Ion channels, guidance molecules, intracellular signaling and transcription factors regulating nervous and vascular system development

Tenpei Akita¹ · Tatsuro Kumada² · Sei-ichi Yoshihara³ · Joaquim Egea⁴ · Satoru Yamagishi⁵

Received: 30 September 2015 / Accepted: 30 September 2015 / Published online: 27 October 2015 © The Physiological Society of Japan and Springer Japan 2015

Abstract Our sophisticated thoughts and behaviors are based on the miraculous development of our complex nervous network system, in which many different types of proteins and signaling cascades are regulated in a temporally and spatially ordered manner. Here we review our recent attempts to grasp the principles of nervous system development in terms of general cellular phenomena and molecules, such as volume-regulated anion channels, intracellular Ca²⁺ and cyclic nucleotide signaling, the Npas4 transcription factor and the FLRT family of axon guidance molecules. We also present an example illustrating that the same FLRT family may regulate the development of vascular networks as well. The aim of this review is to open up new vistas for understanding the intricacy of nervous and vascular system development.

Keywords Nervous system development · Anion channel · Intracellular signaling · Transcription factor · Axon guidance · Vascular system development

Introduction

The nervous system has a complex but highly organized structure, providing the basis for our complex and sophisticated thoughts and behaviors. The development of the nervous network system is thus nothing short of a miracle, with many different kinds of proteins and signaling cascades being up- and downregulated in a temporally and spatially ordered manner during this process. One effective approach to gaining an understanding of the principles of nervous system development is to explore commonalities and variations in general cellular phenomena and molecules. In this review, we focus on the functions of ion channels, intracellular calcium ion (Ca²⁺) and cyclic nucleotide signaling, transcription factors and axon guidance molecules to understand the rules of nervous system development. Interestingly, we also describe how some of these rules are applicable to the development of vascular networks as well. Our aim is to open up new vistas for understanding the intricacy of nervous and vascular system development.
Roles of volume-regulated anion channels during neuronal migration in the developing brain

During proliferation, migration, neurite extension and retraction, among other processes, the cell volume of developing neurons changes dynamically. Cell volume regulation is an essential function in all types of cell and is achieved through regulation of the net influx or efflux of solutes, which is accompanied by passive water movement, across the plasma membrane. The volume-sensitive outwardly rectifying (VSOR) anion channel is a major regulator of anion flux during cell volume regulation and is present on all types of vertebrate cells as the most dominant form of volume-regulated anion channels [1–5]. The VSOR channel is activated in response to cell swelling induced by hypotonic or excitotoxic stimuli or, alternatively, to receptor activation without cell swelling. Opening of the channel provides anions, mainly chloride ions (Cl\(^{-}\)), with a pathway through the cell membrane, which in turn may induce either the net influx or net efflux of solutes across the membrane with the help of the electrochemical driving forces of other cations, such as sodium (Na\(^{+}\)) ions for influx or potassium ions (K\(^{+}\)) for efflux; this process results in an increase or a decrease in cell volume, respectively. The VSOR channel is also permeable to excitatory amino acids, such as glutamate and aspartate, and as such may also play a role in signaling between adjacent cells, especially in the central nervous system (CNS) [5, 6]. Activation of the VSOR channel through triggering by receptor activation is regulated in the immediate vicinity of the activated receptor molecules. In earlier studies carried out by our group (T. Akita) in astrocytes, this activation was found to be mediated by Ca\(^{2+}\)-dependent kinases that are activated in regions containing a very high concentration of intracellular Ca\(^{2+}\) within nanometers of the Ca\(^{2+}\)-permeable ion channels—the so-called Ca\(^{2+}\) nanodomains [7, 8]. This mechanism provides a basis for selective activation of a limited number of VSOR channels located on a part of the cell surface where a small number of receptors are locally activated, and it is this local activation which may drive the changes in local cell volume required for morphological changes of the cell [5].

Biophysical and pharmacological characteristics of the VSOR anion channel differ somewhat between cell and tissue types [3, 5]. These characteristics and the roles of the channel have been well explored in the mature CNS, whereas they are still poorly understood in the immature nervous system [5]. Critical roles of the VSOR anion channel in cell proliferation, migration, differentiation and programmed cell death have been elucidated during development in other biological systems [9–11], strongly indicating that it also plays critical roles during nervous system development, as recently found during cerebral cortex development. There are two major streams of neuronal migration in the developing cortex: tangential migration of gamma-aminobutyric acid (GABA)ergic interneurons originating from the ganglionic eminences in the ventral forebrain and radial migration of glutamatergic pyramidal and spiny stellate neurons from the adjacent ventricular zone lining the dorsal telencephalic ventricles in the fetal brain (Fig. 1a). It has long been suggested that GABA released from migrating interneurons regulates the speed of radial migration of glutamatergic neurons [12–15]. We recently confirmed that blockade of GABA\(_{A}\) receptors with SR95531 accelerates radial migration [16], leading to the conclusion that activation of GABA\(_{A}\) receptors slows the migration, presumably for proper development of the cerebral cortex. Interestingly, we further found that the major endogenous agonist of GABA\(_{A}\) receptors during the radial migration is taurine released through the VSOR channels from neurons—and not GABA [16]. Taurine is known to act as an agonist of the receptors for glycine and GABA [17–19]. In the mature brain, the cytoplasm of both neurons and glia contain taurine at concentrations of 10–20 mM [20], whereas in the embryonic brain the taurine content is five- to tenfold higher [21, 22]. We also observed that taurine is enriched in the marginal zone (MZ) and in the subplate (SP) of the fetal neocortex [16] (Fig. 1b), suggesting that both the Cajal-Retzius neuron in the MZ and the SP neuron secrete taurine. Permeability of the VSOR channel to taurine has been demonstrated in mature neurons and glia [23–26] and, more recently, also in neural stem/progenitor cells obtained from both embryonic and adult brains [21]. However, the mechanism of just how VSOR channels are activated to release taurine during the radial migration is not yet resolved. Activation of GABA\(_{A}\) receptor Cl\(^{-}\) channels depolarizes the membrane of migrating embryonic neurons due to their reversal voltage for Cl\(^{-}\) being higher than their resting membrane voltage [27], and this induces the opening of voltage-gated Ca\(^{2+}\) channels and intracellular oscillatory Ca\(^{2+}\) signaling [12, 15, 27]. If the VSOR channels located at the leading edges of migrating neurons are activated through Ca\(^{2+}\) nanodomains of the oscillatory signaling process, the resulting Cl\(^{-}\) efflux with the help of K\(^{+}\) efflux would promote shrinkage of the edges. Therefore, taurine-mediated GABA\(_{A}\) receptor activation might enhance VSOR...
control activation at the edges of migrating neurons when the edges reach the taurine-rich regions, and this might put a brake on the migration ("VSOR channel hypothesis"; Fig. 1b).

As a regulator of cell volume and intercellular signaling, the VSOR anion channel is likely to play important roles not only in radial migration, but also in tangential migration, proliferation and differentiation of the networks of neurons and glia in the developing brain. It has been demonstrated that normal brain assembly in embryos, including cortical layer formation, axonal fiber projection and morphological synapse formation, depends on neurotransmitters that must be released through as yet unidentified mechanisms that differ from vesicular exocytosis [28, 29]. Therefore, exploring in detail the modes of activation of the VSOR channel and how these relate to the developing processes would shed light on the mystery of fetal brain development in the near future.

**Control of neuronal migration by extrinsic and intrinsic programs through modulating Ca\(^{2+}\) and cyclic nucleotide signaling**

Newborn neurons often exhibit spontaneous elevations of intracellular Ca\(^{2+}\) level. In this section, T. Kumada discusses results showing that both extrinsically and intrinsically determined patterns of intracellular Ca\(^{2+}\) fluctuations and their interactions with cyclic nucleotide signaling control the speed, mode and direction of neuronal migration. Komuro and Rakic originally demonstrated the relationship between Ca\(^{2+}\) fluctuations and neuronal migration using time-lapse Ca\(^{2+}\) imaging of granule cells migrating from a microexplant of the postnatal cerebellum in culture [30]. These authors noted that the amplitude and frequency of Ca\(^{2+}\) transients in isolated granule cells are positively correlated with the rate of cell movement. In the cerebellar tissue, granule cells change their mode of migration during their trajectory; after they tangentially migrate at the fastest rate in the external granular layer, they change the direction from tangential to radial toward the internal granular layer, where they terminate their migration [31] (Fig. 2a). Using cerebellar slice culture preparations, Kumada and Komuro confirmed that granule cells exhibited distinct patterns of Ca\(^{2+}\) transients and rates of cell movement in different cerebellar cortical layers, that the frequency of Ca\(^{2+}\) transients was indeed positively correlated with the migration speed during the whole trajectory and finally that the Ca\(^{2+}\) transients ceased before termination of their
migration [32, 33] (Fig. 2a). Increasing and decreasing the frequency of Ca\(^{2+}\) transients by experimentally altering the Ca\(^{2+}\) influx increased and decreased the migration speed, respectively, and experimentally eliminating the Ca\(^{2+}\) transients induced a premature termination of migration [32]. Thus, the results confirmed that the layer-specific pattern of Ca\(^{2+}\) transients controls the speed of granule cell migration in each cortical layer. Similar regulation has also been reported in tangentially migrating GABAergic interneurons in the developing neocortex [34]. However, in contrast to these observations, an increase in Ca\(^{2+}\) oscillation frequency has been shown to induce termination of the radial migration of glutamatergic neurons in the developing neocortex [15, 35, 36]. Ca\(^{2+}\) signaling dynamically interacts with the signaling cascades mediated by other second messengers, such as cyclic adenosine monophosphate (cAMP) [37]. By pharmacologically manipulating cAMP and cyclic guanosine monophosphate (cGMP) levels, our group revealed that cAMP signaling acts as a brake on granule cell movement, while cGMP signaling acts as an accelerator [38]. Activation of cAMP signaling decreases the frequency of Ca\(^{2+}\) transients, and this is one of the causes of the decrease in granule cell motility, while activation of cGMP signaling does not affect this frequency [39].

Depolarizing neuronal activity mediated by neurotransmitters increases the frequency of Ca\(^{2+}\) transients through activation of voltage-gated Ca\(^{2+}\) channels (VGCC), and this may regulate neuronal development prior to synaptogenesis [40, 41]. Komuro and Rakic were the first to show that the N-type VGCC and the NMDA (N-methyl-D-aspartate) subtype of glutamate receptor facilitate granule cell migration in the cerebellar tissue [42, 43]. In immature neurons, GABA also acts as an excitatory neurotransmitter and increases the Ca\(^{2+}\) transient frequency through VGCC activation [15, 27, 35]. The excitatory GABA action is based on raised intracellular Cl\(^{-}\) concentrations due to Cl\(^{-}\) uptake through the Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransporter 1 (NKCC1) in immature neurons [44]. Developmental or experimental expression of Cl\(^{-}\)-extruding K\(^{+}\)-Cl\(^{-}\) cotransporter 2 (KCC2) converts the excitatory action of GABA into an inhibitory action, which reduces the Ca\(^{2+}\) fluctuation frequency and may vary the mode of neuronal migration [34, 45, 46]. This developmental switch in the action of GABA is controlled by cell-intrinsic programs during neuronal differentiation [47]. In addition to VGCC, the release of Ca\(^{2+}\) from internal Ca\(^{2+}\) stores is also involved in the regulation of neuronal migration [48]. Although Ca\(^{2+}\) signaling is known to play a pivotal role in organizing the cytoskeleton, precise detailed knowledge of just how Ca\(^{2+}\) signaling determines the dynamic behavior of developing neurons and the mode of neuronal migration is still lacking.

**Fig. 2** Control of cerebellar granule cell migration by Ca\(^{2+}\) transients and cyclic nucleotide signaling in a layer-specific manner. a Schematic diagram showing the relationship between the sequence of cerebellar granule cell migration and the pattern of Ca\(^{2+}\) transients. 1–5 enclosed within black circles Stages of granule cell differentiation as it proceeds in ascending order along the migratory pathway: 1 the cell tangentially migrates at the fastest rate in the external granular layer (EGL), 2 the cell changes the direction of migration from tangential to radial, i.e. turns, in two different modes at the boundary between EGL and the molecular layer (ML), 3 the cell radially migrates along the Bergmann glial process, 4 the cell radially migrates within the internal granular layer (IGL), 5 the cell completes migration at the bottom of the IGL. Green arrow at each stage indicates the direction of migration, and its length reflects the migration speed. Red traces Typical patterns of Ca\(^{2+}\) transients at corresponding stage. The speed positively correlates with the frequency of Ca\(^{2+}\) transients. The mode of turning at stage 2 is determined as shown in b. At stage 5, the migration terminates approximately 10 min after loss of Ca\(^{2+}\) transients. Dotted line in the Ca\(^{2+}\) trace Time of termination. b Different modes of granule cell turning controlled by Ca\(^{2+}\) and cyclic nucleotide signaling at stage 2. Stimulating Ca\(^{2+}\) release from internal Ca\(^{2+}\) stores or cyclic guanosine monophosphate (cGMP) signaling increases the occurrence of L-shape turning, which is characterized by turning of the tip of the leading process to a new direction. Movement of the soma follows the direction of the tip turning. In contrast, increasing Ca\(^{2+}\) influx via the voltage-gated Ca\(^{2+}\) channel (VGCC) or glutamate receptors (N-methyl-D-aspartate receptor, NMDAR) or stimulating cyclic adenosine monophosphate (cAMP) signaling increases the occurrence of T- or Y-shape turning, characterized by bifurcation of the leading process [55]. The relationship between the turning modes and the Ca\(^{2+}\) or cyclic nucleotide signaling is essentially the same as that in isolated granule cells [55] (color figure online)
External cues provided by axon guidance molecules are also known to play important roles in neuronal migration. Some guidance molecules, such as Slit, may induce Ca\textsuperscript{2+} elevation in the leading processes of migrating neurons in a manner similar to that observed in axonal growth cones [48–50]. Slit-2, a chemorepellent to cerebellar granule cell migration, induces a propagating Ca\textsuperscript{2+} wave from the leading process to the soma, which is mediated by Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release from the internal stores [49]. Sonic hedgehog, another guidance molecule, also acts as an important trigger of Ca\textsuperscript{2+} signaling-mediated neural activity, inducing neuronal differentiation [51]. These processes suggest the importance of links between individual external cues and distinct modes of Ca\textsuperscript{2+} signaling. At specific turning points, the migrating neurons may change their responsiveness to an external cue, as seen in axonal guidance [52]. Interestingly, the intracellular balance between Ca\textsuperscript{2+} levels and different cyclic nucleotide signaling activities may determine the responsiveness of the migrating neuron to a guidance cue. For example, in embryonic spinal neurons of Xenopus laevis, a higher ratio of cAMP/cGMP in response to netrin-1 binding to DCC (deleted in colorectal cancer) increases Ca\textsuperscript{2+} currents through VGCCs and intracellular Ca\textsuperscript{2+} release, resulting in growth cones being attracted to the guidance cue, whereas a lower ratio in response to netrin-1 binding to the DCC–Unc5 complex decreases the Ca\textsuperscript{2+} currents and Ca\textsuperscript{2+} release, resulting in repulsion from the cue [53].

In addition to external cues, which are essential for the selection of migratory paths, cell-intrinsic programs also control neuronal migration. The speed and mode of migration of isolated granule cells dynamically change even without cell–cell contact and in the absence of external guidance cues [54, 55]. The migrating isolated cells exhibit autonomous changes in the direction of migration (i.e. turning) and in the shape of their leading and trailing edges. The changes can be subdivided into several distinct modes of turning, and the cellular morphology in each mode is similar to that observed in the cerebellar tissue [55] (Fig. 2b). Thus, the changes must be regulated by cell-intrinsic (internal clock) programs, and we have found that Ca\textsuperscript{2+} and cyclic nucleotide signaling cascades are also involved in these programs [39, 55]. For example, in isolated granule cells, the frequency of Ca\textsuperscript{2+} transients, which positively correlates with their migration rate, depends on the elapsed time after plating, and termination of their migration is always preceded by a loss of Ca\textsuperscript{2+} transients, as occurs after 50–60 h of migration in the cerebellar tissue [32] (Fig. 2a). Importantly, experimental manipulations of Ca\textsuperscript{2+} and cyclic nucleotide signaling pathways in isolated cells vary the speed of migration, the frequency of turning and the preference for a turning mode in the same way as in the tissue [55] (Fig. 2b). Thus, the Ca\textsuperscript{2+} and cyclic nucleotide signaling cascades driven by intrinsic programs regulate the autonomous turning of migrating granule cells in isolated conditions. The mechanism by which the intrinsic programs drive the signaling cascades, however, remains to be elucidated. The autonomous changes in Ca\textsuperscript{2+} and cyclic nucleotide signaling by intrinsic programs suggest that the programs may also affect the role of external guidance molecules in directing neuronal migration through regulating the basal activity of these signaling pathways. The mechanism of how intrinsic programs interact with external cues through Ca\textsuperscript{2+} and cyclic nucleotide signaling is an important issue which needs to be addressed.

Taken together, Ca\textsuperscript{2+} and cyclic nucleotide signaling not only mediate the responses to external guidance cues, but they also modulate the responsiveness to guidance cues and control neuronal migration even in the absence of external cues based on cell-intrinsic programs. The intrinsic programs may change the role of external guidance molecules by altering Ca\textsuperscript{2+} and cyclic nucleotide signaling pathways. Thus, both the speed and the fate of neuronal development must be determined by orchestrated interactions between intrinsic programs and external cues through Ca\textsuperscript{2+} and cyclic nucleotide signaling.

**Transcription factor neuronal Per/Arnt/Sim domain protein 4 (Npas4) regulates sensory experience-dependent development of dendritic spines in newborn olfactory bulb interneurons**

Sensory experience regulates neural development in a variety of species and in various brain structures, including the cortex, hippocampus and olfactory bulb (OB) [56–59]. OB interneurons are a good model for studying the modification of neural circuits by sensory inputs from the external world during the postnatal stages. OB interneurons are generated throughout life in the subventricular zone, migrate along the rostral migratory stream and differentiate into GABA-releasing inhibitory interneurons, such as granule cells (GCs) and periglomerular cells (PGCs), in the OB [60–64] (Fig. 3a). Olfactory sensory deprivation or, alternatively, an odor-rich environment can promote suppression or acceleration, respectively, of dendritic morphogenesis and spine formation in newborn OB interneurons [65–71]. However, little is known about the molecular players or the precise mechanisms that integrate odor-induced activity with the developmental processes of OB interneurons.

Using DNA microarray technology and in situ hybridization screenings, our group (S. Yoshihara) newly identified a transcription factor coding gene, neuronal Per/Arnt/Sim domain protein 4 (Npas4), which is expressed in...
a subset of OB GCs following sensory experience [71]. Npas4 is an immediate early gene activated by neuronal activity via Ca$^{2+}$-dependent signaling pathways [72]. The transcription factor Npas4 promotes the formation of inhibitory synapses in the developing visual system [72] and controls the homeostatic inhibitory/excitatory balance that regulates visual cortical plasticity [73]. Moreover, Npas4 interacts with several promoters that are regulated by neuronal activity and mediates BDNF (brain-derived neurotrophic factor) expression in cortical pyramidal and hippocampal CA3 neurons [72, 74–76]. We performed gain- and loss-of-function experiments for the Npas4 gene in OB GCs, based on lentiviral gene transfer. Npas4 overexpression in newborn OB GCs increased the spine density even under sensory deprivation [71]. Furthermore, Npas4 overexpression increased the number of puncta stained by either post-synaptic [post-synaptic density protein 95 (PSD-95) or gephyrin] or pre-synaptic (synaptoporin) markers in the distal region of GC dendrites [71]. Conversely, both Npas4 knockdown and knockout significantly reduced the spine density of OB GCs [71]. These results strongly suggest that Npas4 is both necessary and sufficient on its own for increasing sensory input-dependent synaptogenesis in OB GCs.

To identify the target genes through which Npas4 regulates synaptogenesis in OB interneurons, we performed ChIP-Seq analysis in homogenized OB tissues using the antibody against Npas4 and searched for the gene promoter regions that Npas4 can bind to. As a result, we identified, as a novel target of Npas4, an oncogenic E3 ubiquitin ligase Mdm2 gene that is expressed at low levels in the wild-type OB but at higher levels in the Npas4-knockout OB [71]. Thus, we expected that Npas4 would suppress Mdm2 gene expression. The results of gain-of-function and knockdown experiments for Mdm2 using the lentiviral vector system showed that overexpression and knockdown of Mdm2 reduced and enhanced, respectively, the spine density of GC dendrites compared with controls [71], thereby confirming Mdm2 as a bona fide target gene of Npas4 whose expression is indeed suppressed by Npas4. To identify the protein targets through which Mdm2 regulates synaptogenesis in the OB interneurons, we next searched for those proteins expressed differentially between wild-type and two Npas4-knockout OB by iTRAQ quantitative proteomics analysis. Proteomics and cell-line analysis revealed that Mdm2 ubiquitinates and promotes degradation of a microtubule-associated protein, doublecortin (Dcx) [71]. Immunohistochemistry studies revealed that the expression of Dcx protein in GC dendrites within both the external proximal layer and the GC layer was twofold lower in the Npas4-knockout OB than in the wild-type OB [71]. Furthermore, overexpression and knockdown of Dcx using the lentiviral vector system enhanced and reduced, respectively, the spine density of GC dendrites compared with controls [71]. These results reveal that Dcx plays an important role in increasing the dendritic spine density of the OB GCs. Taken together, our findings suggest that Npas4 inhibits Mdm2 expression, which in turn prevents ubiquitination and degradation of Dcx, thereby promoting the development of dendritic spines of OB GCs after sensory experience (Fig. 3b).

To reveal the physiological function of spine formation in OB GCs, we examined the odor discrimination learning...
and memory in OB-specific Npas4-knockout mice generated by the Cre and loxP system. Loss of Npas4 in OB GCs was found to impair the learning of discrimination between two related odors without affecting the olfactory detection ability [71]. This result suggests that spine formation in interneuronal dendrites in the OB neural circuitry is required for odor-associated learning and memory. Hence, through the regulation of activity-dependent synapse development in OB GCs, Npas4 can play an important role in the formation of functional olfactory learning neuronal circuits.

**FLRT3 is a Robo1-interacting protein that determines Netrin-1 attraction in developing axons**

Axon guidance is the process by which axons navigate to encounter their appropriate synaptic partner within the developing nervous system. Together with neuron migration, axon guidance is one of the most important hallmarks of nervous system development, and much study on this process has rendered many important clues of how it is wired and functions. Results obtained from different animal models have revealed many of the basic principles and the molecular mechanisms involved in the process of axon guidance [77–79]. For example, the tip of the axon has a protrusion, called the “growth-cone,” which is a very motile structure actively exploring the environment for cues that “guide” the axons to reach the proper target. Many of these axon guidance cues have been characterized, and they include five large families, namely, the Netrin, Slit, Ephrin, Semaphorin and classical morphogen families. These cues are sensed by the growth cone through specific receptors located at the surface of the cone, and the receptors activate intracellular signaling cascades which ultimately modify cytoskeleton dynamics. This leads to an attractive or a repulsive response, depending on the cellular context and the type of axon guidance cue and receptor involved. Axon guidance is not continuous but rather a step-wise process in which axons encounter intermediate targets (e.g. the nervous system midline), where they have to make guidance decisions. One intermediate target affects the response to the next one by changing, for example, the composition of receptors at the surface of the growth cone [77, 80]. Moreover, the guidance of a particular set of axons is tightly controlled in time and space and very often is exquisitely coordinated with the migration of other axons or neurons [81]. Interestingly, the molecular mechanisms of axon guidance are not restricted to the nervous system, and they have also been found to be involved in the development of other systems, such as the vascular system (see following section).

Strikingly, the number of cues and receptors involved in axon guidance is relatively low compared to the enormous complexity of the nervous system. Mechanisms of proteolytic cleavage of these proteins, posttranslational modifications, alternative splicing or the presence of co-receptors have been described to fine tune and expand the responses of a particular axon to the environment [80, 82, 83]. More recently, it has been reported in many different contexts that the combination of several axon guidance cues triggers a response that cannot be predicted by the response to any of the cues individually, indicating that axon guidance cues are able to crosstalk. The mechanisms underlying such crosstalk are not known, but this is currently a field of very active research in molecular neurobiology since this is a scenario that resembles more accurately the in vivo situation where axons are challenged with a combination of cues [84]. Also, these studies most probably will provide new insights which will further our understanding of the process of axon guidance and nervous system wiring and function. In this section, recent research carried out by our group (J. Egea; see [85] for further information) will be discussed. This work has elucidated the crosstalk mechanism of two of the most well-characterized axon guidance cues, netrin and slit, in the development of the thalamocortical axons (TCAs). TCAs convey sensory (with the exception of olfaction) and motor information to the neocortex. They are arranged topographically, with rostral thalamic neurons projecting their axons into the motor cortex and intermediate and caudal thalamic neurons projecting their axons into the somatosensory and visual cortex, respectively. This arrangement is controlled by gradients of axon guidance cues in a ventral structure called the corridor which sorts the axons according to their final destination [86] (Fig. 4). Rostral TCAs (rTCAs) cross an area in the corridor with the higher expression levels of Netrin, which can be either repulsive if Netrin interacts with Unc5 receptors or attractive if it interacts with DCC receptors, and of Slit, which interacts with Robo receptors, typically triggering repulsion. In order to understand how rTCAs integrate these two signals, we challenged in vitro explant cultures of rostral thalamus with Netrin and Slit, either individually or in combination (Netrin + Slit). Surprisingly, Netrin did not induce any effect, whereas Slit induced repulsion on these axons. Interestingly, Netrin + Slit triggered a net attractive response on rTCAs but not on intermediate axons (iTCAs), a result that may explain the segregation of the two types of projection in vivo [87]. In order to understand the mechanisms involved in the specific attraction of rTCAs towards Netrin + Slit, we performed several in vivo and in vitro experiments. We demonstrated that the axon guidance receptor Robo1, but not Robo2, was necessary for the attractive effect, suggesting that Slit was activating its
canonical receptor in this context. We also observed that Netrin + Slit induced a strong increase of the DCC levels on the surface of the growth cone on rTCAs but not on Robo1 knockout rTCAs, suggesting that Robo1 signaling is required. This effect was specific since it was neither observed after the stimulation with Slit1 and Netrin in the corridor and FLRT3 in the thalamus. rTCAs require high levels of FLRT3 that in combination with Robo1 activates protein kinase A (PKA) signaling and triggers an increase in DCC (deleted in colorectal cancer) levels at the surface of the growth cone to permit the attraction of these axons by Netrin towards the rostral neocortex. iTCAs Intermediate axons. Adapted from Leyva-Díaz et al. [85] (color figure online)

In summary, these results demonstrate the key role of FLRT3 in the control of, specifically, rTCA projections (see Fig. 4). The presence of FLRT3 and the modulation of Robo1 signaling results in Slit being a permissive cue for Netrin to induce attraction. These axons respond to Netrin + Slit in a manner (attraction) that cannot be predicted by the effects of individual cues (Netrin, no effect; Slit, repulsion). This observation reinforces the notion that mechanisms of crosstalk between axon guidance cues are important to expand the repertoire of axonal responses during development of the nervous system. Crosstalk depends on the presence of specific molecular identities as,
for example, FLRT3 in the rTCAs, which are regulated in time and space. It is important to point out that crosstalk is also context dependent since the same molecules can exert different effects in different tissues. For example, Netrin and Slit interact differently in callosal and commissural axons compared to rTCAs. In callosal axons in the forebrain, DCC, while crossing the midline to project into the contralateral cortex, exerts a hierarchical effect over Robo1 by inhibiting Robo1 signaling to allow crossing of the midline, a territory highly enriched with Slit [98]. In contrast, in the commissural axons of the spinal cord that have crossed the floor-plate, a territory highly enriched with Netrin, into the contralateral side, Robo1 exerts a hierarchical effect over DCC by inhibiting Netrin attraction to avoid re-crossing of axons [99].

Roles of the FLRT and Unc5 protein families proteins in the development of the nervous and vascular systems

The vasculature forms an elaborated and massive network, much like the nervous system, through which oxygen and nutrients are supplied to all body tissues. During development and remodeling, arteries and veins are first formed by vascular endothelial cells and then supported by smooth muscle cells and pericytes [100, 101]. Tip cells, a subset of endothelial cells, undergo sprouting angiogenesis, leading to the formation of capillaries. Similar to the growth cone in neurons, these cells sense the extracellular environment (e.g. extracellular matrix, growth factors and guidance molecules) to determine their fate (e.g. growth/extension, retraction or diversion). It is well known that canonical axon guidance molecules, such as Netrin and Unc5, Slit and Robo, Semaphorins and Neuropilin/Plexin, Ephrin and Eph, are also commonly identified during vascular system development [79, 102].

Unc5 family proteins (Unc5A-D) are known as the receptors for Netrin family proteins to induce axon repulsion during nervous system development (Fig. 5a). In the vascular system, the Unc5B receptor is expressed on endothelial cells, including arterial tip cells (Fig. 5b) [103]. However, the endogenous ligand for Unc5B in the vascular system is still unknown (see below), and its identification has been a major focus of ongoing research in the field of vascular development since the discovery of the importance of Unc5B in vascular morphogenesis more than a decade ago [103]. In this section of the review, potential ligands for Unc5B during vascular development based on the phenotypes of various knockout mice are discussed by S. Yamagishi.

Lu et al. reported that Unc5B is specifically expressed on the endothelial cells of arteries and that disruption of Unc5B genes in the mouse and zebrafish induces an aberrant morphology of tip-cell fillopodia, increased vessel branching and abnormal navigation of vascular network development [103]. Larrivée et al. demonstrated that Unc5B is transiently expressed during the sprouting of capillaries from tumor implants, indicating involvement of Unc5B in angiogenesis [104]. In retinal blood vessels, loss-of-function studies using the antibody or gene knockout against Unc5B demonstrated hypervascularization [105]. Furthermore, most of the conventional Unc5B knockout mice die because of heart failure at embryonic day (E) 12.5 [103]. Navankasattusas et al. subsequently reported that even selective knockout of Unc5B in vascular endothelial cells, using Tie2-Cre mice and Unc5B-floxed mice, is lethal at the same stage as the conventional one because of a placental problem: the number of robust vertical vessel stalks in the placental labyrinth layer is decreased in the conditional Unc5B knockout mice [106]. Taken together, these results support a critical role for Unc5B in vascular formation. By contrast, although in vitro experiments have shown that classical Unc5B ligands, namely Netrin-1, -4 and Robo-4, exert an Unc5B-dependent repulsive effect on tip cells, knockout mice of these ligands demonstrate only minor hypervascularization [103, 105, 107–109] and do not suffer from heart failure as do Unc5B knockout mice [103]. These results imply the existence of other endogenous ligands for the Unc5B receptor for proper vascular development.

In the context of nervous system development, in 2011 we identified FLRT2 and FLRT3 as axon guidance molecules that produce a repulsive effect by binding to the Unc5B and 5D receptors (Fig. 5a; see preceding section) [94]. Structural and biochemical bases for the FLRT–Unc5 binding were recently resolved by crystallography and the surface plasmon resonance assay [95]. FLRT2 and FLRT3 are cleaved at their extracellular juxtamembrane region by matrix metalloproteinases, and the ectodomains are shed into the extracellular space (Fig. 5b). Binding of the FLRT2 ectodomain to the Unc5D receptor is a repulsive cue for axon guidance and neuronal migration. At E15.5 in mice, FLRT2 is strongly expressed in the cortical plate of the developing neocortex, while its receptor Unc5D is expressed in multipolar neurons, namely, future upper layer neurons, in the subventricular zone. Knockout of FLRT2 facilitates the radial migration of multipolar neurons whereas overexpression of Unc5D slows the migration [94]. Thus, the repulsive cue produced by the FLRT2–Unc5D interaction would slow radial migration. Intriguingly, Unc5D expression in multipolar neurons is transiently downregulated when the neurons migrate through the FLRT2-rich cortical plate, presumably in order to avoid FLRT2 binding [94]. In contrast, FLRT3 is expressed especially in the intermediate zone of the neocortex and the rostral part of the thalamus in E15 mice [85, 95]. Unc5B-
positive thalamic neurons are strongly repelled by FLRT3 [95]. The FLRT3–Robo1 coupling in rostral thalamocortical neurons plays an important role in the proper topographical projection of their axons into the neocortex (see preceding section).

In the embryonic cardiovascular system, FLRT2 is expressed in the epicardial sheet and the myocardium of the heart from E9.5 to birth, whereas FLRT3 is transiently expressed in these structures between E9.5 and E12.5 [90]. FLRT3 is also expressed in the postnatal retina during development of Unc5B-expressing retinal blood vessels [95]. Mice with a loss-of-function mutation in FLRT2 die at E11.5/12.5 due to blood pooling, superficial hemorrhage and pericardial edema [90]. In FLRT2-deficient mice, the basement membrane between the epicardium and the myocardium is disorganized, and the epicardial layer is detached from the myocardium, indicating loss of epicardial integrity [90]. Electron microscopy analysis showed that the surface of the epicardium in the FLRT2 mutant is ruffled and uneven and has small hollows [90]. This phenotype shares similarity with that of the FLRT3 knockout embryo in more primitive embryonic structures, in which the basement membrane in the anterior visceral endoderm is highly disorganized and the hollows on the boundary between the visceral endoderm and the definitive endoderm are visible at the mid-streak stage at around E7 [88]. When FLRT2 was ectopically overexpressed in the FLRT3-deficient embryoid bodies, the basement membrane disorganization was clearly improved [90]. Thus, these results indicate that FLRT2 and FLRT3 have an identical function.

Although both Unc5B and FLRT2 mutant mice die at similar developmental stages, the phenotypes of these mutant mice differ somewhat. For example, the thin heart muscle wall in the FLRT2 mutant is not observed in Unc5B knockout mice, whereas the placental vascular defect in Unc5B knockout mice is absent in the FLRT2 mutant [90, 106]. We have also observed that overexpression of a dominant negative form of Unc5B, lacking its intracellular domain, in arterial endothelial cells in vitro does not block repulsive responses of the cells induced by FLRT2 (unpublished data). Conditional knockout mice of FLRT3, another candidate for the Unc5B ligand, produced using Sox2–Cre, which removes all of the FLRT3 genes in the entire embryo and in the labyrinth layer of the placenta, showed a hypervascular phenotype in the retina, similar to that seen in Unc5B knockout postnatal escaper mice [95, 107]. However, these FLRT3 conditional knockout mice are more likely to be born beyond the critical period when

---

**Fig. 5**  
(a) Binding partners of FLRT and Unc5 family proteins. FLRT2, -3, Robo4 and Netrin1, -4 are the ligands for Unc5 receptors inducing repulsive responses in cells. *FNIII* Fibronectin type III domain, *LRR* leucine-rich repeat domain.  
(b) Interaction of FLRT2, -3 with Unc5B, -D on blood vessels and neurons. FLRT2, -3 are cleaved by matrix metalloproteinase (at the site indicated by the scissors). Binding of the ligands to Unc5 receptors normally induces repulsive reactions in tip cells and neurons (color figure online).
most Unc5B knockout mice die [95]. Therefore, neither FLRT2 nor FLRT3 seems to be the unique endogenous ligand for Unc5B. Further analysis using knockout mice of both FLRT2 and FLRT3 or other unknown molecules is needed to identify the endogenous ligand for Unc5B.

Conclusion

In this review, we have mainly focused on nervous system development and attempted to elucidate its principles in terms of ion channels, intracellular signaling, transcription factors and axon guidance molecules. We have also discussed a similar regulation of vascular system development mediated by the same axon guidance molecules. For each molecular category mentioned in this review, there are a tremendous number of molecular subtypes. Nevertheless, some common schemes and principles can clearly be seen in the modes of crosstalk and interactions between different proteins and/or signaling cascades and also in the modes of cellular movement and morphological changes during the system development. We hope that the topics discussed in this review also provide some clues to the developing processes of other biological systems, such as the lymphatic, respiratory, digestive systems, among others, which are also complex and highly regulated in amazing ways.

Acknowledgments  We are grateful to the members of Department of Neurophysiology in Hamamatsu University School of Medicine for useful discussions and suggestions on this work and to Gandhervin Kesavamoorthy for his critical reading and constructive comments on an early version of the manuscript. This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (21790216, 23612008 and 26460293 to T.A.; 23111510 23500444 and 26350594 to T.K.; 20163368 and 25430010 to S.Yo.; 23700412, 25122707 and 26670090 to S.Ya.), from the FP7-PEOPLE-2011-CIG (PCIG9-GA-2011-293980 to J.E.). J.E. was a recipient of a short-term fellowship from the Japan Society for the Promotion of Science (S-14197).

Compliance with ethical standards

Conflict of interest  The authors declare that they have no conflict of interest.

References

1. Strange K, Emma F, Jackson PS (1996) Cellular and molecular physiology of volume-sensitive anion channels. Am J Physiol 270:C711–C730
2. Nilius B, Eggermont J, Voets T, Buysse G, Manolopoulos V, Droogmans G (1997) Properties of volume-regulated anion channels in mammalian cells. Prog Biophys Mol Biol 68:69–119. doi:10.1016/S0079-6107(97)00021-7
3. Okada Y (1997) Volume expansion-sensing outward-rectifier Cl⁻ channel: fresh start to the molecular identity and volume sensor. Am J Physiol 273:C755–C789
4. Okada Y, Sato K, Numata T (2009) Pathophysiology and puzzles of the volume-sensitive outwardly rectifying anion channel. J Physiol 587:2141–2149. doi:10.1113/jphysiol.2008.165076
5. Akita T, Okada Y (2014) Characteristics and roles of the volume-sensitive outwardly rectifying (VSOR) anion channel in the central nervous system. Neuroscience 275:211–231. doi:10.1016/j.neuroscience.2014.06.015
6. Liu H-T, Akita T, Shimizu T, Sabirov RZ, Okada Y (2009) Bradykinin-induced astrocyte-neuron signalling: glutamate release is mediated by ROS-activated volume-sensitive outwardly rectifying anion channels. J Physiol 587:2197–2209. doi:10.1113/jphysiol.2008.165084
7. Akita T, Okada Y (2011) Regulation of bradykinin-induced activation of volume-sensitive outwardly rectifying anion channels by Ca²⁺ nanodomains in mouse astrocytes. J Physiol 589:3909–3927. doi:10.1113/jphysiol.2011.208173
8. Akita T, Fedorovich SV, Okada Y (2011) Ca²⁺ nanodomain-mediated component of swelling-induced volume-sensitive outwardly rectifying anion current triggered by autocrine action of ATP in mouse astrocytes. Cell Physiol Biochem 28:1181–1190. doi:10.1159/000335867
9. Lang F, Busch GL, Ritter M, Völki H, Waldegg S, Gulbins E, Häussinger D (1998) Functional significance of cell volume regulatory mechanisms. Physiol Rev 78:247–306
10. Okada Y, Maeno E, Shimizu T, Dezaki K, Wang J, Morishima S (2001) Receptor-mediated control of regulatory volume decrease (RVD) and apoptotic volume decrease (AVD). J Physiol 532:3–16. doi:10.1111/j.1469-7793.2001.0003g.x
11. Hoffmann EK, Lambert IH, Pedersen SF (2009) Physiology of cell volume regulation in vertebrates. Physiol Rev 89:193–277. doi:10.1152/physrev.00003.2007
12. Behar TN, Li YX, Tran HT, Ma W, Dunlap V, Scott C, Barker JL (1996) GABA stimulates chemotaxis and chemokinesis of embryonic cortical neurons via calcium-dependent mechanisms. J Neurosci 16:1808–1818
13. Behar TN, Schaffner AE, Scott CA, Greene CL, Barker JL (2000) GABA receptor antagonists modulate postmitotic cell migration in slice cultures of embryonic rat cortex. Cereb Cortex 10:899–909. doi:10.1093/cercor/10.9.899
14. Behar TN, Smith SV, Kennedy RT, McKenzie JM, Maric I, Barker JL (2001) GABA_A receptors mediate motility signals for migrating embryonic cortical cells. Cereb Cortex 11:744–753. doi:10.1093/cercor/11.8.744
15. Heck N, Kilb W, Reiprich P, Kubota H, Furukawa T, Fukuda A (2014) Roles of taurine-mediated tonic GABA_A receptor activation in the radial migration of neurons in the fetal mouse cerebral cortex. Front Cell Neurosci 8:88. doi:10.3389/fncel.2014.00088
16. Furukawa T, Yamada J, Akita T, Matsushima Y, Yanagawa Y, Fukuda A (2014) Roles of taurine-mediated tonic GABA_A receptor activation in the radial migration of neurons in the fetal mouse cerebral cortex. Front Cell Neurosci 8:88. doi:10.3389/fncel.2014.00088
17. Hussy N, Deleuze C, Pantaloni A, Desamérien MG, Moos F (1997) Agonist action of taurine on glycine receptors in rat supraoptic magnocellular neurons: possible role in osmoregulation. J Physiol 502:609–621. doi:10.1111/j.1469-7793.1997.609bj.x
18. Sergeeva OA, Haas HL (2001) Expression and function of glycine receptors in striatal cholinergic interneurons from rat and mouse. Neuroscience 104:1043–1055. doi:10.1016/S0306-4522(01)00130-0
19. Albrecht J, Schousboe A (2005) Taurine interaction with neurotransmitter receptors in the CNS: an update. Neurochem Res 30:1615–1621. doi:10.1007/s11064-005-8986-6
20. Walz W, Allen AF (1987) Evaluation of the osmoregulatory function of taurine in brain cells. Exp Brain Res 68:290–298. doi:10.1007/BF00248794
21. Hernández-Benítez R, Sedeño-Cortés A, Ramos-Mandujano G, Pasantes-Morales H (2014) Regulatory volume decrease in neural precursor cells: taurine efflux and gene microarray analysis. Cell Physiol Biochem 34:2038–2048. doi:10.1159/000366399

22. Benítez-Díaz P, Miranda-Contreras L, Mendoza-Bricévo RV, Peña-Contreras Z, Palacios-Prü E (2003) Prenatal and postnatal contents of amino acid neurotransmitters in mouse parietal cortex. Dev Neurosci 25:366–374. doi:10.1159/000073514

23. Pasantes-Morales H, Moran J, Schousboe A (1990) Volume-sensitive release of taurine from cultured astrocytes: properties and mechanism. Glia 3:427–432. doi:10.1002/glia.440030514

24. Jackson PS, Strange K (1993) Volume-sensitive anion channels mediate swelling-activated mositol and taurine efflux. Am J Physiol 265:C1489–C1500

25. Feustel PJ, Jin Y, Kimelberg HK (2004) Volume-regulated anion channels are the predominant contributors to release of excitatory amino acids in the ischemic cortical penumbra. Stroke 35:1164–1168. doi:10.1161/01.STR.0000124127.59464_a1

26. Haskew-Layton RE, Rudkowskaya A, Jin Y, Feustel PJ, Kimelberg HK, Mongin AA (2008) Two distinct modes of hypoosmotic medium-induced release of excitatory amino acids and taurine in the rat brain in vivo. PLoS ONE 3:e3543. doi:10.1371/journal.pone.0003543

27. Owens DF, Boyce LH, Davis MB, Kriegstein AR (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci 16:6414–6423

28. Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, Vermeer H, Toonen RF, Hammer RE, van den Berg TK, Missler M, Geuze HJ, Suðhof TC (2000) Synaptic assembly of the brain by controlling second-messenger signalings. J Neurosci 20:6561–6573

29. Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, Vermeer H, Toonen RF, Hammer RE, van den Berg TK, Missler M, Geuze HJ, Suðhof TC (2000) Synaptic assembly of the brain by controlling second-messenger signalings. J Neurosci 20:6561–6573

30. Komuro H, Rakic P (1996) Distinct modes of neuronal migration in different domains of developing cerebellar cortex. J Neurosci 16:6414–6423

31. Komuro H, Rakic P (1998) Distinct modes of neuronal migration in different domains of developing cerebellar cortex. J Neurosci 18:1478–1490

32. Demarque M, Represa A, Becq H, Khalilov I, Ben-Ari Y, Aniksztejn L (2002) Paracrine communication by a Ca\(^{2+}\)- and SNARE-independent release of GABA and glutamate prior to synapse formation. Neuron 36:1051–1061. doi:10.1016/S0896-6273(02)01053-X

33. Komuro H, Rakic P (1996) Intracellular Ca\(^{2+}\) fluctuations modulate the rate of neuronal migration. Neuron 17:275–285. doi:10.1016/S0896-6273(00)81359-2

34. Komuro H, Rakic P (1996) Distinct modes of neuronal migration in different domains of developing cerebellar cortex. J Neurosci 18:1478–1490

35. Kumada T, Ohno N, Vaudry D, Komuro H (2015) The role of calcium and cyclic nucleotide signaling in cerebellar granule cell migration under normal and pathological conditions. Dev Neurobiol 75:369–387. doi:10.1002/dneu.22219

36. Spitzer NC (2006) Electrical activity in early neuronal development. Nature 444:707–712. doi:10.1038/nature05300

37. Rosenberg SS, Spitzer NC (2011) Calcium signaling in neuronal development. Cold Spring Harb Perspect Biol 3:a004259. doi:10.1101/cshperspect.a004259

38. Komuro H, Rakic P (1992) Selective role of N-type calcium channels in neuronal migration. Science 257:806–809. doi:10.1126/science.1323145

39. Komuro H, Rakic P (1993) Modulation of neuronal migration by NMDA receptors. Science 260:95–97. doi:10.1126/science.8096653

40. Spitzer NC (2006) Electrical activity in early neuronal development. Nature 444:707–712. doi:10.1038/nature05300

41. Rosenberg SS, Spitzer NC (2011) Calcium signaling in neuronal development. Cold Spring Harb Perspect Biol 3:a004259. doi:10.1101/cshperspect.a004259

42. Komuro H, Rakic P (1996) Modulation of neuronal migration by NMDA receptors. Science 260:95–97. doi:10.1126/science.8096653

43. Komuro H, Rakic P (1996) Intracellular Ca\(^{2+}\) fluctuations modulate the rate of neuronal migration. Neuron 17:275–285. doi:10.1016/S0896-6273(00)81359-2

44. Komuro H, Rakic P (1998) Distinct modes of neuronal migration in different domains of developing cerebellar cortex. J Neurosci 18:1478–1490

45. Kumada T, Komuro H (2004) Long-range Ca\(^{2+}\) signaling from growth cone to soma mediates reversal of neuronal migration induced by slit-2. Cell 129:385–395. doi:10.1016/j.cell.2007.01.051

46. Huang Z, Wang Y, Su Z, Geng J-G, Chen Y, Yuan X-B, He C (2011) Slit-2 represses the migration of olfactory ensheathing cells by triggering Ca\(^{2+}\)-dependent cofilin activation and RhoA inhibition. J Cell Sci 124:186–197. doi:10.1242/jcs.071357

47. Belgacem YH, Borodinsky R, Murakami F, Komuro H (2003) Prenatal and postnatal contents of amino acid neurotransmitters in mouse parietal cortex. J Neurosci 22:5966–5981

48. Shirasaka R, Katsumata R, Murakami F (1998) Change in calcium and cytosolic GABA concentrations in cerebellar granule cells in vitro. J Neurosci 22:5966–5981
55. Kumada T, Jiang Y, Kawanami A, Cameron DB, Komuro H (2009) Autonomous turning of cerebellar granule cells in vitro by intrinsic programs. Dev Biol 326:237–249. doi:10.1016/j.ydbio.2008.11.012
56. Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133–1138. doi:10.1126/science.274.5290.1133
57. Sanes JR, Lichtman JW (2001) Induction, assembly, maturation and maintenance of a postsynaptic apparatus. Nat Rev Neurosci 2:791–805. doi:10.1038/sj.nrn7557
58. Nithianantharajah J, Hannan AJ (2006) Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci 7:697–709. doi:10.1038/nrn1970
59. Lepousez G, Valley MT, Lledo P-M (2013) The impact of adult neurogenesis on olfactory bulb circuits and computations. Annu Rev Physiol 75:339–363. doi:10.1146/annurev-physiol-030212-183731
60. Lledo P-M, Merkle FT, Alvarez-Buylla A (2008) Origin and function of olfactory bulb interneuron diversity. Trends Neurosci 31:392–400. doi:10.1016/j.tins.2008.05.006
61. Whitman MC, Greer CA (2009) Adult neurogenesis and the olfactory system. Prog Neurobiol 89:162–175. doi:10.1016/j.pneurobio.2009.07.003
62. Adam Y, Mizrahi A (2010) Circuit formation and maintenance-perspectives from the mammalian olfactory bulb. Curr Opin Neurobiol 20:134–140. doi:10.1016/j.conb.2009.11.001
63. Kaneko N, Marin O, Koike M, Hirota Y, Uchiyama Y, Wu JY, Lu Q, Tessier-Lavigne M, Alvarez-Buylla A, Okano H, Rubenstein JLR, Sawamoto K (2010) New neurons clear the path of astrocitic processes for their rapid migration in the adult brain. Neuron 67:213–223. doi:10.1016/j.neuron.2010.06.018
64. Sakamoto M, Imayoshi I, Ohsutka T, Yamaguchi M, Mori K, Kageyama R (2011) Continuous neurogenesis in the adult forebrain is required for innate olfactory responses. Proc Natl Acad Sci USA 108:8479–8484. doi:10.1073/pnas.1018782108
65. Rochefort C, Gheusi G, Vincent J-D, Lledo P-M (2002) Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. J Neurosci 22:2679–2689
66. Saghatelian A, Roux P, Migliore M, Rochefort C, Desmaisons D, Charneau P, Shepherd GM, Lledo PM (2005) Activity-dependent adjustments of the inhibitory network in the olfactory bulb following early postnatal deprivation. Neuron 46:103–116. doi:10.1016/n.euron.2005.02.016
67. Yamaguchi M, Mori K (2005) Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb. Proc Natl Acad Sci USA 102:9697–9702. doi:10.1073/pnas.0406082102
68. Livneh Y, Feinstein N, Klein M, Mizrahi A (2009) Sensory input enhances synaptogenesis of adult-born neurons. J Neurosci 29:86–97. doi:10.1523/JNEUROSCI.4105-08.2009
69. Lin CW, Sim S, Ainsworth A, Okada M, Kelsch W, Lois C, Yoshihara S, Takahashi H, Nishimura N, Naritsuka H, Shirao T, Livneh Y, Feinstein N, Klein M, Mizrahi A (2012) Experience-dependent expression of Npas4 regulates plasticity in adult visual cortex. J Physiol 590:4777–4787. doi:10.1111/j.1469-772X.2012.34237
70. Prunsil P, Sepp M, Orav E, Koppel I, Timmus T (2011) Identification of cis-elements and transcription factors regulating neuronal activity-dependent transcription of human BDNF gene. J Neurosci 31:3295–3308. doi:10.1523/JNEUROSCI.4540-10.2011
71. Ramamoothri K, Fropf R, Belfort GM, Fitzmaurice HL, McKinney RM, Neve RL, Otto T, Lin Y (2011) Npas4 regulates a transcriptional program in CA3 required for contextual memory formation. Science 334:1669–1675. doi:10.1126/science.1208049
72. Bloodood BL, Sharma N, Browne HA, Trepan AZ, Greenberg ME (2013) The activity-dependent transcription factor Npas4 regulates domain-specific inhibition. Nature 503:121–125. doi:10.1038/nature12743
73. Dickson BJ, Zou Y (2010) Navigating intermediate targets: the nervous system midline. Cold Spring Harb Perspect Biol 2:a002055. doi:10.1101/cshperspect.a002055
74. Schnorrer F, Dickson BJ (2004) Axon guidance: morphogens show the way. Curr Biol 14:R19–R21. doi:10.1016/j.cub.2003.12.016
75. Dickson BJ (2002) Molecular mechanisms of axon guidance. Science 298:1599–1604. doi:10.1126/science.1072165
76. Black DL, Zipursky SL (2008) To cross or not to cross: alternatively spliced forms of the Robo3 receptor regulate discrete steps in axonal midline crossing. Neuron 58:297–298. doi:10.1016/j.neuron.2008.04.019
77. López-Bendito G, Cautain A, Sánchez JA, Bielle F, Flames N, Garratt AN, Talmage DA, Role LW, Charnay P, Marin O, Garel S (2006) Tangential neuronal migration controls axon guidance: a role for Neuregulin-1 in thalamocortical axon navigation. Cell 125:127–142. doi:10.1016/j.cell.2006.01.042
78. Schmid RS, Maness PF (2008) L1 and NCAM adhesion molecules as signaling coreceptors in neuronal migration and process outgrowth. Curr Opin Neurobiol 18:245–250. doi:10.1016/j.conb.2008.07.015
79. Bai G, Pfaff SL (2011) Protease regulation: the Yin and Yang of neural development and disease. Neuron 72:9–21. doi:10.1016/j.neuron.2011.09.012
80. Dudanova I, Klein R (2013) Integration of guidance cues: parallel signaling and crosstalk. Trends Neurosci 36:295–304. doi:10.1016/j.tins.2013.01.007
81. Leyva-Díaz E, Del Toro D, MenaL MJ, Cambray S, Susin R, Tessier-Lavigne M, Klein R, Egea J, López-Bendito G (2014) FLRT3 is a Robo1-interacting protein that determines netrin-1 attraction in developing axons. Curr Biol 24:494–508. doi:10.1016/j.cub.2014.01.042
82. Molnár Z, Garel S, López-Bendito G, Maness P, Price DJ (2012) Mechanisms controlling the guidance of thalamocortical axons through the embryonic forebrain. Eur J Neurosci 35:1573–1585. doi:10.1111/j.1460-9568.2012.08119.x
83. Bielle F, Marcel-Mondéjar P, Leyva-Díaz E, Lokmane L, Mire E, Mailhes C, Keita M, García N, Tessier-Lavigne M, Garel S, López-Bendito G (2011) Emergent growth cone responses to combinations of Slit1 and Netrin 1 in thalamocortical axon topography. Curr Biol 21:1748–1755. doi:10.1016/j.cub.2011.09.008
90. Müller P-S, Schulz R, Maretto S, Costello I, Srinivas S, Bikoff EK, Robinson M, Parsons Perez MC, Tebar L, Palmer J, Patel A, Seiradake E, del Toro D, Nagel D, Cop F, Härtil R, Ruff T, Seyit-Fothergill T, Donahoo ALS, Douglass A, Zalucki O, Yuan J, Bouchard J-F, Moore SW, Tritsch NX, Roux PP, Shekarabi M, O'Sullivan ML, de Wit J, Savas JN, Comoletti D, Otto-Hitt S, Karaulanov E, Böttcher RT, Stannek P, Wei W, Rau M, Ogata SP (2004) FLRT3 is expressed in sensory neurons after peripheral nerve injury and regulates neurite outgrowth. Mol Cell Neurosci 27:202–214. doi:10.1016/j.mcn.2004.06.008

91. Tsuji L, Yamashita T, Kubo T, Madura T, Tanaka H, Hosokawa K, Tohyama M, Hampel F, Sanchez R, Rodriguez-Manzaneque MT, Bosl MR, Fässler R, Lickert H, Klein R (2008) Genetic ablation of M, Hampel F, Sanchez R, Rodriguez-Manzaneque MT, Bosl MR, Fässler R, Lickert H, Klein R (2008) Genetic ablation of FLRT3 reveals a novel morphogenetic function for the anterior visceral endoderm in suppressing mesoderm differentiation. Genes Dev 22:3349–3362. doi:10.1101/gad.486708

92. Lu X, Le Noble F, Yuan L, Jiang Q, De Lafarge B, Sugiyama D, Bréant C, Claes F, Scales SJ, Brezzi C, Michaud A, Carmeliet P, Tessier-Lavigne M, Eichmann A (2007) UNC5B receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. Nature 432:179–186. doi:10.1038/nature03080

93. Larrivée B, Freitas C, Tombe M, Ly X, DeLafarge B, Yuan L, Bovrévée K, Bréant C, Del Toro R, Bréchot N, Germain S, Bono F, Dol F, Claes F, Fischer C, Autiero M, Thomas JL, Carmeliet P, Tessier-Lavigne M, Eichmann A (2004) The netrin receptor UNC5B inhibits sprouting angiogenesis. Dev Genes Dev 24:233–247. doi:10.1242/dev.013623

94. Stein E, Tessier-Lavigne M (2001) Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. Science 291:1928–1938. doi:10.1126/science.1058445

95. Adams RH, Altalool K (2007) Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol 8:464–478. doi:10.1038/nrm2183

96. Eichmann A, Makinen T, Altalool K (2005) Neural guidance molecules regulate vascular remodeling and vessel navigation. Genes Dev 19:1013–1021. doi:10.1101/gad.1305405

97. Bremer G, Harlos K, Border EC, Acker-Palmer A, Jones EY, Marks D, Sheasby A, De Felipe C, Coffin R, Livesey FJ, Hunt P, Corvol P, Tessier-Lavigne M, Eichmann A (2004) The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. Cold Spring Harb Perspect Biol 2:a001875. doi:10.1101/cshperspect.a001875