Chapter 4
Semaphorins and Cell Migration in the Central Nervous System

Alain Chédotal

Abstract In the central nervous system (CNS), neuroblasts, postmitotic neurons, and glial cells migrate along stereotyped routes from their birthplace to their final destination. Two main types of neuronal migration have been distinguished: a radial one for neurons that migrate along radial glia in a direction perpendicular to the pial surface and a tangential mode for neurons which migrate along other neurons or axons, independently of radial glia (Marin O, Rubenstein JL, Annu Rev Neurosci 26:441–483, 2003; Metin et al, J Neurosci 28(46):11746–11752, 2008). The initiation of migration, the direction followed by migrating neurons or glia, and their decision at specific choice points are influenced by molecules in the environment of the neurons and by intrinsic developmental programs. Many studies in various systems have shown that semaphorins and their receptors play an essential role in this process. Semaphorins influence the motility of neurons and oligodendrocytes and also shape the pathway they follow during their migration. Here, I review these results, focusing on the vertebrate CNS and a few model systems.

Keywords Neural stem cells • Cerebellum • Granule cells • Centrosome • Oligodendrocytes • GnRH

4.1 Semaphorins Control the Radial Migration of Cortical Neurons

The six-layered mammalian neocortex contains two main types of neurons: (1) pyramidal neurons that primarily project outside the cortex and originate from the ventricular zone of the dorsal telencephalon, or pallium; and (2) interneurons, which make local connections between different layers or across layers and originate from the ganglionic eminence in the ventral telencephalon or subpallium (Rakic 2009;

A. Chédotal (✉)
INSERM, UMRS_968, Institut de la Vision, Paris F-75012, France

Sorbonne Universités, UPMC Univ Paris 06, Institut de la Vision, Paris F-75012, France

CNRS, UMR_7210, Paris F-75012, France
e-mail: alain.chedotal@inserm.fr

A. Kumanogoh (ed.), *Semaphorins*, DOI 10.1007/978-4-431-54385-5_4

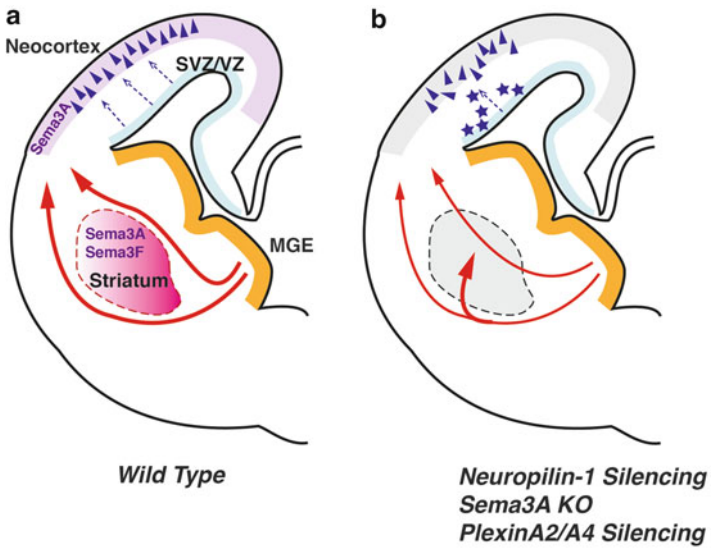

Fig. 4.1 Role of secreted semaphorins in cortical neuron migration. (**a**) In the embryonic forebrain of wild-type mice, cortical interneurons originate from the medial ganglionic eminence (*MGE*) and migrate tangentially to the cortex avoiding the striatum where Sema3A and Sema3F are expressed (*red arrows*). Sema3A is also expressed in the upper part of the cortical plate and promotes the radial migration of pyramidal neurons (*blue triangles*) from the subventricular/ventricular zone (*SVZ/VZ*) and controls their polarity. (**b**) Sema3A, neuropilin-1, and Plexin-A2 loss of function perturb the migration of both types of neurons. A large fraction of cortical interneurons invade the striatum. In the cortex, the radial migration of layer II/III neurons from the SVZ/VZ is affected and many neurons differentiate at an ectopic location. Their polarity and morphology are also abnormal

Bartolini et al. 2013). There is a variety of interneurons differing in morphology (dendritic arborization) and neurotransmitter content (Ascoli et al. 2008). Several studies have shown that semaphorins control the migration of both types of cortical neurons (Fig. 4.1). Pyramidal neurons are known to migrate along the processes of radial glia cells, which extend from the ventricular surface to the cortical surface (Metin et al. 2008). There is little dispersion of the pyramidal neurons during their radial migration, which at least partially explains the columnar organization of the cortex (Rakic et al. 2009).

Several studies showed that Sema3A is expressed in the superficial layers of the cortical plate (Bagnard et al. 1998; Polleux et al. 1998; Chen et al. 2007) in a decreasing gradient from the surface to the depth of the cortex. Cortical neurons also express the neuropilin-1 receptor during radial migration (Chen et al. 2007). In the embryonic day 16 (E16) mouse cortex, the silencing of neuropilin-1 expression using RNA interference or Sema3A overexpression strongly impaired the ability of layer II/II neurons to reach the cortical plate, although they could enter the intermediate zone and proliferated normally (Chen et al. 2007; Shelly et al. 2011) (Fig. 4.1). The same defects were seen using

recombinase-mediated inactivation of neuropilin-1 in cortical neurons. Co-culture experiments mixing cortical slices and sema3A-expressing cells showed that Sema3A has a chemoattractive activity on radially migrating neurons (Chen et al. 2007). Neuropilin-2 loss of function also significantly perturbed radial migration. Similarly, the silencing of Plexin-A4 and Plexin-A2 prevented cortical neurons from leaving the intermediate zone, suggesting that they interact with neuropilin-1 to mediate Sema3A activity. The polarity of the ectopic neuropilin-1-deficient neurons was severely affected, and they adopted a multipolar morphology with multiple neurites rather than a bipolar morphology with a single and unbranched leading process oriented toward the cortical surface (Chen et al. 2007; Shelly et al. 2011). A role for Sema3A in the polarization of newly generated neurons has been also demonstrated for hippocampal neurons, which also migrate radially (Shelly et al. 2011). This step is essential to initiate radial migration (Namba et al. 2014), and therefore Sema3A could act first to polarize neurons and initiate migration and later to attract the neurons toward the surface. Interestingly, similar migration defects were also observed after inactivation of various molecules that are known to act downstream of Sema3A/neuropilin-1 such as the cycling-dependent kinase Cdk5 (Gupta et al. 2003), the Src Kynase Fyn (Sasaki et al. 2002), or Tag-1 (Law et al. 2008; Namba et al. 2014). In addition, Sema3A inhibits the activity of protein kinase A on the phosphorylation of GSK3β and LKB1, two kinases acting on radial migration and neuronal polarity (Asada et al. 2007; Asada and Sanada 2010). However, the exact mechanism of action of Sema3A remains unknown, and the apparently normal cortical layering in the Sema3A knockout (Catalano et al. 1998) suggests that Sema3A acts redundantly with other factors. Sema3A not only controls the migration of pyramidal neurons but also regulates the growth of their apical and basal dendrites and guides their axons in the white matter (Polleux et al. 1998, 2000; Sasaki et al. 2002; Zhou et al. 2013). Surprisingly, much less is known about the function of transmembrane semaphorins in the migration of cortical neurons. A recent study (Azzarelli et al. 2014) revealed that Plexin-B2 is required for radial migration. In the mouse embryo, Plexin-B2 is expressed in the ventricular zone, subventricular zone, and cortical plate. ShRNA-mediated knockdown of Plexin-B2 prevents most neurons from leaving the ventricular zone/subventricular zone (VZ/SVZ) and perturbs the acquisition of cortical neuronal morphology. It was shown that the small GTP-binding protein, Rnd3, which inhibits RhoA signaling through p190RhoGAP (Wennerberg et al. 2003; Pacary et al. 2013), acts downstream of Plexin-B2 during radial migration, confirming previous studies that had shown that Rnds are partners of type-B plexins (Oinuma et al. 2004; Azzarelli et al. 2014). Plexin-B2 inhibits Rnd3, which results in the activation of RhoA activity. The antagonistic activity of Plexin-B2 and Rnd3 allows stabilizing the level of active RhoA during radial migration. Several class 4 semaphorins bind to Plexin-B2, and Sema4D enhances cortical neuron motility in vitro (Hirschberg et al. 2010). However, a role for transmembrane semaphorins in radial migration has not yet been confirmed in vivo. Worthy of note, the organization of the embryonic cortex is severely disturbed in the constitutive Plexin-B2 knockout, suggesting that

Plexin-B2 function in cortical development extends beyond radial migration and that it also acts on cell proliferation and neuronal differentiation (Hirschberg et al. 2010).

4.2 Semaphorin Function in the Tangential Migration of Cortical Neurons

Most inhibitory interneurons [using gamma-aminobutyric acid (GABA) as a neurotransmitter] are produced from progenitors located in the medial ganglionic eminence that migrate tangentially (between E12 and E16 in the mouse), independently of radial glia fibers, across the basal forebrain and then colonize the cortical wall (de Carlos et al. 1996; Anderson et al. 1997; Tamamaki et al. 1997). In the subpallium, cortical interneurons avoid the striatum where Sema3A and Sema3F are highly expressed (Fig. 4.1). During their migration, GABAergic interneurons express neuropilin-1 or neuropilin-2 receptors and are repelled from the nascent striatum by Sema3A and Sema3F. In the absence of neuropilin-1 or neuropilin-2 signaling, many interneurons enter the striatum (Marin et al. 2001; Tamamaki et al. 2003; Zimmer et al. 2010). Ectopic interneurons are also detected after silencing Plexin-A1 in cortical interneurons (Andrews et al. 2013). Lim kinases (Limk) control actin dynamics through cofilin and were previously seen to be involved in semaphorin signaling (Aizawa et al. 2001; Scott et al. 2009). Limk2 was detected in cortical interneurons, and loss-of-function studies using electroporation of Limk2 siRNA in forebrain slices and medial ganglionic eminence (MGE) suggest that it acts downstream of Sema3A/neuropilin-1/Plexin-A1 during migration (Andrews et al. 2013).

Migrating interneurons also express Roundabout 1 (Robo1), one of the receptors of Slit repellents, but Slits are not required for cortical interneuron migration in the subpallium (Marin et al. 2003). However, in Robo1 knockout mice, some GABAergic interneurons abnormally enter into the striatal anlage, and their density is also increased in the cortex (Hernandez-Miranda et al. 2011), a phenotype not observed in *Slit* knockouts (Marin et al. 2003; Andrews et al. 2008). It was recently shown that Robo1-deficient interneurons are less responsive to Sema3A/3F repulsion in migration assays and express a lower level of neuropilin-1 and Plexin-A1. This result suggests that there could be crosstalk between the semaphorin/plexin and Slit/Robo pathways in migrating neurons. Accordingly, Robo1 could be co-immunoprecipitated with neuropilins, suggesting that they are in the same receptor complex. Moreover, chondroitin sulfate proteoglycans (CSPGs), which bind to many extracellular matrix components, regulate the spatial distribution of Sema3A in the basal forebrain and potentiate its repulsive activity for migrating cortical interneurons (Zimmer et al. 2010).

Interestingly, neuropilin-2 expression in striatal interneurons is repressed by the transcription factor Nkx2.1 (Nobrega-Pereira et al. 2008), which directly binds to regulatory elements in the neuropilin-2 promoter. In *Nkx2.1* knockout, neuropilin-

2 is upregulated and there is a reduction in the number of striatal interneurons (Nobrega-Pereira et al. 2008). Therefore, the differential expression of neuropilin-2 by MGE-derived interneurons controls their cortical versus striatal fate. The Dlx1/2 TFs, which control MGE development, are also candidate repressors of neuropilin-2, but the possible influence of this interaction on cortical interneuron migration is unknown (Le et al. 2007).

Neuropilin-2 was also involved in the migration of another type of forebrain neurons, the "LOT cells." The lateral olfactory tract (LOT) contains axons projecting from the olfactory bulb to the pyriform cortex and conveys olfactory inputs. The LOT extends caudally along a region of the forebrain at the interface between the pallium (cortex) and subpallium (MGE) (Sato et al. 1998). It was shown that LOT axons follow a path that is delineated by a specific set of cells, called the LOT cells. It was initially thought that the LOT cells were acting as guidepost cells for LOT axons, but these can be misguided even if the LOT cells are properly positioned, suggesting that these are not sufficient for guiding LOT axons (Fouquet et al. 2007). Cell-labeling studies showed that LOT cells are among the first to be born in the dorsal telencephalon and that they migrate tangentially (between E9.5 and E11.5 in the mouse) in a ventral direction to their final position at the surface of the forebrain (Tomioka et al. 2000; Kawasaki et al. 2006). Sema3F (and not other secreted semaphorins) has a repulsive activity on migrating LOT cells, mediated by neuropilin-2 (Ito et al. 2008). In the final phase of LOT cell migration, Sema3F is expressed in the subpallium in a domain that is surrounding the LOT cells. In neuropilin-2- and Sema3F-knockout embryos, LOT cells migrate more deeply in the forebrain rather than being confined to the surface. Together, these data suggest that neuropilin-2/sema3F act at the end of LOT cell migration to confine them to their superficial position. In parallel, other guidance cues such as Netrin-1 are controlling the dorsoventral patterning of the LOT cells (Kawasaki et al. 2006).

As illustrated by these examples, semaphorins have an important function in the control of neuronal migration in the neocortex, suggesting that defects in semaphorin signaling might be linked to various human neurological diseases in which ectopic or mislocalized neurons were detected, such as schizophrenia and autism spectrum disorders. This hypothesis is supported by preliminary studies indicating increase of Sema3A expression in the cerebellum of schizophrenic patients (Eastwood et al. 2003) and the existence of single-nucleotide polymorphisms (SNPs) in a Japanese cohort of schizophrenics (Fujii et al. 2011). Similarly, there is also evidence for SNPs in Plexin-A2 (Mah et al. 2006; Allen et al. 2008) associated with schizophrenia, but this is debated (Fujii et al. 2007).

4.3 Semaphorins and the Migration of GnRH Neurons

Gonadotropin-releasing hormone (GnRH) has a key role in reproduction by controlling the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland. Hypothalamic neuroendocrine cells secreting GnRH

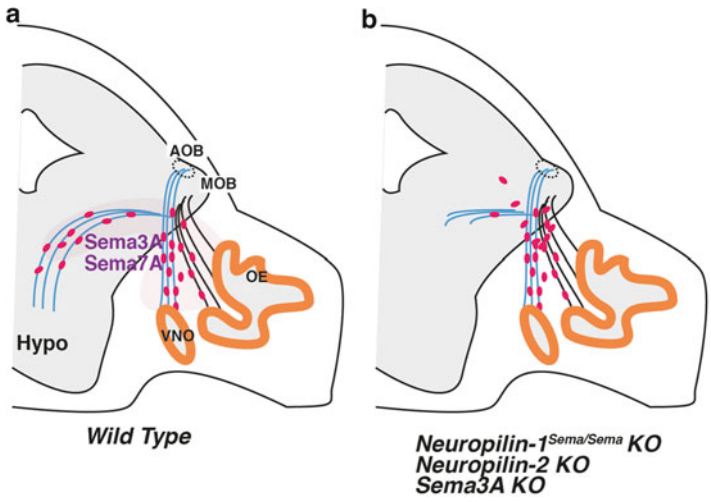

Fig. 4.2 Control of GnRH neuron migration by secreted semaphorins. (**a**) In the wild-type embryo, GnRH neurons (*red*) migrate along vomeronasal axons (*blue*) and olfactory axons (*black*) to the basal forebrain and hypothalamus. Sema3A and Sema7A are expressed along their migration pathway. (**b**) In neuropilin and Sema3A knockouts, the growth of olfactory axons and vomeronasal axons is perturbed, which results in the misrouting of GnRN neurons. Abbreviations: *VNO* vomeronasal organ, *OE* olfactory epithelium, *Hypo* hypothalamus, *MOB* main olfactory bulb, *AOB* accessory olfactory bulb

originate outside the central nervous system (CNS) in the nasal placode (Fig. 4.2) and migrate (from about E11 in the mouse) into the median eminence in the central nervous system along olfactory/vomeronasal axons (Cariboni et al. 2007b). This particular type of neuronal migration is called axophilic (Schwanzel-Fukuda and Pfaff 1989). The abnormal migration of GnrH neurons, such as in Kallmann syndrome, causes a depletion of GnRH neurons, leading to hypogonadism and infertility. Many Kallmann patients are also anosmic. So far, ten causative genes have been identified, only accounting for about one third of Kallmann cases: KAL1, which is X-linked and encodes Anosmin-1 (Legouis et al. 1991), fibroblast growth factor 8 (FGF8) and its receptor FGFR1 (Falardeau et al. 2008), HS6ST1 (Tornberg et al. 2011), WDR11 (Kim et al. 2010), CHD7 (Jongmans et al. 2009), Prokineticin-2 (PROK2, and its receptor PROKR2) (Dode et al. 2006; Pitteloud et al. 2007), and more recently SEMA3A (Hanchate et al. 2012). In mouse and human, olfactory and vomeronasal axons express neuropilin-1, neuropilin-2, and Plexin-A1 during GnRH neuron migration (Giger et al. 1996; Murakami et al. 2001; Cloutier et al. 2002; Cariboni et al. 2007a, b; Hanchate et al. 2012). Sema3A and Sema3F are found in the olfactory epithelium and vomeronasal organ (Cloutier et al. 2002). GnRH cells also express neuropilin-1/2, Plexin-A1 mRNA, Sema3A, and Sema3F (Cariboni et al. 2007a).

Many studies suggest that semaphorin/neuropilin are required for guiding vomeronasal/olfactory axons to the CNS. In the *Sema3A* knockout, olfactory axon

projections to the olfactory bulb are disorganized (Schwarting et al. 2000) and most GnRH neurons accumulate ectopically close to the olfactory bulb (OB) (Cariboni et al. 2011b), which likely explains hypogonadism in adult *Sema3A*$^{-/-}$ (Cariboni et al. 2011b; Hanchate et al. 2012). In *Neuropilin-1*$^{Sema/Sema}$ knock-in mice, a large fraction of olfactory axons fails to enter the OB, and vomeronasal axons are also misrouted (Cariboni et al. 2011b). In these mice, about half the GnRH neurons still migrate along these axons and, as a result, settle in the cortex and thalamus instead of the hypothalamus (Fig. 4.2). Puberty is delayed in *Neuropilin-1*$^{Sema/Sema}$ knock-in mice, and fertility is severely affected; this is also true for *Neuropilin-2* knockout mice, which have reduced gonadal size (Cariboni et al. 2007a). Their phenotypic analysis revealed that the number of GnRH neurons in the hypothalamus is significantly reduced (Cariboni et al. 2007a) and that many migrate into the dorsal telencephalon or accumulate at the nasal septum. In *Sema3F* knockouts, vomeronasal axons defasciculate at the surface of the CNS (Cloutier et al. 2002, 2004), but GnRH migrate normally (Cariboni et al. 2011b). Surprisingly, in this system, Sema3A seems to signal through neuropilin-1 and neuropilin-2 as a *Neuropilin-1*$^{Sema/Sema}$; *Neuropilin-2* double-mutant phenocopy of *Sema3A* knockouts (Cariboni et al. 2011b).

Interestingly, exon sequencing in Kallmann patients identified families with heterozygous nonsense and missense mutations in the *SEMA3A* gene (Hanchate et al. 2012; Young et al. 2012). These mutations either prevent Sema3A from being produced or result in the secretion of an inactive Sema3 protein (Hanchate et al. 2012; Young et al. 2012). It was also proposed that Sema3A heterozygous mutations alone are not sufficient to induce Kallmann syndrome but that it acts synergistically with other proteins or pathways also mutated in the patients (Hanchate et al. 2012). Together, these observations suggest that the inability of GnRH to enter the brain when semaphorin/neuropilin signaling is altered is probably secondary to a failure of olfactory axons to penetrate into the olfactory bulb.

However, there is also evidence for a cell-autonomous action of neuropilin-1 in GnRH neurons. In chemotactic assays, Sema3A and Sema3F have a repulsive activity on the migration of the GnRH cell line (GN11) whereas vascular endothelial growth factor (VEGF) has an attractive activity (Cariboni et al. 2007a). VEGF also controls their survival (Cariboni et al. 2011a). Interestingly, both activities appear to be mediated by neuropilin-1, at least in vitro.

The phenotypic analysis of *Neuropilin-1* null embryos (Cariboni et al. 2007a) revealed a severe decrease of the number of GnRH neurons in the forebrain, which could be explained by a combination of increased cell death and abnormal migration. This reduction was also observed following conditional deletion of neuropilin-1 in neuronal precursors (Cariboni et al. 2011a). However, it was also reported that the conditional inactivation of neuropilin-1 in GnRH neurons (using a *GnRH*:*cre* line) does not perturb their migration (Hanchate et al. 2012), which would argue in favor of the non-cell-autonomous model of action of neuropilin-1 in GnRH neurons.

Transmembrane semaphorins have also been involved in the migration of GnRH neurons. The GPI-linked Sema7A is expressed along the migratory pathway

followed by GnRH neurons to the olfactory bulb. GnRH neurons express two of its receptors, integrin-β1 and Plexin-C1, and their migration is altered in Sema7A knockout mice, which have fertility defects (Messina et al. 2011). GnRH neurons and olfactory axons also express Plexin-B1 during their migration, and its ligand Sema4D promotes, at least in vitro, the migration of GnRH cells by coupling B1 to methylethyltryptamine (*N*-methyl, *N*-ethyltryptamine, MET), a tyrosine kinase receptor for hepatocyte growth factor (HGF) (Giacobini et al. 2008). The migration of GnRH neurons is perturbed in *Plexin-B1* knockouts although the olfactory projections look normal. However, GnRH neurons are not affected in *Sema4D* knockouts, suggesting that other class 4 semaphorins might act redundantly. Interestingly, at later stages, semaphorin signaling (such as 4D/B1) regulates cellular rearrangements that accompany the cyclic production of the pituitary hormones (see Messina and Giacobini 2013, for review).

4.4 When Semaphorins Restrain Neuronal Migration at CNS Boundaries

In the hindbrain and spinal cord, several studies showed that semaphorins act at boundaries between CNS compartments to restrict cell migration.

Boundary cap (BC) cells are neural crest cell derivatives localized at the motor axon exit points at the border between the CNS and PNS in vertebrate embryos (Vermeren et al. 2003). Following genetic or surgical ablation of BC cells, motor axons exit the brain at their normal position, but some motor neuron cell bodies migrate out of the CNS inside the motor nerves, which suggests that BC cells constitute a repulsive barrier for motor neurons (Fig. 4.3). It was later found that in mouse and chick embryos BC cells express Sema6A, Sema3B, and Sema3G (Bron et al. 2007; Mauti et al. 2007). Motor neurons express neuropilin-2 (a Sema3B receptor) and Plexin-A2. In neuropilin-2 knockouts, a significant fraction of the motor neurons enter the nerve roots (Bron et al. 2007), but this is not seen when sema3B is inactivated, suggesting that BCs express another neuropilin-2 ligand. Motor neuron emigration in the peripheral nervous system (PNS) was also observed following silencing of Sema6A expression in BC cells and of its receptor plexin-A2 in motor neurons (Bron et al. 2007).

During early development, the hindbrain is transiently segmented into cellular units called rhombomeres (Lumsden and Keynes 1989; Kiecker and Lumsden 2005). Each rhombomere expresses specific combinations of transcription factors and contain progenitors that generate various neuronal populations. There is little cell dispersion and mixing between neighboring rhombomeres, and specialized cells, with distinct molecular properties and that do not produce neurons, delineate rhombomere boundaries (Lumsden and Krumlauf 1996). In zebrafish embryos, a group of neurons expressing fibroblast growth factor 20a (fbfg20a) occupy the center of each rhombomere, where it represses neuronal differentiation (Gonzalez-

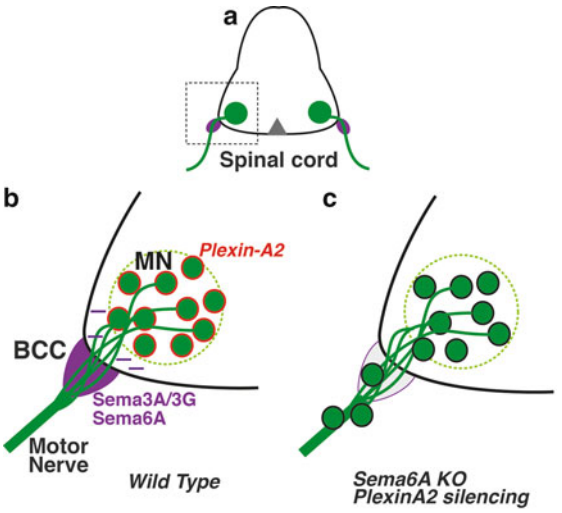

Fig. 4.3 Semaphorins and boundary cap cells. (**a**) Schematic representation of the embryonic spinal cord. Motor neurons (*green*) are localized in the ventral part of the spinal cord and send their axons outside the cord. (**b**) Region outlined in (**a**). In the wild type, motor neurons (*MN*) express Plexin-A2 and their cell bodies are prevented from leaving the spinal cord by semaphorins, expressed by boundary cap cells (*BCC*). (**c**) After Sema6A or Plexin-A2 loss of function, some MN exit the spinal cord and migrate inside motor nerves

Quevedo et al. 2010). Fgf20a neurons were found to express neuropilin-2 whereas boundary cells express its ligands, Sema3fb and Sema3gb (Terriente et al. 2012). The knockdown of either neuropilin-2 or Sema3fb/Sema3gb leads to the dispersion of Fgf20 neurons inside the rhombomere and increased neurogenesis; this suggests that Sema3fb/Sema3gb, secreted by boundary cells, repels fgf20 cells and confines them to the center of each rhombomere, thereby influencing neurogenesis.

Neuropilin-1 also influences neuronal migration between rhombomeres. In the mouse, neurons that will form the facial motor nucleus are generated in rhombomere 4 but migrate caudally to settle in rhombomere 6 (Studer et al. 1996). In neuropilin-1-null mice, the tangential migration of facial motor neurons is compromised and most fail to reach rhombomere 6 (Schwarz et al. 2004). In this case, it was found that VEGF, and not class 3 semaphorins, is the neuropilin-1 ligand.

4.5 Semaphorins and Plexins Control the Migration of Granule Cells in the Cerebellum and Olfactory Bulb

In the developing cerebellum of most vertebrates, granule cells (GCs) derive from precursors localized in the external granular layer (EGL), which covers the surface cerebellar cortex (Chedotal 2010). In mice, GCs are generated during the first 3 postnatal weeks. They first migrate tangentially in the EGL and then radially across the molecular layer to reach the internal granule cell layer (IGL) (Ramon and Cajal 1911; Altman 1972) (Fig. 4.4).

Previous studies showed that during their tangential migration GCs express the transmembrane semaphorin Sema6A (Kerjan et al. 2005), which is downregulated at the onset of radial migration. Migrating GCs also express Plexin-A2, one of

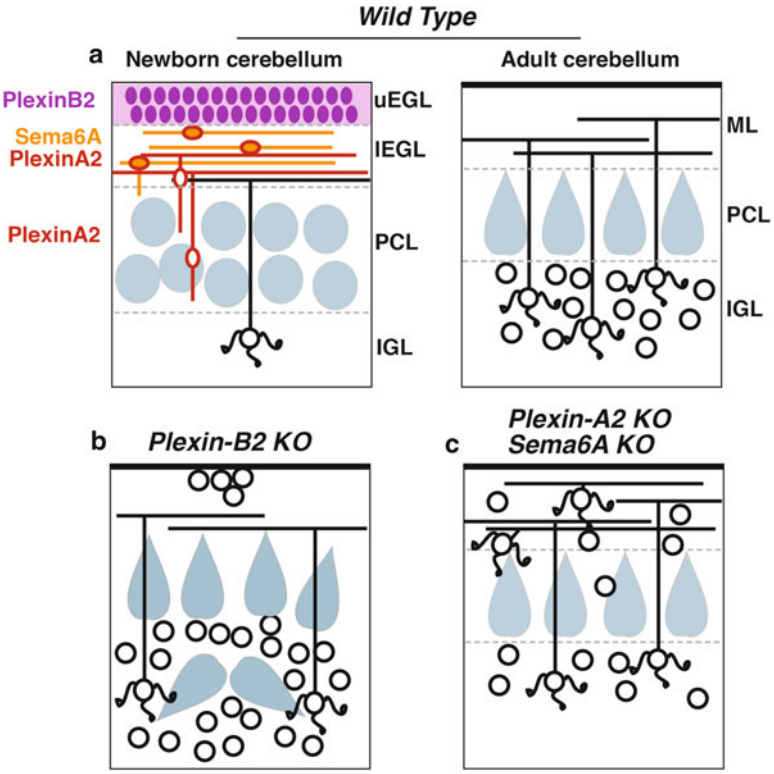

Fig. 4.4 Control of cerebellar granule cell migration by transmembrane semaphorins. (**a**) In newborn wild-type mice, granule cell progenitors (in *purple*) proliferate exclusively in the upper external granule cell layer (*uEGL*) and express Plexin-B2. Postmitotic granule cells start differentiating and migrate tangentially in the lower EGL (*lEGL*). During this phase, they express Sema6A and Plexin-A2. Next, they migrate radially through the molecular layer (*ML*) and the Purkinje cell layer (*PCL*) to the internal granule cell layer (*IGL*). Sema6A is downregulated at the onset of radial migration. At this stage Purkinje cells (in *blue*) are still distributed in multiple layers. In adult mice, Purkinje cells are aligned in a monolayer, above differentiated granule cell bodies all localized in the inner granular layer (*IGL*). (**b**) In *Plexin-B2* mutants, some ectopic granule cells are found at the top of the molecular layer and the cerebellar cortex is fragmented. Islands of Purkinje cells (*arrow*) are embedded in differentiated granule cells. (**c**) In *Sema6A* and *Plexin-A2* knockouts, about half the granule cells fail to migrate radially and are found in the molecular layer

the receptors for Sema6A (Renaud et al. 2008). Phenotypic analysis of *Sema6A* knockout and *PlexinA2* knockout mice revealed that migration from the EGL of about half of the GC population is aborted and that their cell body remains trapped inside the molecular layer (Kerjan et al. 2005; Renaud et al. 2008). Time-lapse studies of GC migration in postnatal EGL explants showed that, in both *Sema6A* and *PlexinA2* knockouts, the migration defects are probably related to an anomaly of nuclear translocation (Renaud et al. 2008). In *Sema6A* knockouts,

the centrosome appears unable to detach from the nucleus, whereas in *Plexin-A2* knockouts, the distance separating the centrosome from the nucleus becomes significantly increased. Therefore, in both cases, an abnormal coupling of the nucleus and centrosome might block migration. However, this could just illustrate a more global disorganization of the migration machinery, in particular of the actin dynamics. In the current working model, Sema6A–Plexin-A2 interaction is thought to have a role in the control of the switch from a tangential to radial mode of migration (Chédotal 2010) as this is correlated with the downregulation of Sema6A expression. The analysis of GC migration in mouse chimeras combining the wild type and either Plexin-A2 or Sema6A knockouts suggests that Sema6A is a ligand for Plexin-A2 in migrating GCs (Renaud et al. 2008). In vitro assays have also shown that GC migration is impaired on Sema6A-expressing cells (Janssen et al. 2010), suggesting that Sema6A could repel GCs away from the EGL. However, it might promote cell motility rather than simply inhibiting tangential migration. In vitro data suggest that the guanine nucleotide exchange factor (GEF) trio might act downstream of Plexin-A2/Sema6A (Peng et al. 2010). Rac1 might also be involved, because the conditional inactivation of Rac1 in migrating GCs somehow phenocopies the defects observed in *Sema6A* and *Plexin-A2* knockouts (Tahirovic et al. 2010). As Sema6A and Plexin-A2 are also coexpressed in GCs, this suggests that they could also signal in *cis* in tangentially migrating GCs, as described for Sema6A and Plexin-A4 in sensory axons (Haklai-Topper et al. 2010).

During their proliferation in the upper part of the EGL, GC precursors express Plexin-B2, which is downregulated in postmitotic GCs entering the lower EGL (Fig. 4.4). Therefore, there is a correlation between Plexin-B2 extinction and the initiation of tangential migration and cell-cycle exit (Friedel et al. 2007). In Plexin-B2 knockout mice, GC differentiation is initiated and the GCs migrate away from the cerebellar surface along radial glia. However, mutant GCs do not stop their proliferation and keep dividing outside the upper EGL, within the molecular layer, and even in the IGL, which profoundly disorganizes the structure of the adult cerebellar cortex (Deng et al. 2007; Friedel et al. 2007). The Plexin-B2 mechanism of action and its physiological ligand(s) in the developing cerebellum are still unknown, although genetic and in vitro evidence suggests that Sema4C and Sema4G are involved (Maier et al. 2011). Both semaphorins are expressed in migrating GCs; they bind to Plexin-B2, and in Sema4C/sema4G double knockouts a subset of GCs does not migrate properly. However, the cerebellar defects are much more severe in Plexin-B2 knockouts, supporting the existence of other ligands.

Interestingly, Plexin-B2 is also expressed in the postnatal and adult brain in the precursors of olfactory bulb (OB) granule cells (Saha et al. 2012). These interneurons are generated throughout life from neural stem cells located in the subventricular zone lining the cerebral ventricles. These cells produce neuroblasts that migrate tangentially to the OB along the rostral migratory stream (RMS) (Lois and Alvarez-Buylla 1994; Sanai et al. 2011). These cells differentiate and switch to a radial mode of migration on entering the OB (Bovetti et al. 2007; Snapyan et al. 2009). As in the cerebellum, the downregulation of Plexin-B2 expression is correlated with the final cell division and the initiation of the radial

migration of postmitotic granule cells. However, in this case, Plexin-B2 promotes the proliferation of SVZ neuroblasts in addition to acting on GC migration (Saha et al. 2012). It also acts on periglomerular cells, the second type of OB interneurons generated by SVZ neuroblasts. RMS neuroblasts migrate more rapidly in *Plexin-B2* knockout than in wild-type mice. Similarly, macrophages and dendritic cells purified from Plexin-B2 knockout mice migrate more rapidly (Roney et al. 2011), suggesting that in various cell types, including cerebellar cortical and OB neurons, Plexin-B2 could provide a molecular tuning mechanism allowing migrating cells to control migration/proliferation choices at specific locations and time points. Again, the Plexin-B2 ligands involved in this process remain to be characterized.

4.6 Semaphorins Control the Migration of Oligodendrocyte Precursors During Myelination and Remyelination

Oligodendrocytes are the myelinating cells in the central nervous system of jawed vertebrates (Zalc et al. 2008). These cells extend processes that wrap around the axon and form the myelin sheath, interrupted at the level of the nodes of Ranvier where voltage-gated sodium channels accumulate, which allows the rapid saltatory conduction of action potentials (Sherman and Brophy 2005 for a review).

Oligodendrocytes are generated across the CNS from precursors (or OPCs, oligodendrocyte precursors) localized in multiple neuroepithelial foci of the ventricular zone (Pringle and Richardson 1993; Vallstedt et al. 2005). Starting at embryonic ages, OPCs migrate from the ventricular zone to colonize the CNS. In rodents, OPCs start to produce postmitotic oligodendrocytes after birth, but myelination continues for several weeks. Moreover, some OPCs are still present in the adult CNS and produce new oligodendrocytes throughout life. In demyelinating diseases, such as multiple sclerosis (MS), the myelin sheath is destroyed and oligodendrocytes die, leading to myelin-poor areas called plaques. OPCs also exist in the adult CNS and can proliferate to generate new oligodendrocytes in response to injury (Franklin and Ffrench-Constant 2008). These adult OPCs appear to migrate into demyelinated regions, and their recruitment is required for myelin repair. The migration and proliferation of OPCs during development is influenced by trophic/growth factors such as platelet-derived growth factor (PDGF) and fibroblast growth factor 2 (FGF-2). However, mounting evidence indicates that axon guidance molecules, including secreted semaphorins, also pattern their migration.

OPCs invade the optic nerve (ON) from the preoptic area in the brain, around 2 weeks of gestation, and reach the optic nerve head before birth (Spassky et al. 2002). In mammals, OPCs do not enter the retina. In mouse and rat embryos, ON nerve OPCs express neuropilin-1 and neuropilin-2 (Sugimoto et al. 2001; Spassky et al. 2002) as well as postnatal rat OPCs (Cohen et al. 2003). Neuropilin-1 is still expressed in mature oligodendrocytes (Ricard et al. 2001), unlike neuropilin-2 (Xiang et al. 2012). In three-dimensional (3D) collagen gel cultures, OPCs

migrating from ON explants are repelled by cells secreting Sema3A, and this repulsive activity is blocked by anti-neuropilin-1 antibodies (Sugimoto et al. 2001; Spassky et al. 2002). By contrast, OPCs are attracted by cells secreting Sema3F via a receptor complex comprising neuropilin-2 (Spassky et al. 2002) and Plexin-A3 (Xiang et al. 2012). These chemotropic activities appear specific, as migrating OPCs do not respond to gradients of soluble Sema3C or Sema3E. However, postnatal OPCs avoid substrate-bound Sema3C and Sema3B (Cohen et al. 2003). Sema3A is enriched in cells that surround the ON and might prevent OPCs from leaving the ON. Sema3F, which is expressed by retinal ganglion cells, might attract OPCs toward their axons. Moreover, oligodendrocytes themselves express several secreted and transmembrane semaphorins (Cohen et al. 2003; Moreau-Fauvarque et al. 2003; Bernard et al. 2012), which might also act influence cell–cell interaction during oligodendrocyte migration. For instance, Sema4F is a transmembrane semaphorin (Encinas et al. 1999) expressed by OPCs in the mouse CNS (Armendariz et al. 2012). In vitro migration assays suggest that Sema4F inhibits the migration of ON OPCs, possibly in a paracrine/autocrine manner. Of note, direct in vivo support for these models is still lacking as neither the distribution of oligodendrocytes nor myelination seems to be perturbed in *Sema3A*, *Sema3F*, or *neuropilin-1/2* knockouts (Kitsukawa et al. 1997; Taniguchi et al. 1997; Chen et al. 2000; Giger et al. 2000). A recent study suggests that the distribution of OPCs is uneven in the neocortex of newborn Plexin-A4 knockout mice (Okada and Tomooka 2012). Plexin-A4 forms, with neuropilin-1, a receptor complex for Sema3A, but it is also a receptor for Sema6A. Sema6A is expressed during myelination by postnatal oligodendrocytes and could therefore influence the migration of OPCs. Accordingly, 3T3 cells expressing Sema6A have a repulsive activity for cells of an OPC line, which is mediated by Plexin-A4 (Okada et al. 2007; Okada and Tomooka 2013). However, these cells also express Sema6A and are repelled by Plexin-A4, suggesting that Sema6A might act as a receptor in OPCs. Although the analysis of *Sema6A* knockout revealed a myelination delay of CNS white matter tracts, including the ON, the recruitment of oligodendrocytes to these axonal tracts does not seem to be altered (Bernard et al. 2012), which would support a ligand function of Sema6A in OPC migration, if any.

Recent data suggest that secreted semaphorins might also influence the remyelination of demyelinating lesions by acting on the migration of adult OPCs. It has been proposed that impaired remyelination in chronic MS lesions is linked to a poor recruitment of OPCs to the plaques. Adult OPcs still express neuropilins and Plexin-A1/-A3 receptors, suggesting that these and OPCs could still be attracted or repelled by Sema3F and Sema3A, respectively (Piaton et al. 2011). It was shown that the expression of Sema3A and Sema3A transcripts is enriched and upregulated in neurons and glia (mostly astrocytes) at active inflammatory MS lesions and in demyelinating lesions induced by lysophosphatidylcholine (LPC) in the rat spinal cord (Williams et al. 2007). Therefore, abnormal semaphorin expression might perturb the ability of OPCs and new oligodendrocytes to disseminate inside the lesion, opening new therapeutic perspectives for MS treatment. A first step toward the in vivo validation of this strategy has been obtained in LPC mouse models

in which lentiviral vectors expressing Sema3A or Sema3F were injected into the demyelinated lesion. Sema3F overexpression increased significantly the number of OPCs at the lesion whereas Sema3A decreased it (Piaton et al. 2011). In addition, OPC recruitment after LPC lesions was impaired in *neuropilin-1*$^{Sema/Sema}$ knock-in mice, which are unable to bind Sema3A but still bind vascular endothelial growth factor (VEGF) (Gu et al. 2003). Although another study has confirmed the inhibitory action of Sema3A on remyelination in a model of ethidium bromide-induced demyelination (Syed et al. 2011), it was suggested that this was primarily through an action on OPC differentiation rather than on their migration. In conclusion, the physiological function of semaphorins in myelination and remyelination is still an open question. Another transmembrane semaphorin, Sema5A, is expressed by cells of the oligodendrocyte lineage (Goldberg et al. 2004; Hilario et al. 2009), as well as its receptor Plexin-B3 (Artigiani et al. 2004). However, no myelin defects were detected in Plexin-B3 knockout mice (Worzfeld et al. 2009). By contrast, Sema5A was found to inhibit the motility of glioma cells through Plexin-B3 and the inactivation of Rac1. Sema5A also promotes the glial differentiation of glioma cells, suggesting that a downregulation of Sema5A expression, as observed in human astrocytomas, might influence the dissemination of tumor cells within the CNS (Li and Lee 2010; Li et al. 2012).

References

Aizawa H, Wakatsuki S, Ishii A, Moriyama K, Sasaki Y, Ohashi K, Sekine-Aizawa Y, Sehara-Fujisawa A, Mizuno K, Goshima Y, Yahara I (2001) Phosphorylation of cofilin by LIM-kinase is necessary for semaphorin 3A-induced growth cone collapse. Nat Neurosci 4(4):367–373. doi:10.1038/86011

Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, Tanzi RE, Bertram L (2008) Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet 40(7):827–834. doi:10.1038/ng.171

Altman J (1972) Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. J Comp Neurol 145(3):353–397

Anderson SA, Eisenstat DD, Shi L, Rubenstein JL (1997) Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science 278(5337):474–476

Andrews W, Barber M, Hernadez-Miranda LR, Xian J, Rakic S, Sundaresan V, Rabbitts TH, Pannell R, Rabbitts P, Thompson H, Erskine L, Murakami F, Parnavelas JG (2008) The role of Slit-Robo signaling in the generation, migration and morphological differentiation of cortical interneurons. Dev Biol 313(2):648–658

Andrews WD, Zito A, Memi F, Jones G, Tamamaki N, Parnavelas JG (2013) Limk2 mediates semaphorin signalling in cortical interneurons migrating through the subpallium. Biol Open 2(3):277–282. doi:10.1242/bio.20133202

Armendariz BG, Bribian A, Perez-Martinez E, Martinez A, de Castro F, Soriano E, Burgaya F (2012) Expression of Semaphorin 4F in neurons and brain oligodendrocytes and the regulation of oligodendrocyte precursor migration in the optic nerve. Mol Cell Neurosci 49(1):54–67. doi:10.1016/j.mcn.2011.09.003

Artigiani S, Conrotto P, Fazzari P, Gilestro GF, Barberis D, Giordano S, Comoglio PM, Tamagnone L (2004) Plexin-B3 is a functional receptor for semaphorin 5A. EMBO Rep 5(7):710–714

Asada N, Sanada K (2010) LKB1-mediated spatial control of GSK3beta and adenomatous polyposis coli contributes to centrosomal forward movement and neuronal migration in the developing neocortex. J Neurosci 30(26):8852–8865. doi:10.1523/JNEUROSCI.6140-09.2010

Asada N, Sanada K, Fukada Y (2007) LKB1 regulates neuronal migration and neuronal differentiation in the developing neocortex through centrosomal positioning. J Neurosci 27(43):11769–11775. doi:10.1523/JNEUROSCI.1938-07.2007

Ascoli GA, Alonso-Nanclares L, Anderson SA, Barrionuevo G, Benavides-Piccione R, Burkhalter A, Buzsaki G, Cauli B, Defelipe J, Fairen A, Feldmeyer D, Fishell G, Fregnac Y, Freund TF, Gardner D, Gardner EP, Goldberg JH, Helmstaedter M, Hestrin S, Karube F, Kisvarday ZF, Lambolez B, Lewis DA, Marin O, Markram H, Munoz A, Packer A, Petersen CC, Rockland KS, Rossier J, Rudy B, Somogyi P, Staiger JF, Tamas G, Thomson AM, Toledo-Rodriguez M, Wang Y, West DC, Yuste R (2008) Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat Rev Neurosci 9(7):557–568. doi:10.1038/nrn2402

Azzarelli R, Pacary E, Garg R, Garcez P, van den Berg D, Riou P, Ridley AJ, Friedel RH, Parsons M, Guillemot F (2014) An antagonistic interaction between PlexinB2 and Rnd3 controls RhoA activity and cortical neuron migration. Nat Commun 5:3405. doi:10.1038/ncomms4405

Bagnard D, Lohrum M, Uziel D, Puschel AW, Bolz J (1998) Semaphorins act as attractive and repulsive guidance signals during the development of cortical projections. Development 125(24):5043–5053

Bartolini G, Ciceri G, Marin O (2013) Integration of GABAergic interneurons into cortical cell assemblies: lessons from embryos and adults. Neuron 79(5):849–864. doi:10.1016/j.neuron.2013.08.014

Bernard F, Moreau-Fauvarque C, Heitz-Marchaland C, Zagar Y, Dumas L, Fouquet S, Lee X, Shao Z, Mi S, Chedotal A (2012) Role of transmembrane semaphorin Sema6A in oligodendrocyte differentiation and myelination. Glia 60(10):1590–1604. doi:10.1002/glia.22378

Bovetti S, Bovolin P, Perroteau I, Puche AC (2007) Subventricular zone-derived neuroblast migration to the olfactory bulb is modulated by matrix remodelling. Eur J Neurosci 25(7):2021–2033. doi:10.1111/j.1460-9568.2007.05441.x

Bron R, Vermeren M, Kokot N, Andrews W, Little GE, Mitchell KJ, Cohen J (2007) Boundary cap cells constrain spinal motor neuron somal migration at motor exit points by a semaphorin-plexin mechanism. Neural Dev 2:21. doi:1749-8104-2-21 [pii] 10.1186/1749-8104-2-21

Cariboni A, Hickok J, Rakic S, Andrews W, Maggi R, Tischkau S, Parnavelas JG (2007a) Neuropilins and their ligands are important in the migration of gonadotropin-releasing hormone neurons. J Neurosci 27(9):2387–2395. doi:10.1523/JNEUROSCI.5075-06.2007

Cariboni A, Maggi R, Parnavelas JG (2007b) From nose to fertility: the long migratory journey of gonadotropin-releasing hormone neurons. Trends Neurosci 30(12):638–644. doi:10.1016/j.tins.2007.09.002

Cariboni A, Davidson K, Dozio E, Memi F, Schwarz Q, Stossi F, Parnavelas JG, Ruhrberg C (2011a) VEGF signalling controls GnRH neuron survival via NRP1 independently of KDR and blood vessels. Development 138(17):3723–3733. doi:10.1242/dev.063362

Cariboni A, Davidson K, Rakic S, Maggi R, Parnavelas JG, Ruhrberg C (2011b) Defective gonadotropin-releasing hormone neuron migration in mice lacking SEMA3A signalling through NRP1 and NRP2: implications for the aetiology of hypogonadotropic hypogonadism. Hum Mol Genet 20(2):336–344. doi:10.1093/hmg/ddq468

Catalano SM, Messersmith EK, Goodman CS, Shatz CJ, Chedotal A (1998) Many major CNS axon projections develop normally in the absence of semaphorin III. Mol Cell Neurosci 11(4):173–182

Chedotal A (2010) Should I stay or should I go? Becoming a granule cell. Trends Neurosci 33(4):163–172

Chen H, Bagri A, Zupicich JA, Zou Y, Stoeckli E, Pleasure SJ, Lowenstein DH, Skarnes WC, Chedotal A, Tessier-Lavigne M (2000) Neuropilin-2 regulates the development of selective cranial and sensory nerves and hippocampal mossy fiber projections. Neuron 25(1):43–56

Chen G, Sima J, Jin M, Wang KY, Xue XJ, Zheng W, Ding YQ, Yuan XB (2007) Semaphorin-3A guides radial migration of cortical neurons during development. Nat Neurosci 11(1):36–44

Cloutier JF, Giger RJ, Koentges G, Dulac C, Kolodkin AL, Ginty DD (2002) Neuropilin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. Neuron 33(6):877–892

Cloutier JF, Sahay A, Chang EC, Tessier-Lavigne M, Dulac C, Kolodkin AL, Ginty DD (2004) Differential requirements for semaphorin 3F and Slit-1 in axonal targeting, fasciculation, and segregation of olfactory sensory neuron projections. J Neurosci 24(41):9087–9096

Cohen RI, Rottkamp DM, Maric D, Barker JL, Hudson LD (2003) A role for semaphorins and neuropilins in oligodendrocyte guidance. J Neurochem 85(5):1262–1278

de Carlos JA, Lopez-Mascaraque L, Valverde F (1996) Dynamics of cell migration from the lateral ganglionic eminence in the rat. J Neurosci 16(19):6146–6156

Deng S, Hirschberg A, Worzfeld T, Penachioni JY, Korostylev A, Swiercz JM, Vodrazka P, Mauti O, Stoeckli ET, Tamagnone L, Offermanns S, Kuner R (2007) Plexin-B2, but not Plexin-B1, critically modulates neuronal migration and patterning of the developing nervous system in vivo. J Neurosci 27(23):6333–6347

Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP (2006) Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet 2(10):e175

Eastwood SL, Law AJ, Everall IP, Harrison PJ (2003) The axonal chemorepellant semaphorin 3A is increased in the cerebellum in schizophrenia and may contribute to its synaptic pathology. Mol Psychiatry 8(2):148–155

Encinas JA, Kikuchi K, Chedotal A, de Castro F, Goodman CS, Kimura T (1999) Cloning, expression, and genetic mapping of Sema W, a member of the semaphorin family. Proc Natl Acad Sci U S A 96(5):2491–2496

Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N (2008) Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest 118(8):2822–2831. doi:10.1172/JCI34538

Fouquet C, Di Meglio T, Ma L, Kawasaki T, Long H, Hirata T, Tessier-Lavigne M, Chedotal A, Nguyen-Ba-Charvet KT (2007) Robo1 and robo2 control the development of the lateral olfactory tract. J Neurosci 27(11):3037–3045

Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9(11):839–855. doi:10.1038/nrn2480

Friedel RH, Kerjan G, Rayburn H, Schuller U, Sotelo C, Tessier-Lavigne M, Chedotal A (2007) Plexin-B2 controls the development of cerebellar granule cells. J Neurosci 27(14):3921–3932

Fujii T, Iijima Y, Kondo H, Shizuno T, Hori H, Nakabayashi T, Arima K, Saitoh O, Kunugi H (2007) Failure to confirm an association between the PLXNA2 gene and schizophrenia in a Japanese population. Prog Neuropsychopharmacol Biol Psychiatry 31(4):873–877

Fujii T, Uchiyama H, Yamamoto N, Hori H, Tatsumi M, Ishikawa M, Arima K, Higuchi T, Kunugi H (2011) Possible association of the semaphorin 3D gene (SEMA3D) with schizophrenia. J Psychiatry Res 45(1):47–53. doi:10.1016/j.jpsychires.2010.05.004

Giacobini P, Messina A, Morello F, Ferraris N, Corso S, Penachioni J, Giordano S, Tamagnone L, Fasolo A (2008) Semaphorin 4D regulates gonadotropin hormone-releasing hormone-1 neuronal migration through PlexinB1-Met complex. J Cell Biol 183(3):555–566. doi:10.1083/jcb.200806160

Giger RJ, Wolfer DP, De Wit GM, Verhaagen J (1996) Anatomy of rat semaphorin III/collapsin-1 mRNA expression and relationship to developing nerve tracts during neuroembryogenesis. J Comp Neurol 375(3):378–392. doi:10.1002/(SICI)1096-9861(19961118)375:3<378::AID-CNE3>3.0.CO;2-# [pii] 10.1002/(SICI)1096-9861(19961118)375:3<378::AID-CNE3>3.0.CO;2-#

Giger RJ, Cloutier JF, Sahay A, Prinjha RK, Levengood DV, Moore SE, Pickering S, Simmons D, Rastan S, Walsh FS, Kolodkin AL, Ginty DD, Geppert M (2000) Neuropilin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. Neuron 25(1):29–41

Goldberg JL, Vargas ME, Wang JT, Mandemakers W, Oster SF, Sretavan DW, Barres BA (2004) An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J Neurosci 24(21):4989–4999

Gonzalez-Quevedo R, Lee Y, Poss KD, Wilkinson DG (2010) Neuronal regulation of the spatial patterning of neurogenesis. Dev Cell 18(1):136–147. doi:10.1016/j.devcel.2009.11.010

Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, Richards LJ, Kolodkin AL, Ginty DD (2003) Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Dev Cell 5(1):45–57

Gupta A, Sanada K, Miyamoto DT, Rovelstad S, Nadarajah B, Pearlman AL, Brunstrom J, Tsai LH (2003) Layering defect in p35 deficiency is linked to improper neuronal-glial interaction in radial migration. Nat Neurosci 6(12):1284–1291. doi:10.1038/nn1151

Haklai-Topper L, Mlechkovich G, Savariego D, Gokhman I, Yaron A (2010) Cis interaction between Semaphorin6A and Plexin-A4 modulates the repulsive response to Sema6A. EMBO J 29(15):2635–2645. doi:emboj2010147 [pii] 10.1038/emboj.2010.147

Hanchate NK, Giacobini P, Lhuillier P, Parkash J, Espy C, Fouveaut C, Leroy C, Baron S, Campagne C, Vanacker C, Collier F, Cruaud C, Meyer V, Garcia-Pinero A, Dewailly D, Cortet-Rudelli C, Gersak K, Metz C, Chabrier G, Pugeat M, Young J, Hardelin JP, Prevot V, Dode C (2012) SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome. PLoS Genet 8(8):e1002896. doi:10.1371/journal.pgen.1002896

Hernandez-Miranda LR, Cariboni A, Faux C, Ruhrberg C, Cho JH, Cloutier JF, Eickholt BJ, Parnavelas JG, Andrews WD (2011) Robo1 regulates semaphorin signaling to guide the migration of cortical interneurons through the ventral forebrain. J Neurosci 31(16):6174–6187. doi:10.1523/JNEUROSCI.5464-10.2011

Hilario JD, Rodino-Klapac LR, Wang C, Beattie CE (2009) Semaphorin 5A is a bifunctional axon guidance cue for axial motoneurons in vivo. Dev Biol 326(1):190–200. doi:10.1016/j.ydbio.2008.11.007

Hirschberg A, Deng S, Korostylev A, Paldy E, Costa MR, Worzfeld T, Vodrazka P, Wizenmann A, Gotz M, Offermanns S, Kuner R (2010) Gene deletion mutants reveal a role for semaphorin receptors of the plexin-B family in mechanisms underlying corticogenesis. Mol Cell Biol 30(3):764–780. doi:10.1128/MCB.01458-09

Ito K, Kawasaki T, Takashima S, Matsuda I, Aiba A, Hirata T (2008) Semaphorin 3F confines ventral tangential migration of lateral olfactory tract neurons onto the telencephalon surface. J Neurosci 28(17):4414–4422

Janssen BJ, Robinson RA, Perez-Branguli F, Bell CH, Mitchell KJ, Siebold C, Jones EY (2010) Structural basis of semaphorin-plexin signalling. Nature 467(7319):1118–1122. doi:nature09468 [pii] 10.1038/nature09468

Jongmans MC, van Ravenswaaij-Arts CM, Pitteloud N, Ogata T, Sato N, Claahsen-van der Grinten HL, van der Donk K, Seminara S, Bergman JE, Brunner HG, Crowley WF Jr, Hoefsloot LH (2009) CHD7 mutations in patients initially diagnosed with Kallmann syndrome – the clinical overlap with CHARGE syndrome. Clin Genet 75(1):65–71. doi:10.1111/j.1399-0004.2008.01107.x

Kawasaki T, Ito K, Hirata T (2006) Netrin 1 regulates ventral tangential migration of guidepost neurons in the lateral olfactory tract. Development 133(5):845–853

Kerjan G, Dolan J, Haumaitre C, Schneider-Maunoury S, Fujisawa H, Mitchell KJ, Chedotal A (2005) The transmembrane semaphorin Sema6A controls cerebellar granule cell migration. Nat Neurosci 8(11):1516–1524

Kiecker C, Lumsden A (2005) Compartments and their boundaries in vertebrate brain development. Nat Rev Neurosci 6(7):553–564

Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, Ha KS, Itokawa Y, Meliciani I, Wenzel W, Lee D, Rosenberger G, Ozata M, Bick DP, Sherins RJ, Nagase T, Tekin M, Kim SH, Kim CH, Ropers HH, Gusella JF, Kalscheuer V, Choi CY, Layman LC (2010) WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet 87(4):465–479. doi:10.1016/j.ajhg.2010.08.018

Kitsukawa T, Shimizu M, Sanbo M, Hirata T, Taniguchi M, Bekku Y, Yagi T, Fujisawa H (1997) Neuropilin-semaphorin III/D-mediated chemorepulsive signals play a crucial role in peripheral nerve projection in mice. Neuron 19(5):995–1005

Law CO, Kirby RJ, Aghamohammadzadeh S, Furley AJ (2008) The neural adhesion molecule TAG-1 modulates responses of sensory axons to diffusible guidance signals. Development 135(14):2361–2371

Le TN, Du G, Fonseca M, Zhou QP, Wigle JT, Eisenstat DD (2007) Dlx homeobox genes promote cortical interneuron migration from the basal forebrain by direct repression of the semaphorin receptor neuropilin-2. J Biol Chem 282(26):19071–19081. doi:10.1074/jbc.M607486200

Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, Millasseau P, Le Paslier D, Cohen D, Caterina D et al (1991) The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. Cell 67(2):423–435

Li X, Lee AY (2010) Semaphorin 5A and plexin-B3 inhibit human glioma cell motility through RhoGDI alpha-mediated inactivation of Rac1 GTPase. J Biol Chem 285(42):32436–32445. doi:10.1074/jbc.M110.120451

Li X, Law JW, Lee AY (2012) Semaphorin 5A and plexin-B3 regulate human glioma cell motility and morphology through Rac1 and the actin cytoskeleton. Oncogene 31(5):595–610. doi:10.1038/onc.2011.256

Lois C, Alvarez-Buylla A (1994) Long-distance neuronal migration in the adult mammalian brain. Science 264(5162):1145–1148

Lumsden A, Keynes R (1989) Segmental patterns of neuronal development in the chick hindbrain. Nature 337(6206):424–428

Lumsden A, Krumlauf R (1996) Patterning the vertebrate neuraxis. Science 274(5290):1109–1115

Mah S, Nelson MR, Delisi LE, Reneland RH, Markward N, James MR, Nyholt DR, Hayward N, Handoko H, Mowry B, Kammerer S, Braun A (2006) Identification of the semaphorin receptor PLXNA2 as a candidate for susceptibility to schizophrenia. Mol Psychiatry 11(5):471–478

Maier V, Jolicoeur C, Rayburn H, Takegahara N, Kumanogoh A, Kikutani H, Tessier-Lavigne M, Wurst W, Friedel RH (2011) Semaphorin 4C and 4G are ligands of Plexin-B2 required in cerebellar development. Mol Cell Neurosci 46(2):419–431. doi:10.1016/j.mcn.2010.11.005

Marin O, Rubenstein JL (2003) Cell migration in the forebrain. Annu Rev Neurosci 26:441–483

Marin O, Yaron A, Bagri A, Tessier-Lavigne M, Rubenstein JL (2001) Sorting of striatal and cortical interneurons regulated by semaphorin–neuropilin interactions. Science 293(5531):872–875

Marin O, Plump AS, Flames N, Sanchez-Camacho C, Tessier-Lavigne M, Rubenstein JL (2003) Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction. Development 130(9):1889–1901

Mauti O, Domanitskaya E, Andermatt I, Sadhu R, Stoeckli ET (2007) Semaphorin6A acts as a gate keeper between the central and the peripheral nervous system. Neural Dev 2:28. doi:1749-8104-2-28 [pii] 10.1186/1749-8104-2-28

Messina A, Giacobini P (2013) Semaphorin signaling in the development and function of the gonadotropin hormone-releasing hormone system. Front Endocrinol (Lausanne) 4:133. doi:10.3389/fendo.2013.00133

Messina A, Ferraris N, Wray S, Cagnoni G, Donohue DE, Casoni F, Kramer PR, Derijck AA, Adolfs Y, Fasolo A, Pasterkamp RJ, Giacobini P (2011) Dysregulation of Semaphorin 7A/beta1-integrin signaling leads to defective GnRH-1 cell migration, abnormal gonadal development and altered fertility. Hum Mol Genet 20(24):4759–4774. doi:10.1093/hmg/ddr403

Metin C, Vallee RB, Rakic P, Bhide PG (2008) Modes and mishaps of neuronal migration in the mammalian brain. J Neurosci 28(46):11746–11752

Moreau-Fauvarque C, Kumanogoh A, Camand E, Jaillard C, Barbin G, Boquet I, Love C, Jones EY, Kikutani H, Lubetzki C, Dusart I, Chedotal A (2003) The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J Neurosci 23(27):9229–9239

Murakami Y, Suto F, Shimizu M, Shinoda T, Kameyama T, Fujisawa H (2001) Differential expression of plexin-A subfamily members in the mouse nervous system. Dev Dyn 220(3):246–258

Namba T, Kibe Y, Funahashi Y, Nakamuta S, Takano T, Ueno T, Shimada A, Kozawa S, Okamoto M, Shimoda Y, Oda K, Wada Y, Masuda T, Sakakibara A, Igarashi M, Miyata T, Faivre-Sarrailh C, Takeuchi K, Kaibuchi K (2014) Pioneering axons regulate neuronal polarization in the developing cerebral cortex. Neuron 81(4):814–829. doi:10.1016/j.neuron.2013.12.015

Nobrega-Pereira S, Kessaris N, Du T, Kimura S, Anderson SA, Marin O (2008) Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. Neuron 59(5):733–745

Oinuma I, Ishikawa Y, Katoh H, Negishi M (2004) The Semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. Science 305(5685):862–865

Okada A, Tomooka Y (2012) Possible roles of Plexin-A4 in positioning of oligodendrocyte precursor cells in developing cerebral cortex. Neurosci Lett 516(2):259–264. doi:10.1016/j.neulet.2012.04.005

Okada A, Tomooka Y (2013) A role of Sema6A expressed in oligodendrocyte precursor cells. Neurosci Lett 539:48–53. doi:10.1016/j.neulet.2013.01.026

Okada A, Tominaga M, Horiuchi M, Tomooka Y (2007) Plexin-A4 is expressed in oligodendrocyte precursor cells and acts as a mediator of semaphorin signals. Biochem Biophys Res Commun 352(1):158–163. doi:S0006-291X(06)02438-7 [pii] 10.1016/j.bbrc.2006.10.176

Pacary E, Azzarelli R, Guillemot F (2013) Rnd3 coordinates early steps of cortical neurogenesis through actin-dependent and -independent mechanisms. Nat Commun 4:1635. doi:10.1038/ncomms2614

Peng YJ, He WQ, Tang J, Tao T, Chen C, Gao YQ, Zhang WC, He XY, Dai YY, Zhu NC, Lv N, Zhang CH, Qiao YN, Zhao LP, Gao X, Zhu MS (2010) Trio is a key guanine nucleotide exchange factor coordinating regulation of the migration and morphogenesis of granule cells in the developing cerebellum. J Biol Chem 285(32):24834–24844. doi:10.1074/jbc.M109.096537

Piaton G, Aigrot MS, Williams A, Moyon S, Tepavcevic V, Moutkine I, Gras J, Matho KS, Schmitt A, Soellner H, Huber AB, Ravassard P, Lubetzki C (2011) Class 3 semaphorins influence oligodendrocyte precursor recruitment and remyelination in adult central nervous system. Brain 134(pt 4):1156–1167. doi:awr022 [pii] 10.1093/brain/awr022

Pitteloud N, Zhang C, Pignatelli D, Li JD, Raivio T, Cole LW, Plummer L, Jacobson-Dickman EE, Mellon PL, Zhou QY, Crowley WF Jr (2007) Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A 104(44):17447–17452. doi:10.1073/pnas.0707173104

Polleux F, Giger RJ, Ginty DD, Kolodkin AL, Ghosh A (1998) Patterning of cortical efferent projections by semaphorin-neuropilin interactions. Science 282(5395):1904–1906

Polleux F, Morrow T, Ghosh A (2000) Semaphorin 3A is a chemoattractant for cortical apical dendrites. Nature 404(6778):567–573. doi:10.1038/35007001

Pringle NP, Richardson WD (1993) A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. Development 117(2):525–533

Rakic P (2009) Evolution of the neocortex: a perspective from developmental biology. Nat Rev Neurosci 10(10):724–735. doi:10.1038/nrn2719

Rakic P, Ayoub AE, Breunig JJ, Dominguez MH (2009) Decision by division: making cortical maps. Trends Neurosci 32(5):291–301. doi:10.1016/j.tins.2009.01.007

Ramon Y, Cajal S (1911) Histologie système nerveux de l'homme et des vertébrés, vol 2. Maloine, Paris

Renaud J, Kerjan G, Sumita I, Zagar Y, Georget V, Kim D, Fouquet C, Suda K, Sanbo M, Suto F, Ackerman SL, Mitchell KJ, Fujisawa H, Chedotal A (2008) Plexin-A2 and its ligand, Sema6A, control nucleus-centrosome coupling in migrating granule cells. Nat Neurosci 11(4):440–449

Ricard D, Rogemond V, Charrier E, Aguera M, Bagnard D, Belin MF, Thomasset N, Honnorat J (2001) Isolation and expression pattern of human Unc-33-like phosphoprotein 6/collapsin response mediator protein 5 (Ulip6/CRMP5): coexistence with Ulip2/CRMP2 in Sema3a-sensitive oligodendrocytes. J Neurosci 21(18):7203–7214

Roney KE, O'Connor BP, Wen H, Holl EK, Guthrie EH, Davis BK, Jones SW, Jha S, Sharek L, Garcia-Mata R, Bear JE, Ting JP (2011) Plexin-B2 negatively regulates macrophage motility, Rac, and Cdc42 activation. PLoS ONE 6(9):e24795. doi:10.1371/journal.pone.0024795

Saha B, Ypsilanti AR, Boutin C, Cremer H, Chedotal A (2012) Plexin-B2 regulates the proliferation and migration of neuroblasts in the postnatal and adult subventricular zone. J Neurosci 32(47):16892–16905. doi:10.1523/JNEUROSCI.0344-12.2012

Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai HH, Wong M, Gupta N, Berger MS, Huang E, Garcia-Verdugo JM, Rowitch DH, Alvarez-Buylla A (2011) Corridors of migrating neurons in the human brain and their decline during infancy. Nature 478(7369):382–386. doi:10.1038/nature10487

Sasaki Y, Cheng C, Uchida Y, Nakajima O, Ohshima T, Yagi T, Taniguchi M, Nakayama T, Kishida R, Kudo Y, Ohno S, Nakamura F, Goshima Y (2002) Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex. Neuron 35(5):907–920

Sato Y, Hirata T, Ogawa M, Fujisawa H (1998) Requirement for early-generated neurons recognized by monoclonal antibody lot1 in the formation of lateral olfactory tract. J Neurosci 18(19):7800–7810

Schwanzel-Fukuda M, Pfaff DW (1989) Origin of luteinizing hormone-releasing hormone neurons. Nature 338(6211):161–164

Schwarting GA, Kostek C, Ahmad N, Dibble C, Pays L, Puschel AW (2000) Semaphorin 3A is required for guidance of olfactory axons in mice. J Neurosci 20(20):7691–7697

Schwarz Q, Gu C, Fujisawa H, Sabelko K, Gertsenstein M, Nagy A, Taniguchi M, Kolodkin AL, Ginty DD, Shima DT, Ruhrberg C (2004) Vascular endothelial growth factor controls neuronal migration and cooperates with Sema3A to pattern distinct compartments of the facial nerve. Genes Dev 18(22):2822–2834

Scott GA, McClelland LA, Fricke AF, Fender A (2009) Plexin C1, a receptor for semaphorin 7a, inactivates cofilin and is a potential tumor suppressor for melanoma progression. J Invest Dermatol 129(4):954–963. doi:10.1038/jid.2008.329

Shelly M, Cancedda L, Lim BK, Popescu AT, Cheng PL, Gao H, Poo MM (2011) Semaphorin3A regulates neuronal polarization by suppressing axon formation and promoting dendrite growth. Neuron 71(3):433–446. doi:10.1016/j.neuron.2011.06.041

Sherman DL, Brophy PJ (2005) Mechanisms of axon ensheathment and myelin growth. Nat Rev Neurosci 6(9):683–690

Snapyan M, Lemasson M, Brill MS, Blais M, Massouh M, Ninkovic J, Gravel C, Berthod F, Gotz M, Barker PA, Parent A, Saghatelyan A (2009) Vasculature guides migrating neuronal precursors in the adult mammalian forebrain via brain-derived neurotrophic factor signaling. J Neurosci 29(13):4172–4188. doi:10.1523/JNEUROSCI.4956-08.2009

Spassky N, de Castro F, Le Bras B, Heydon K, Queraud-LeSaux F, Bloch-Gallego E, Chedotal A, Zalc B, Thomas JL (2002) Directional guidance of oligodendroglial migration by class 3 semaphorins and netrin-1. J Neurosci 22(14):5992–6004

Studer M, Lumsden A, Ariza-McNaughton L, Bradley A, Krumlauf R (1996) Altered segmental identity and abnormal migration of motor neurons in mice lacking Hoxb-1. Nature 384(6610):630–634

Sugimoto Y, Taniguchi M, Yagi T, Akagi Y, Nojyo Y, Tamamaki N (2001) Guidance of glial precursor cell migration by secreted cues in the developing optic nerve. Development 128(17):3321–3330

Syed YA, Hand E, Mobius W, Zhao C, Hofer M, Nave KA, Kotter MR (2011) Inhibition of CNS remyelination by the presence of semaphorin 3A. J Neurosci 31(10):3719–3728. doi:31/10/3719 [pii] 10.1523/JNEUROSCI.4930-10.2011

Tahirovic S, Hellal F, Neukirchen D, Hindges R, Garvalov BK, Flynn KC, Stradal TE, Chrostek-Grashoff A, Brakebusch C, Bradke F (2010) Rac1 regulates neuronal polarization through the WAVE complex. J Neurosci 30(20):6930–6943. doi:10.1523/JNEUROSCI.5395-09.2010

Tamamaki N, Fujimori KE, Takauji R (1997) Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. J Neurosci 17(21):8313–8323

Tamamaki N, Fujimori K, Nojyo Y, Kaneko T, Takauji R (2003) Evidence that Sema3A and Sema3F regulate the migration of GABAergic neurons in the developing neocortex. J Comp Neurol 455(2):238–248. doi:10.1002/cne.10476

Taniguchi M, Yuasa S, Fujisawa H, Naruse I, Saga S, Mishina M, Yagi T (1997) Disruption of semaphorin III/D gene causes severe abnormality in peripheral nerve projection. Neuron 19(3):519–530

Terriente J, Gerety SS, Watanabe-Asaka T, Gonzalez-Quevedo R, Wilkinson DG (2012) Signalling from hindbrain boundaries regulates neuronal clustering that patterns neurogenesis. Development 139(16):2978–2987. doi:10.1242/dev.080135

Tomioka N, Osumi N, Sato Y, Inoue T, Nakamura S, Fujisawa H, Hirata T (2000) Neocortical origin and tangential migration of guidepost neurons in the lateral olfactory tract. J Neurosci 20(15):5802–5812

Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, Hall JE, Quinton R, Seminara SB, Hughes V, Van Vliet G, Van Uum S, Crowley WF, Habuchi H, Kimata K, Pitteloud N, Bulow HE (2011) Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism. Proc Natl Acad Sci U S A 108(28):11524–11529. doi:10.1073/pnas.1102284108

Vallstedt A, Klos JM, Ericson J (2005) Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain. Neuron 45(1):55–67. doi:10.1016/j.neuron.2004.12.026

Vermeren M, Maro GS, Bron R, McGonnell IM, Charnay P, Topilko P, Cohen J (2003) Integrity of developing spinal motor columns is regulated by neural crest derivatives at motor exit points. Neuron 37(3):403–415

Wennerberg K, Forget MA, Ellerbroek SM, Arthur WT, Burridge K, Settleman J, Der CJ, Hansen SH (2003) Rnd proteins function as RhoA antagonists by activating p190 RhoGAP. Curr Biol 13(13):1106–1115

Williams A, Piaton G, Aigrot MS, Belhadi A, Theaudin M, Petermann F, Thomas JL, Zalc B, Lubetzki C (2007) Semaphorin 3A and 3F: key players in myelin repair in multiple sclerosis? Brain 130(pt 10):2554–2565. doi:awm202 [pii] 10.1093/brain/awm202

Worzfeld T, Rauch P, Karram K, Trotter J, Kuner R, Offermanns S (2009) Mice lacking Plexin-B3 display normal CNS morphology and behaviour. Mol Cell Neurosci 42(4):372–381. doi:10.1016/j.mcn.2009.08.008

Xiang X, Zhang X, Huang QL (2012) Plexin A3 is involved in semaphorin 3F-mediated oligodendrocyte precursor cell migration. Neurosci Lett 530(2):127–132. doi:10.1016/j.neulet.2012.09.058

Young J, Metay C, Bouligand J, Tou B, Francou B, Maione L, Tosca L, Sarfati J, Brioude F, Esteva B, Briand-Suleau A, Brisset S, Goossens M, Tachdjian G, Guiochon-Mantel A (2012) SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. Hum Reprod 27(5):1460–1465. doi:10.1093/humrep/des022

Zalc B, Goujet D, Colman D (2008) The origin of the myelination program in vertebrates. Curr Biol 18(12):R511–R512

Zhou J, Wen Y, She L, Sui YN, Liu L, Richards LJ, Poo MM (2013) Axon position within the corpus callosum determines contralateral cortical projection. Proc Natl Acad Sci U S A 110(29):E2714–E2723. doi:10.1073/pnas.1310233110

Zimmer G, Schanuel SM, Burger S, Weth F, Steinecke A, Bolz J, Lent R (2010) Chondroitin sulfate acts in concert with semaphorin 3A to guide tangential migration of cortical interneurons in the ventral telencephalon. Cereb Cortex 20(10):2411–2422. doi:10.1093/cercor/bhp309