Should I Stay or Should I Go? Ephs and Ephrins in Neuronal Migration

Jennifer Rodger^a Lorena Salvatore^b Paolo Migani^c

^aExperimental and Regenerative Neurosciences, School of Animal Biology M317, University of Western Australia, Crawley, W.A., Australia; ^bLaboratorio del Metabolismo Lipidico e Tumorale, Consorzio Mario Negri Sud, Santa Maria Imbaro, and ^cDipartimento di Biochimica, Biologia e Genetica, Università Politecnica delle Marche, Ancona, Italy

Key Words

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Abstract

In neuroscience, Ephs and ephrins are perhaps best known for their role in axon guidance. It was first shown in the visual system that graded expression of these proteins is instrumental in providing molecular coordinates that define topographic maps, particularly in the visual system, but also in the auditory, vomeronasal and somatosensory systems as well as in the hippocampus, cerebellum and other structures. Perhaps unsurprisingly, the role of these proteins in regulating cell-cell interactions also has an impact on cell mobility, with evidence that Eph-ephrin interactions segregate cell populations based on contact-mediated attraction or repulsion. Consistent with these studies, evidence has accumulated that Ephs and ephrins play important roles in the migration of specific cell populations in the developing and adult brain. This review focusses on two examples of neuronal migration that require Eph/ephrin signalling – radial and tangential migration of neurons in cortical development and the migration of newly generated neurons along the rostral migratory stream to the olfactory bulb in the adult brain. We discuss the challenge involved in understanding how cells determine whether they respond to signals by migration or axon guidance.

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Ephs and Ephrins in Neuronal Migration

Cell migration is a key process in nervous system development, requiring precisely coordinated movements in time and space [1]. Most newly generated neurons, both during development and in the adult brain, arise from the proliferative epithelium surrounding the ventricles known as the ventricular zone. They then adopt either radial (towards the pial surface) or tangential (other directions) modes of migration to reach their final destinations within the central nervous system. The key cellular and molecular events that mediate migration are reviewed elsewhere [1] and will not be described here in detail. The focus of this review is to bring together recent studies describing the role of Eph/ephrins in neuronal cell migration and to consider these against a background of axonal guidance literature. Although Ephs and ephrins have been implicated in the migration of a range of neuronal and non-neuronal cells (e.g. motor neurons [2], inner ear cells [3], vascular elements [4], Schwann cells and oligodendrocytes [5, 6]), we will discuss emerging evidence for their role in guiding the migration of newly generated cortical progenitors and neurons [7–11]. In addition, we will discuss the sometimes contradictory evidence suggesting that Eph/ephrins guide migrating neuroblasts along the rostral migratory stream (RMS) to the olfactory bulb in the adult brain [12, 13].

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Jennifer Rodger
Experimental and Regenerative Neurosciences
School of Animal Biology M317, University of Western Australia
35 Stirling Highway, Crawley, WA 6009 (Australia)
Tel. +61 8 6488 2245, E-Mail jennifer.rodger@uwa.edu.au

Introduction to Ephs and Ephrins

Ephrins are membrane-bound proteins which are expressed in many regions of the developing brain. Through their interaction with Eph receptors, they guide cells and their processes to form highly organised brain regions and projections [14, 15]. As versatile signalling molecules, they are involved in many biological processes both during development and in the adult. In addition to their roles in cell proliferation [16], migration [11], neurite extension [17] and branching [18], regeneration [19, 20] and apoptosis [21] in the central nervous system, Ephs and ephrins contribute to vascular development [22–24], embryo implantation [25] and recently have become therapeutic targets in cancer [26, 27]. Their versatility comes from their regulation of a remarkably wide range of intracellular signalling pathways, resulting in precise and context-dependent regulation of cell-cell interactions [28–30].

Ephrins are divided into A-type components, which are connected to the cell membrane by a glycosylphosphatidylinositol linkage, and B-type components, with a transmembrane sequence [31–33]. Ephrin-Eph receptor binding is mainly type specific although within-type specificity is low; however, between-type interactions have been documented for 2 receptors (EphA4 and EphB2) [34, 35]. Very early in Eph/ephrin studies, it was suggested that despite this apparent promiscuity, there was the possibility for signalling arising from different combinations of ligands and receptors to result in unique outcomes [33]. Such a specificity may be associated with protein interactions, cell-type-specific or compartmentalised expression of Eph/ephrins, as well as differential recruitment of intracellular signalling pathways [36, 37].

Eph-ephrin signalling is exceptionally powerful because it is bidirectional: through the receptor and the ligand [38]. Eph receptors are receptor tyrosine kinases and after ligand binding, the receptors phosphorylate intracellular proteins that mediate changes in the cytoskeleton, modifying cell shape and movement [39, 40]. The outcome can be repulsive or attractive for the entire cell (regulating migration) or cellular structure (controlling growth of axons, dendrites or spines) [41, 42]. The ephrin ligands also transmit intracellular signals in response to Eph receptor binding: ephrins type B possess a cytoplasmic tail which triggers intracellular signalling via tyrosine phosphorylation sites and a PDZ-binding site [43, 44]. These processes are known as ‘reverse signalling’ and in some cases are found to have very different outcomes from the ‘forward’, Eph receptor-mediated signal [45, 46]. Reverse signalling by ephrins type A is less well charac-

terised and is thought to involve membrane-associated Src-related kinases such as Fyn and Src [38, 47]. Interpretation of signals can be further complicated by co-expression of Ephs and ephrins on the same cell, resulting in *cis*-interactions that have complex modulatory effects on signalling [48–52].

A key aspect of Eph and ephrin signalling is the ability of signals to be either repulsive or attractive depending on the cellular context. Molecular switches have been identified that determine whether responses will be attractive or repulsive: neurotrophins [51, 53], intracellular kinases [7], cAMP [54–56], cytoplasmic structure [57] and expression levels of the Eph/ephrin proteins themselves [17] are all implicated in the signalling outcome. There is also evidence for feedback regulation of Eph and ephrin expression following Eph-ephrin binding in fish and mouse models. In goldfish during optic nerve regeneration, injection of recombinant Eph receptor proteins resulted in altered expression of ephrin ligands in the visual system [19]. Similarly, studies of Eph and ephrin gene expression levels in the cortex of ephrin A5 knockout mice demonstrate upregulation of ephrin A2, EphA5 and EphB within distinct cortical layers [58]. A question raised by these diverse mechanisms is the chronological aspect of the switch between attraction and repulsion. Some mechanisms, such as phosphorylation and protein-protein interactions are rapid with relatively short half-lives, allowing for rapid switching, perhaps in response to transient environmental changes such as neurotransmitter release or growth factor secretion to facilitate plastic changes. By contrast, changes in gene expression would take longer to develop, but have a long-lasting impact on cellular responses to the environment, potentially making significant changes to cell structure and behaviour.

Repulsive Signalling: How and Why?

The concept of repulsive signalling has been described as paradoxical: Eph-ephrin binding brings cells together – how can they then be separated? A number of mechanisms have been shown to underpin this behaviour. Subsequent to EphA-ephrin-A binding, ephrins A are cleaved by a metalloprotease in hippocampal cells *in vitro*, allowing ingrowing EphA receptor-expressing axons to retract [59, 60]. In cortical neurons *in vitro*, Eph-ephrin binding results in internalisation of the Eph receptor with ephrin still attached [30, 61, 62]. Finally, membrane ripping has been suggested to remove the Eph receptor from the cell surface during migration of cortical cells [7].

The concept of repulsive signalling raises another apparent paradox: how can repulsive interactions build a brain with complex circuitry and regionalisation, when attraction would appear to be more useful, particularly in axonal guidance? The advantage of repulsive signalling in cell migration is that it mediates segregation of cell populations [63, 64]. Similarly, repulsive signalling in axon guidance may provide benefits in patterning axonal projections. In a purely attractive environment, connections would form in the first available 'attractive' location, increasing the probability of projection errors as observed in ephrin A^{-/-} mice [65, 66]. The presence of repulsive cues may promote growth cone motility and searching within a target region, allowing other processes such as contributions from other guidance cues or activity-dependent mechanisms to come into play.

Eph-Ephrin Interactions in Cortical Development

The mammalian neocortex consists of two main neuronal classes, the excitatory glutamatergic pyramidal cells and the inhibitory GABAergic interneurons. The pyramidal cells arise in the cortical proliferative zone (known as the ventricular zone, VZ) of the dorsal telencephalon and migrate radially along the processes of radial glia to form the basic laminar structure of the neocortex [67]. These neurons are the first arrivals in the neocortex and together with Cajal-Retzius cells [68] form a transient structure known as the preplate around embryonic day (E) 10 in mice [1]. The next wave of pyramidal cells splits the preplate into two layers: the superficial marginal zone (layer 1) and a deeper layer, the subplate, forming the cortical plate in between [69]. Subsequently, waves of pyramidal cells enter the cortical plate and form layers that are progressively more superficial as development progresses [70]. At the same time, GABAergic interneurons originating primarily from the VZ of the subpallium, the medial (MGE) and caudal ganglionic eminences in the basal telencephalon, arrive in the developing cortex by tangential migration (fig. 1). They continue to migrate tangentially within the lower subplate and marginal zone of the cortex, some dive down to the surface of the ventricle, dispersing across the full extent of the cortex. Finally, they adopt a radial migration pattern and enter the cortical plate [71, 72] (fig. 1).

As the pyramidal and interneurons migrate into the developing cortical plate, they establish radially oriented columns based on the distribution of pyramidal cell precursors in the VZ [73] (fig. 1, inset). There is some inter-

Fig. 1. Migration of cortical neurons during development. Summary of the migration of cortical interneurons (blue; colours in the online version only) and pyramidal projection neurons (orange) into the developing cortex. Interneurons (ovals) express EphA4, are born in the VZ in the MGE and migrate through 'corridors' defined by ephrin A3 (in the striatum) and ephrin A5 (in the VZ and area entopeduncularis, AEP) expression. They enter the cortical plate in the marginal zone, SVZ and VZ and migrate tangentially and radially to attain their final locations in the cortical plate (**inset** right). Migration of pyramidal (projection) neurons within the cortex is shown in the **inset**. Developmental gradients of Ephs and ephrins are expressed as shown by wedge shapes above the cortex. Pyramidal neurons (circles) are born within the VZ and migrate (vertical arrows) following the processes of radial glia (RG) using ephrin-B-reelin signalling to form a radially oriented column of clonally related neurons. However, a subset of cells also migrate or 'jump' tangentially (horizontal arrows) between columns, a process mediated by EphA7-ephrin-A signalling. The diagram is based on and summarises data from several publications [8–11]. LGE = Lateral ganglionic eminence; CP = cortical plate; MZ = marginal zone.

mixing between pyramidal cells from adjacent columns as a subset of these cells undergo a brief tangential migration or lateral 'jump' [9, 74]. These structures known as microcolumns are highly structured and contain cortical circuits thought to underpin the integration of sensory inputs [75].

Consistent with the widespread and highly regulated expression of Eph receptors and ephrins during cortical development [58, 76–78], there is evidence that ephrins

are involved in 3 aspects of this complex process: radial migration of pyramidal cells [11], tangential migration of interneurons [7, 8, 10] and tangential migration of pyramidal cells during formation of microcolumns [9] (fig. 1).

Evidence for ephrins in radial migration comes primarily from studies of the relationship between ephrin B and reelin signalling pathways [11]. Reelin^{-/-} mice (also known as reeler mice) display a striking phenotype of inverted cortical lamination [67, 79]. Mice with abnormal reelin signalling show disrupted lamination and neuronal morphology in other brain regions including the hippocampus and cerebellum [70, 79, 80]. Reelin is an extracellular matrix protein which exerts its effects by binding to membrane-bound receptors apolipoprotein E/very low-density lipoprotein receptor on neurons [81], resulting in phosphorylation of Dab1 by Src-related kinases [82, 83] and guiding migration, most likely by regulating attachment and detachment from radial glia [79]. However, apolipoprotein E/very low-density lipoprotein receptor does not possess intrinsic kinase activity, requiring a reelin coreceptor molecule to mediate the phosphorylation of Dab1. A recent study has identified ephrins B as coreceptors as they bind directly to reelin and apolipoprotein E/very low-density lipoprotein receptor [11], recruiting Src [84], resulting in phosphorylation of Dab1 [11]. The study used a series of compound mutants that were heterozygous knockout for reelin, but homozygous knockout for one or more ephrin B types. Heterozygous reelin mutants have a normal phenotype, but in combination with an ephrin B knockout, the mice display a typical reelin-like inversion of cortical layers. Furthermore, the ephrin B1/B2/B3 triple knockout mice have a reelin-like phenotype. Spectacular rescue experiments confirmed that the reelin^{-/-} phenotype could be fully rescued by exogenous clustered EphB-Fc (to activate ephrin B signalling), resulting in appropriate migration to their final destinations of cortical neurons that had accumulated at the ventricular surface [11]. Interestingly, although ephrin B1, B2 and B3 all influenced migration of cortical neurons, other brain regions appeared to be more selective for specific family members [11], suggesting that different migration behaviours in these brain regions may recruit different signalling partners to the reelin-ephrin-B complex. The integral role of Src-related kinases in the reelin signalling pathway may also suggest an involvement of ephrins A [47].

In contrast to the role for EphB-ephrin-B signalling in radial migration of pyramidal neurons, tangential migration of inhibitory interneurons has primarily implicated EphA-ephrin-A signalling. The migration of interneu-

rons is over relatively long distances and is known to rely on a range of short- and long-distance cues including neuregulins, slits and semaphorins [1, 85–87], as well as growth factors that initiate and accelerate their migration [88]. A recent addition to this list is EphA4-ephrin-A5 binding, which guides the earliest stages of GABAergic interneuron migration from the MGE into the cortex [8, 10], but not within the cortex [9, 72]. Early born interneurons (E12–E13) migrate into the cortex via a deep migration path that runs ventral to the striatum, whereas later born neurons (E13–E17) migrate dorsally to the striatum through the subventricular zone (SVZ) of the telencephalon, running adjacent to the VZ [10, 89, 90] (fig. 1). Expression patterns of EphA and ephrin A in this system have been clearly described: calbindin-positive interneurons express EphA4 throughout their migration [8, 10], whereas ephrin A3 is expressed in the striatum [10] and ephrin A5 in the VZ of the MGE [8], defining the two corridors (ventral and dorsal) that are permissive for the migration of EphA4-positive interneurons (fig. 1). The repulsive action of ephrin A3 and ephrin A5 for EphA4-expressing interneurons was confirmed by stripe assays, coculture experiments using siRNA downregulation and blockade of ephrin A ligands with recombinant fusion proteins. The latter experiment additionally provided unexpected information about EphA and ephrin A expression: experiments where wild-type and transgenic brain slices were cocultured to visualise migrating interneurons did not initially show repulsive activity of ephrin A5 in the VZ, due to a downregulation of ephrin A5 in vitro [8]. However, addition of exogenous ephrin-A5-Fc restored the normal migration pattern. The experiments suggest that Eph and ephrin expression is tightly regulated not only cell-intrinsically, but also by environmental cues including circuitry and neuronal firing patterns as suggested by studies of brain injury [19, 20, 91, 92] and plasticity [93, 94]. Of consequence is the result that when exogenous ephrin-A5-Fc was added to the slice cultures, it bound preferentially to the VZ, suggesting that an Eph receptor may be co-expressed in this location, as described in the visual system [42].

Later stages of neuronal migration in the developing cortex involve the multipolar migration of pyramidal cells [95, 96], a process which has recently been shown to involve EphA-ephrin signalling [9] (fig. 1). This study is important because it suggests that Eph-ephrin interactions may be attractive or repulsive in neuronal migration, as they are in axon guidance. Previous studies have illustrated how repulsive interactions define and segregate cell populations, whereas the study by Torii et

Fig. 2. Ephrin B1 immunoreactivity in SVZ and RMS. Ephrin B1 expression in adult mouse brain longitudinal sections including the SVZ and RMS pathway. The panel is a photomontage of 2 sections: one cut longitudinally at 1.3 mm from the midline (left, centre) and the other cut at 1.7 mm from the midline (right). Immunoreactivity is detected in the SVZ beneath the lateral ventricle (LV), which is displayed at the anterior and posterior aspects of the striatum/caudoputamen (CPu), under the corpus callosum (cc) and in front of the fimbria (fi), respectively (***). Immunore-

activity is also detected in the main section of the RMS, at the entrance and along the olfactory peduncle (**) and within the olfactory bulb (*). MOB = Main olfactory bulb; AOB = accessory olfactory bulb. Methods for ephrin B1 immunohistochemistry are as described in Migani et al. [78], and additional details are provided in the supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000333784). Scale bar = 400 μ m.

al. [9] demonstrates Eph-ephrin-mediated cell dispersion, a process that may involve attraction. The study shows that ephrin A2/A3/A5 triple knockout mice have significant disruption of cell organisation in the tangential but not radial orientation, with areas of highly irregular neuronal distributions within layers. Although the authors definitively show that EphA7-ephrin-A interactions are important for regulating this tangential dispersion, it remains unclear why areas of abnormal lamination are patchy and apparently randomly distributed throughout the cortex in ephrin triple knockout mice. Possibilities include that these proteins may contribute to cortical arealisation in ways that remain to be determined. It is interesting to note that the individual variation in cortical laminar disruption in ephrin triple knockout mice is reminiscent of the defects in axon guidance in the visual system of ephrin A single, double and triple knockout mice, which are detected in only a proportion of animals and affect only some cells [65, 66, 97–99]. This individual variation may reflect the inherent ability of Eph-ephrin signalling to integrate with other molecular cues, as well as with activity-dependent mechanisms [30, 100].

Eph-Ephrin Interactions in the Migration of Neuroblasts along the RMS

In the adult rodent brain, neurogenesis takes place primarily in two zones: the SVZ, which lies below the medioventral striatal surface of the lateral ventricle, and the subgranular zone of the hippocampal fascia dentata [101]. Neuroblasts produced in the SVZ migrate along the RMS to their final destination in the olfactory bulb where they differentiate and integrate into the bulb layers [102] (fig. 2). The cells remain strictly within the RMS for the duration of this long migration, suggesting that powerful mechanisms are at work to define the boundaries of the migration path.

In the SVZ, neuroblasts (A cells) originate in cell clusters under the ventricular epithelium. Each cluster is wrapped by glial-type cells (B cells), which are the principal self-generating (stem) cells. The B cells also generate neuroblasts and rapidly dividing C cells, which amplify the production of neuroblasts [103, 104]. Neuroblasts migrate out of the SVZ in chains which are aligned mainly in the rostrocaudal direction under the ventricular epithelium, at the surface of the corpus striatum. These

chains converge at the anterior angle of the ventricle and form the RMS, a compact single pathway which enters the core of the olfactory bulb (fig. 2) [105–107]. In addition to neuroblasts, the RMS also contains a dense meshwork of astroglial cells forming long tube-like structures, which contain and presumably guide the migrating cells [108]. Self-generating cells may also be present in the RMS [109] but their ability to produce neuroblasts in this location has not been assessed. When neuroblasts reach the central part of the olfactory bulb, they make a final radial turn and develop into olfactory granule and periglomerular cells, which integrate into primary olfactory circuits as interneurons [110, 111].

Several studies have identified factors which guide neuroblasts along their SVZ-RMS path, but the process remains poorly understood. The vasculature has been suggested to provide structural guidance, both at the level of individual cells migrating into the olfactory bulb [112] and for the bulk of neuroblasts along the RMS [113, 114]. Alternatively, neuroblasts may be self-guided, associating into chains along the RMS via homophilic mechanisms whereby cells follow signals generated by cells of the same type [115]. However, a key requirement for guidance along the RMS is the presence of repelling factors in the tissue surrounding the path, as well as the expression of factors with attracting power in the final target area [113]. Possible candidates for these roles are extracellular matrix components [112, 116, 117] and soluble or membrane-bound factors with chemorepulsive or chemo-attractive effects [118–121].

Our work and that of others has implicated EphB-ephrin-B interactions in regulating neurogenesis within the SVZ and in guiding neuroblast migration. The clearest evidence for a role for EphB-ephrin-B interactions comes from a study which infused recombinant EphB2 and ephrin B2 fusion proteins into the SVZ of adult mice, and showed disrupted migration and proliferation of neural stem cells in the SVZ. Subsequent studies have confirmed the role of EphB-ephrin-B interactions in regulating cell proliferation within the SVZ [122] (ephrin B3 specifically [13]), but also raise doubts about the identity of which ephrins type B are involved, as well as the type of cells in which they are expressed [78], making it difficult to determine mechanisms of action. In situ hybridisation and LacZ constructs have shown that EphB1 and EphB2 are expressed in cells of the SVZ surrounding chains of migrating neuroblasts, although the specific cell type (astrocytes, C cell or ependymal cell) could not be resolved [12]. The same study used combinations of recombinant fusion proteins (-Fc) and PCR to detect ephrin B2 and B3,

but not ephrin B1, in astrocytes in the SVZ [12]. A later study detected ephrin B1 and B2 but not ephrin B3 in the SVZ by immunohistochemistry and confirmed colocalisation of these proteins with glial fibrillary acidic protein (GFAP) [13]. In addition, our previous work confirmed strong immunoreactivity for ephrin B1 in the RMS region of the olfactory bulb in the mouse [78]. We sought to further investigate ephrin B1 expression in the RMS, and our recent unpublished results (fig. 2, 3) suggest that although a proportion of ephrin-B1-positive cells also express GFAP (fig. 3c, g) in the SVZ and RMS, consistent with previous studies in the SVZ [12, 13], a significant number are GFAP negative and are rather double labelled with polysialylated neural cell adhesion molecule-neural cell adhesion molecule and doublecortin (DCX), both markers for migrating neuroblasts (A cells; fig. 3a, b, e, f). The chains of ephrin-B1-positive cells also aligned with blood vessels in the RMS (fig. 3d), consistent with the vasculature contributing to guidance of neuroblasts along this pathway [112–114].

Despite some contradictions in the literature, there is therefore strong evidence that ephrins type B contribute to guiding neuroblasts from their production site in the SVZ to their destination in the olfactory bulb. Moreover, possible expression of ephrin B1 on migrating elements instead of on surrounding tissues and glia would imply that the guidance mechanism involves receptor-like ('reverse') signalling in these cells. A guidance mechanism of this kind is involved in the formation of the corpus callosum, where ephrin-B1-expressing immature cortical axons are forced to cross the midline by the repulsive effect of the EphB2 receptor in the surrounding septal and subcortical tissues; the intracellular signalling within cortical axons is mediated by the PDZ-binding domain on ephrin B1 [123]. Another mechanism which could be triggered by ephrin B1 on neuroblasts is migration by attraction to (or less likely by repulsion from) an Eph receptor expressed on neighbouring cells. A mechanism of this type fits the model of homophilic guidance and implies the existence of subpopulations of ephrin B and EphB receptor-bearing neuroblasts. A possible specialization among neuroblasts is suggested by our data, since we observed two separate populations of neuroblasts: one that was ephrin B1 immunopositive (double-labelled) and the other ephrin B1 immunonegative (single-labelled; fig. 3). Furthermore, a spatial dimension to the separate populations was highlighted by DCX labelling, which revealed that the proportion of double-labelled cells progressively decreased from the SVZ to the olfactory bulb (fig. 3). A possibility is that the ephrin B1 single-labelled cells we

detect could correspond to the DCX-negative neuroblasts previously identified in the SVZ-RMS pathway [124]. The presence of two separate populations could represent the selective loss of ephrin B1 or DCX expression by maturing and differentiating neuroblasts.

Confirmation of the ephrin-B1-dependent guidance mechanisms proposed above requires more information about the expression of B-type Eph receptors in the tissue surrounding the SVZ and the RMS path, as well as on migrating neuroblasts themselves. An online database of specific mRNA distribution (Allen Brain Atlas) has shown that, of the Eph receptors that have been shown to bind ephrin B1, EphA4, EphB1 and EphB2 mRNAs are transcribed in the anterior forebrain and olfactory tissue, however, no signal above background is detected in the SVZ-RMS path. These data contrast with previous studies showing strong EphB2, EphB3 and EphA4 expression in the SVZ in adult mice [12]. Although contradictory, these data suggest that ephrin B1 guidance of neuroblast migration may be independent of EphB receptors. The possibility is consistent with experiments showing that ventricular injection of ephrin B1 ectodomain failed to disturb the formation of neuroblast chains, whereas a similar treatment with ephrin B2 and EphB2 ectodomains produced striking effects [12]. A possible explanation is that the mechanism relies on ephrin B2 binding to EphA4 [33, 34], a mechanism which would not be acti-

vated by ephrin B1 which binds only weakly to EphA4 [40, 125]. Alternatively, EphB and ephrin B may control cell migration via two distinct mechanisms, one that is Eph receptor dependent (via ephrin B2) and the other that is Eph receptor independent via ephrin B1. It has previously been shown that ephrin B1 phosphorylation can be induced by activation of the platelet-derived growth factor receptor [126]. Furthermore other neurotrophic factor receptors, already shown to be involved in neuroblast production and guidance [113, 121, 127], may similarly crosstalk with ephrin B1 signalling as they do for EphAs and ephrins type A [51, 53]. Possible candidates for this role are the intracellular protein Ankyrin Repeat rich Membrane Spanning (ARMS) [128] although its expression in the SVZ/RMS is not described, the p75 neurotrophin receptor, which is expressed in RMS neuroblasts [113], or the tropomyosin receptor kinase (TrkB) receptor, although its expression on RMS neuroblasts is more controversial [113, 121]. The possibility of interaction between ephrin B1 and TrkB at the same cellular site is suggested by the inhibitory effect of ephrin B1 on the mitotic activity of TrkB/brain-derived neurotrophic factor-transfected cells [126]. Furthermore, there is evidence in the RMS-SVZ system that TrkB receptor on glial cells regulates brain-derived neurotrophic factor availability for the p75 neurotrophin receptor on neuroblasts [113]. Future studies of neurotrophic factor receptors and Eph tyrosine kinase receptor expression in this system are required to elucidate the mechanism of Eph/ephrin-mediated neuroblast guidance.

Fig. 3. Colocalisation of ephrin B1 with neuroblasts and glial cells in the RMS and SVZ. **a** Ephrin B1/polysialylated neural cell adhesion molecule-neural cell adhesion molecule (PSA-NCAM) immunoreactivity in the medial part of the RMS. Double-labelled profiles are indicated by arrows. **b** Clusters of cells in posterior positions of the SVZ, showing extensive colocalisation of ephrin B1 and DCX immunoreactivity. **c** Distribution of ephrin B1 and GFAP immunoreactivity in the SVZ and RMS. **d** Chains of ephrin-B1-labelled cellular profiles following the direction of laminin-positive blood vessels in the medial region of the RMS. All scale bars = 50 μ m. Histograms show the percentage of ephrin B1 and neuroblast or glial marker double-labelled cells in RMS and SVZ. Bars represent the percentage of double-labelled cells in the RMS at the olfactory (olf.) bulb level (* in fig. 2), at two levels in the olfactory peduncle (ped.; ** in fig. 2), and in the SVZ (***) in fig. 2). Data are presented as mean values $\pm$ standard error. Significant differences (ANOVA, $p < 0.05$) were observed only for ephrin B1 + DCX, ephrin B1 alone and DCX alone data sets. Post hoc analysis (Student-Newman-Keuls test) revealed significant ($p < 0.05$) differences indicated by the asterisk. Methods for ephrin B1 immunohistochemistry are as described in Migani et al. [78], and additional details of antibodies and analysis are provided as online supplementary material.

Should I Stay or Should I Go? Parallels between Axon Guidance and Migration

In conclusion, Eph-ephrin signalling pathways in axon guidance and neuronal migration have much in common through their shared molecular components and cellular effects [7, 28]. However, these similarities raise a problem: how does the cell know whether to respond to a cue with just a growth cone response, or by a whole cell movement? For example, as discussed above, in the developing mammalian cortex, ephrins type A do not regulate radial positioning in projection neurons, this is accomplished by ephrins B [11], but ephrins A do regulate axon and dendritic targeting and connectivity of these same projection neurons [98, 99, 129–131]. The large number of possible combinations of Ephs and ephrins in vertebrates makes linking individual signalling events to cellular responses (such as axon guidance or

migration) challenging. Furthermore, the difficulties inherent in identifying the different Eph and ephrin family members, particularly in the absence of reliable and specific antibodies to many of the individual proteins, result in contradictions within the field. Thus, early stages of these important experiments may be optimally accomplished in single Eph-ephrin pair models, such as those of some insects. *Manduca sexta* (the tobacco hornworm) and *Drosophila melanogaster* provide a much simpler environment in which to study Ephs and ephrins because a single family member is present for each; the insect ephrin is a glycosylphosphatidylinositol-linked protein similar to vertebrate ephrins A. Most studies show a role for these proteins in axon guidance: in both insects, Eph-ephrin signaling has primarily been implicated in axon guidance related to the processing of olfactory cues. In *Drosophila* the proteins are involved in axon guidance within the mushroom body, a structure implicated in learning memory, particularly for chemical (odorant) stimuli [132], while in *Manduca*, Eph and ephrin may play a similar role in sorting olfactory sensory axons within the antenna [133]. Evidence for Eph-ephrin sig-

naling in neuronal migration comes primarily from larval *Manduca*, in which these proteins restrict the migration of neurons, keeping cells aligned within the railroad-like nervous system of the larval insect [134]. Reverse signalling via ephrin has also been suggested to prevent midline crossing and reduce motility, similar to effects seen in vertebrate brains [135]. Thus, invertebrate Eph-ephrin signalling mediates a similar range of cell behaviours as do their vertebrate counterparts, but does so with a restricted palette that may facilitate elucidation of the intracellular pathways and switches that determine which behaviours are elicited. This fundamental biological understanding would provide the foundations for more targeted interventions in health and disease.

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