the closure of the neuronal tube. In human embryos, the neural plate arises 18 days after fertilisation and the neural tube is formed between the weeks 3 -11 of gastrulation. Neural tube closure defects result in spina bifida or anencephaly. The edges of the neural folds co-express *EPHA7* and *EFNA5 (103)*. EphA7 is spliced in three different forms, one full length and two truncated versions. The expression of the truncated proteins suppresses the tyrosine phosphorylation of the full length EphA7 thereby changing the cellular response from repulsion to adhesion (103). It was proposed that EphA7 in combination with ephrinA5 serves as a molecular switch during neural tube formation. This assumption leads to the intriguing possibility that below the threshold level for repulsion, persistent Eph receptor activation leads to an adhesive response (94).

Ephrins regulate the radial migration of excitatory neurons

After exiting the cell cycle, post-mitotic cells migrate radially towards the pial surface to reach their final position in the developing cortical layers. Thus, radial migration is the major mechanism that controls neuronal positioning (104, 105) and mis-regulation results in cortical malformations like lissencephaly and heterotopia. Ephrin family members are already known to control tangential migration of cortical interneurons (106-110), but they also influence radial migration.

The initiating step after delamination is the multipolar bipolar transition in which new-born neurons acquire neuronal polarity by converting from a multipolar to a bipolar morphology with a leading and trailing process (111). During early neurogenesis, post-mitotic neurons mainly migrate in a glia-independent manner by phasic translocation (112). At later stages of corticogenesis gliophil migration dominates, in which the basal processes of radial glia cells build a scaffold along which neurons migrate to their cortical target layers (113, 114). The morphology and integrity of the glial fibres influences the radial migration (115) and disruption of the processes causes cortical malformations similar to migration defects (9). Hence, one mechanism through which ephrins regulate migration seems to rely on the regulation of adhesive complexes, enabling coordinated interactions between the glial fibres and the migrating neurons required for proper migration.

During locomotion, the leading process extends to the pial surface followed by the nucleokinesis, the movement of the nucleus (116). Thereby, the centrosome serves as a microtubule organising centre that is

proximal to the leading process. From there microtubules extend towards the anterior leading process and posterior towards the nucleus to form a perinuclear cage and to exert pulling forces (117). In contrast, posterior contraction of myosin II generates pushing forces (118). During locomotion, the leading process defines the direction of the movement and extracellular cues instruct the migration. Therefore, the growth cone exhibits a miscellaneous assortment of guidance molecules to extend towards the correct direction. The expression of *EFNB2, EPHA4 and EPHB1* is complementary in the developing cortex and regulate the radial migration (119). EphA4 activation by ephrinB2 inhibits migrating neurons and therefore acts as a stop signal in the intermediate zone (Figure 3). Neurons with a low expression levels of EphA4 can pass, whereas cells with a high EphA4-expression are repelled. This may contribute to the ordered formation of the cortical layers. Consequently, *EPHA4*-deficiency disrupts the barrier constituted by ephrinB2 and results in aberrant migration of cortical neurons (119).

Locomotion terminates when the neurons reach the final destination and detach from the glial fibres. This requires anti-adhesive signals to allow detachment from the radial processes (120) or decreased levels of cell adhesion molecules in the membrane as shown for N-Cadherin (121). Impairments in terminal translocation can lead to over-migration of neurons into the meninges resulting in neocortical dysplasia, polymicrogyria, or cobblestone lissencephaly (9). It has been demonstrated that mutations in the gene coding for Reelin result in severe abnormalities of the cellular organisation of the cortex. The function of Reelin in establishing the cortical layers seems associated to EphB/ephrinB signalling as *EFNB1/B2/B3* triple mutant mice mimic the *Reeler* phenotype with the disrupted laminar organisation (122), suggesting a link between the canonial Reelin pathway and ephrinB signalling. However, other studies did not confirm this crosstalk (123).

Besides its laminar architecture, the cortex is further organised in ontogenetic columns of clonally related neurons that are generated by a single stem cell and are stereotypically interconnected (124). Thereby the glial mother cell serves as a scaffold during migration, ensuring the precise radial location of the generated daughter cells within the ontogenetic column. However, some clonally-related neurons undergo a lateral shift from their original glial cell to an adjacent basal process (125) that might represent an early information processing mechanism in the developing cortex. It has been shown that ephrin family members control this lateral dispersion. EphA/ephrinA signalling is required to generate cortical columns (Figure 3) and deletion of *EFNA5* results in impaired lateral distribution (125). Furthermore, ephrinB1 regulates the lateral dispersion of post-mitotic cells by limiting the neurite extension during the multipolar phase and gain of ephrinB1 function leads to abnormal clustering of neurons (126). Even though defects in the lateral distribution are not verifiable yet in the adult human brain, it is conceivable that this

can result in mental disorders and cognitive deficits. In summary, the Eph/ephrin family is implicated in the regulation of various aspects of cortical migration.

Conclusions

Relating human mental diseases to failures in developmental processes is challenging. Defects in different mechanisms can result in similar phenotypes as most of the developmental and subcellular processes are intimately linked to each other. For instance, adhesion plays a role during proliferation, differentiation and migration. Although, the causative genes for a variety of human syndromes have already been identified, the defects in the cellular processes causing the cortical malformations still remain to be elucidated. Therefore, it is essential to analyse the role of the respective gene in a particular developmental context.

Eph/ephrin family members are key players in regulating complex cell-cell interactions and they are crucially involved in several developmental processes during cortical formation. For that reason, Eph/ephrins are highly relevant candidates causing diverse structural malformations in human brains. Here we discussed their crucial roles in several cellular processes underlying cerebral cortex development. However, deciphering their functions in specific developmental steps remains a challenge due to the tremendous diversity of potential interactions and effects of the pleiotropic Eph/ephrin system in distinct biological contexts. A deeper understanding of the regulatory network controlling Eph/ephrin signalling, their interactions with potential effector proteins and crosstalk with other pathways during fundamental neurodevelopmental processes will help to gain valuable insights in the pathophysiology of human cortical malformations.

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Conflict of Interest

The authors declare no competing interests

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Table 1: Expression of ephrin family members in the cerebral cortex during development, their cellular role during cortical development and their described implications in mental diseases.

Molecul	Expression	Cellular function during brain development	Related brain disease
EphA3	CP, IMZ (postmitotic neurons) (6, 41) Primate SVZ (58)	Cortex expansion (58)	Autism spectrum disorders (129)
EphA4	VZ, CP, IMZ (6, 41)	Cortical stem cell expansion Promotes symmetric divisions (6, 41)	Mental disorders (131)
EphA7	Cortical progenitor cells (65) CP, IMZ, VZ (41)	Pro-apoptotic (65)	Mild neurological disorders and structural symptoms (130) Autism spectrum disorders (129) Exencephalic overgrowth of forebrain (65)
EphA5	Primate SVZ (58) CP (41)	Cortex expansion (58)	
EphB1	IMZ (41)	Activation of ephrinB1 by EphB1 maintains cortical progenitor state (42)	Schizophrenia (132)
EphB2	CP,IMZ (41)	Completion of cytokinesis (86) Apical Integrin-based adhesion (88)	Mental disorders (131)
ephrinA1	CP+IMZ (41)		Microencephaly, developmental delay (66)
ephrinA2	CP+IMZ+proliferative zone (41)	Excitatory neuron differentiation (128)	Microencephaly, developmental delay (66) Autism spectrum disorder (133)
ephrinA4	CP+IMZ (41)		Microencephaly, developmental delay (66) Epilepsy (134)
EphrinA5	IMZ (thalamic fibres) (6)	Maintainance of apical progenitor cells (Symmetric division); control production of IPCs (6) Proapoptotic (65) Lateral dispersion of postmitotic neurons (125)	Schizophrenia (135)
EphrinB1	Proliferative zone (41, 42)	Apical attachment (88) Apical Integrin-based adhesion (88) Maintanance of cortical stem cells (42) Lateral dispersion of postmitotic neurons (126)	Exencephaly (88)
EphrinB2	VZ, IMZ ,CP (127; 128)	Promotes neurogenic division and neuronal maturation (128) Stop signal for migrating neurons (119)	Schizophrenia (87)

