SCIENCE CHINA
Life Sciences

• REVIEW •

March 2010 Vol.53 No.3: 304–314
doi: 10.1007/s11427-010-0068-6

# Axon guidance and neuronal migration research in China

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Received December 30, 2009; accepted January 19, 2010

Proper migration of neuronal somas and axonal growth cones to designated locations in the developing brain is essential for the assembly of functional neuronal circuits. Rapid progress in research of axon guidance and neuronal migration has been made in the last twenty years. Chinese researchers began their exploration in this field ten years ago and have made significant contributions in clarifying the signal transduction of axon guidance and neuronal migration. Several unique experimental approaches, including the migration assay of single isolated neurons in response to locally delivered guidance cues, have been developed by Chinese neuroscientists to investigate the molecular machinery underlying these guidance events.

**axon guidance, neuronal migration, development, neuronal circuits**

**Citation:** Yuan X B. Axon guidance and neuronal migration research in China. Sci China Life Sci, 2010, 53: 304−314, doi: 10.1007/s11427-010-0068-6

Neurons are born in restricted germinal zones of the developing brain and undergo active migration along specific routes, sometimes over very long distances, to reach their designated residences of their cell bodies. Axons initiated from these cell bodies also travel long distances to find their proper target neurons in order to form synaptic connections. In order to properly form neuronal circuits, the migration of either the soma of newborn neurons or their axons and dendrites needs to be guided by spatial and temporal signals in developing tissues, with some guidance mechanisms shared by these two different developmental events [1].

The growing axons and dendrites, also referred to as neurites in general, have a dynamic structure at the tip called growth cone, where newly synthesized materials are assembled for neurite elongation. Growth cones also actively navigate through the surrounding tissues, response to extracellular signals in the tissue environment, and guide the neurite extension toward the right targets [2]. In migrating neurons, a similar growth cone-like structure exists at the tip of leading neurites and may perform the similar pathfinding function [3,4]. In the past two decades, great progress has been made in the understanding of the molecular and cellular mechanisms underlying the pathfinding of neuronal migration and neurite outgrowth [1,5]. A number of axon guidance molecules, i.e. netrins, semphorins, slits and ephrins, have been discovered and their receptor proteins have been identified. Functions of these molecules in guiding axonal projections have been described and intracellular signal transduction underlying the guidance of neurite extension and neuronal migration has also been extensively studied. Based on experimental studies, theoretical computational models have been introduced to depict the growth cone guidance process [6].

This review will highlight the major findings concerning axon guidance and neuronal migration made by Chinese researchers during the past decade. Some regulatory mechanisms for nerve regeneration after the adult nervous system injury are notably similar to that of developmental nerve growth [7]. Axon regeneration has also been studied in China, but the research results are not covered here.

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# 1 Axon guidance

## 1.1 Guidance of retinal axons in the optic chiasm

At the ventral diencephalon of mouse brain, there is an X-shaped nerve bundle known as the optic chiasm, in which axons from the ventral temporal (VT) regions of the retina turn before reaching the midline and join axons coming from the contralateral side to form the ipsilateral optic tract, whereas axons from the nasal dorsal (ND) region of the retina cross the midline and travel in the contralateral optic tract [8]. This segregation of uncrossed from crossed axons is crucial for binocular vision in mammals. In the optic tract there is a chronotopic order of retinal fibers, with the youngest axons closest to and oldest axons farthest from the pial surface of the tract. This order reflects the arrival time of retinal axons in the tract. The axon guidance decision and age-related fiber order at the optic chiasm has been shown to be regulated by cues present on the radial glia and neurons at the vicinity of the chiasm [8]. Chan SunOn and colleagues [9,10] in the Chinese University of Hong Kong have examined the development of the retinal axon projection at the ventral diencephalon (also known as the retinofugal pathway) in the mouse by confocal microscopic observation of the fiber organization at different segments of the retinofugal pathway after labeling axons from different quadrants of the retina with different fluorescent dyes. Consistent with previous observations in other mammalian species, they observed in mice a dynamic age-related order and a quadrant-specific order in the organization of retinal axons in the retinofugal pathway before and after the chiasm. Moreover, two *in vitro* experimental systems have been used by these researchers to examine the potential function of candidate guidance molecules on the retinal axon projection. One experimental system is the slice culture preparation which maintains the integrity of the retinofugal pathway with the convenience of pharmacological treatments of the tissue. The other method is the culturing of the retinal explant adjacent to a spot coated with different molecules to test the potential guidance function of coated molecules on the extension of axons growing out of the explant.

### *1.1.1 Guidance of retinal axons in the optic chiasm by proteoglycans*

Most studies have been focused on the effect of protein factors in axon pathfinding. The potential contributions of glycan molecules present in developing brain tissues have long been overlooked. Chan SunOn and colleagues [10–23] explored the potential role of a group of proteoglycans expressed in the ventral diencephalon and in retina, in the routing and organization of optic fibers at the chiasm. These proteoglycans include sulphated molecules chondroitin sulphate proteoglycans (CS-PGs) and heparan sulfate proteoglycans (HS-PGs), a type of nonsulfated linear glycosaminoglycan hyaluronan (HA), and the cell surface carbohydrate molecule SSEA-1, which is also known as CD15 (leukocyte cluster of differentiation 15) and Lewis-x, a member of the Lewis blood group antigens in humans [24]. By immunohistochemisty using antibodies specific for different glycan epitopes and antibodies against carrier proteins of glycans, they observed specific expression of each of these proteoglycans along the retinofugal pathway [11,13,14,17,19,20,22]. They found that cell adhesion molecules that may recognize these proteoglycans [25,26], including L1, the poly-sialylated form of NCAM (PSA-NCAM) and CD44, are also expressed in a site-specific pattern along the optic pathway at comparable developmental stages [15,16], consistent with the previous reports [27,28]. The specific expression of these proteoglycans and adhesion molecules suggests that they may implement defasciculation, resorting, and subsequent refasciculation of axons at sites of the optic chiasm.

In the slice culture preparation of retinofugal projections, specific enzymatic digestion of the CS or HA sugar epitopes from the ventral diencephalon tissue dramatically perturbed the midline choices and chronic organization of optic fibers [12,20]. Interestingly, the treatment with specific antibodies [15], which disturb the interaction between HA and CD44, or the treatment with exogenous HA [21], which probably displaces the endogenous HA from binding to CD44, also produced similar defects in midline crossing of retinal axons as the enzymatic removal of HA [21]. In a culture of retina explants close to a proteoglycan-coated spot, they observed that CS at a critical concentration was able to inhibit the extension of neurites from VT but not DN retina in a protein kinase C (PKC)-dependent manner [18,23]. Application of soluble Lewis-x/SSEA-1 inhibited the outgrowth of neurites from both VT and DN retina in culture [19]. However, the treatment with HA, either in soluble or in substrate-bound form, did not affect the outgrowth and extension of retinal axons in explant cultures [21]. In a summary, these tissue culture studies showed that (i) CS-PGs expression in the ventral diencephalon directs the turning of uncrossed axons at the chiasmatic midline, and deviates the growth cones from the deep parts of the tract towards the subpial regions during formation of the age-related fiber organization; (ii) the HA/CD44 complex may not directly guide the extension of retinal axons, but modulate activities or accessibility of other axon-guidance factors in the chiasm; (iii) SSEA-1 may help the formation of age-related fiber arrangement by deviating newly arrived growth cones, which arise from all retinal quadrants, away from the deep parts of the optic tract. These studies also suggest that glycan side chains, the sugars of proteoglycan molecules in the optic pathway, are contributing factors in controlling the midline crossing of retinal axons in the mouse chiasm, although the carrier protein of proteoglycans and other guidance molecules associated with the sugars may also contribute to the guidance

processes.

*1.1.2 Guidance of retinal axons in the optic chiasm by other proteins*

Chan SunOn and colleagues discovered a novel developmental function of Nogo, an inhibitory molecule for axon regeneration in the adult central nervous system [7], in the sorting of VT retinal axons into the ipsilateral projection [29,30]. They observed that Nogo is expressed by radial glia in the midline within the optic chiasm, and the Nogo receptor (NgR) is expressed in retinal neurites and growth cones. Nogo inhibited the neurite outgrowth from both DN and VT retina in culture. In a coculture preparation of the retina and optic chiasm, NgR was selectively reduced on neurites and growth cones from DN retina but not VT retina when growth cones contact chiasm cells. In slice cultures of the optic pathway, blocking Nogo effect significantly reduced uncrossed projections. It is the first report that Nogo participates in the pathfinding of retinal axons.

Moreover, Chan SunOn and colleagues found that the morphogen sonic hedgehog (Shh) protein, which has been shown to guide the midline crossing of commissural axons in the spinal cord [31], may also guide the pathfinding of retinofugal projections in mice at different developmental stages [32–34]. They observed that Shh is localized largely in the lateral regions of the chiasm and at the optic tract, and to a lesser extent in the caudal regions of the diencephalon. Pharmacological blockade of Shh signaling in brain slice preparation of the optic pathway reduced the midline crossing and increased the projection of axons to the contralateral optic stalk. Whether Shh exerts its influence on retinal axons directly or indirectly through its effect on glial cells or neurons in the chiasm remains to be clarified.

### 1.2 Hippocampal axon guidance

As the major excitatory input on the hippocampus, the entorhino-hippocampal fiber tract consists of the alvear path, the perforant path, and a midline-crossing commissural projection. Deng JinBo and colleagues in Henan University investigated the development of the three subsets of the entorhino-hippocampal projection in rats by tracing the projections with multiple fluorescent dyes at different developmental stages [35]. By using calretinin immunocytochemistry, the authors discovered transitory Cajal-Retzius (CR) cells in the stratum lacunosum-moleculare of the hippocampus from E16, and there is a close association between CR cells and entorhinal afferents, suggesting that CR cells may serve as a guidepost for entorhinal afferents during cortical development. CR cells are known to secrete an extracellular matrix protein reelin, which is essential for the proper radial migration and lamination of cortical neurons [36]. To test whether reelin guides the extension of entorhinal-hippocampal projections, the authors co-cultured entorhinal slices from GFP-transgenic mice with hippocampal slices of wild type or reelin mutant mice (reeler mice) [37]. Similar to the extension of the perforant projection *in vivo*, the regenerated fibers from the entorhinal cortex in wild type mice grew along the molecular layer of the dentate gyrus, and only a few fibers was found to penetrate through the granular layer into the hilus. By contrast, regenerated fibers leave the molecular layer and continue to aberrantly grow into the granular layer, hilus, pyramidal layer, and even stratum oriens in reeler mice. These results suggest that reelin secreted from CR cells may contribute to the guidance of the perforant axons by restricting their extension in the molecular layer of dentate gyrus.

### 1.3 Regulation of the corpus callosal axon projection by neuronal activity

The corpus callosum is the largest bundle of commissural axons in the mammalian brain, but the guidance mechanisms underlying its development remain largely unknown. Ding YuQiang and colleagues at the Institute of Neuroscience (ION) of Chinese Academy of Sciences (CAS) found that neuronal activity is implicated in the normal development and maintenance of callosal projections in the mouse somatosensory cortex [38]. By using *in utero* electroporation, they labelled a subpopulation of cortical callosal neurons and traced their axon projection at different developmental stages. They observed that callosal axons develop region- and layer-specific termination patterns in the contralateral cortex. Suppressing the neuronal activity by electroporation-aided overexpression of the inward rectifying potassium channel Kir2.1 in these callosal neurons resulted in overgrowth of many axons into layer I and scattering of axon terminals beyond the normal terminating region, the border between the primary and secondary somatosensory cortices. Blocking synaptic transmission via expression of the tetanus toxin light chain (TeNT-LC) in these neurons led to a more severe failure in the projections to the border region, and the eventual disappearance of callosal projections over the entire somatosensory cortex. Co-expression of Kir2.1 and TeNT-LC produced an additive effect of expressing them alone. These results provide very strong support for the notion that spontaneous neuronal activities regulate the development of neural circuitry and suggest that neuronal excitation and synaptic transmission are involved in regulating different aspects of axon projection.

### 1.4 Signal transduction of growth cone guidance

*1.4.1 Nerve growth cone guidance by GPCR*

When growth cones encounter localized extracellular cues that are either attractive or repulsive, receptors on the growth cone surface read the concentration difference of these guidance cues on each side and steer growth cone migration towards the favorite direction. The cytoplasmic signal transduction cascade that mediates the proper growth

cone response to external guidance cues remains largely unclear and has attracted the interest of neurobiologists for quite a long time. Using an *in vitro* "growth cone turning assay" [39], which mimics the nerve growth cone guidance by the gradient of guidance cues *in vivo*, Duan ShuMin and colleagues at the ION have examined the signal transduction of growth cone guidance by several potential guidance molecules [40]. They discovered that stromal-derived factor 1 (SDF-1), a chemokine that attracts migrating leukocytes and cerebellar granule cells via a G protein-coupled receptor (GPCR), also guides the axon extension in cerebellar granule cells through activation of heterotrimeric protein $G_i$ and phospholipase C. At the downstream of phospholipase C activatioin, the $Ca^{2+}$ release from internal stores is essential for the SDF-triggered growth cone turning, either attraction or repulsion. Although chemokines have been shown to guide the cell migration through GPCR signaling under various physiological and pathological conditions, this is the first piece of evidence showing the guidance of nerve extension by a chemokine through GPCR, implying the possibility of axon guidance by a large group of known chemokines which transduce the cytoplasmic signal through GPCR. As SDF-1 guides the migration of cerebellar granule neurons, these results provided further support for the notion that guidance of neuronal migration and guidance of neurite extension may share similar extracellular cues and intracellular signal transduction mechanisms.

### *1.4.2 Rho GTPases signaling in nerve growth cone guidance*

Reorganization of cytoskeletal structures in nerve growth cones is essential for the growth cone turning in response to extracellular guidance cues. Small GTPases of the Rho family (Rho GTPases) have emerged as key regulators for cytoskeletal rearrangements in a variety of cells, including neurons. Using the growth cone turning assay, Yuan Xiao Bing, Duan ShuMin, and their colleagues [41] at the ION have examined whether Rho GTPases are involved in mediating the growth cone guidance by an attractive factor, brain- derived neurotrophic factor (BDNF), and a repulsive factor, lysophosphatidic acid (LPA). They found that the expression of either dominant-negative or constitutively active Cdc42 in cultured *Xenopus* spinal neurons, at a concentration that did not substantially affect filopodial formation and neurite extension, abolished BDNF-induced attractive turning, while the expression of dominant-negative RhoA abolished LPA-induced repulsion. In cultured neurons, BDNF activated Cdc42 and Rac. The authors also observed that a crosstalk between the Cdc42 and RhoA pathways through their converging actions on the myosin activity regulates the polarity of growth cone turning triggered by extracellular factors. These findings provide the direct evidence that Rho GTPases mediate the directional signal of extracellular guidance cues by regulating the cytoskeleton, rather than by simply providing permissive conditions for the neurite outgrowth. This work also implies the interesting possibility that growth cone response to one axon guidance factor may be modulated by another guidance factor through the crosstalk between their intracellular signaling pathways, including Rho GTPases.

In another line of study, Nancy IP and colleagues in the Hongkong University of Science and Technology (HUSK) showed that α2-chimaerin, a member of the GTPase-activating proteins that negatively regulate Rho GTPase activity, interacts with the guidance receptor EphA4 [42]. Activation of EphA4 by its ligand induced a rapid increase of tyrosine phosphorylation of α2-chimaerin and enhanced its GTPase-activating protein (GAP) activity toward Rac1. More significantly, the GAP activity of α2-chimaerin was required for the growth cone collapse effect of EphA4. This study has therefore identified a new α2-chimaerin-dependent signaling mechanism for Rho GTPase regulation by the guidance receptor EphA4 to regulate the actin cytoskeleton and growth cone guidance.

In addition to Rho GTPases, elevation of cytosolic $Ca^{2+}$ ($[Ca^{2+}]_i$) in nerve growth cones is also implicated in growth cone guidance [2]. The polarity of growth cone turning triggered by extracellular guidance molecules depends on the magnitude of $[Ca^{2+}]_i$ elevation and/or the steepness of the $[Ca^{2+}]_i$ gradient across the growth cone [5]. How does the $Ca^{2+}$ signal regulate the cytoskeletal rearrangements during growth cone turning? Yuan XiaoBing, Poo MuMing, and their colleagues at the ION provided direct evidence that $[Ca^{2+}]_i$ elevation activates Cdc42 and Rac in neurons through the $Ca^{2+}$-dependent PKC and the $Ca^{2+}$- and calmodulin-dependent kinase II (CaMK-II) [43]. Using the growth cone turning assay, they found that a gradient of $[Ca^{2+}]_i$ across the growth cone generated by a gradient of an intracellular $Ca^{2+}$ mobilization drug ryanodine triggered attractive growth-cone turning in a Rho GTPases- dependent manner.

Using the similar growth cone turning assay, Nancy Ip and colleagues [44] at the HUSK and ION discovered that agrin, a molecule previously known to induce postsynaptic specializations at neuromuscular junctions, inhibits the neurite extension in cultured *Xenopus* spinal neurons. A gradient of agrin induced repulsive growth cone turning in a $PI_3$-kinase- and $[Ca^{2+}]_i$-dependent way. The authors found that agrin inhibited Rac GTPase activity in neuronal culture, and transfection with dominant-negative Rac1 prevented the agrin-induced growth cone turning. This study clarified an unexpected function of agrin in axon pathfinding. These above studies further showed the significance of Rho GTPases in mediating the guidance signal of extracellular factors.

### *1.4.3 TRPC channels in growth cone guidance*

$Ca^{2+}$ mediates the growth cone turning signal triggered by a wide variety of guidance factors. It is of great interest to understand how the $Ca^{2+}$ signal in the growth cone is trig-

gered by guidance factors. Yuan XaioBing, Wang YiZheng and their colleagues [45] at the ION found that a non-selective cationic channel TRPC may be involved in this process (Figure 1). In cultured cerebellar granule neurons, they found that the growth cone chemoattraction triggered by BDNF was blocked by pharmacological inhibition of TRP channels, by specifically knocking down the expression of endogenous TRPC3 and 6 (TRPC3/6) proteins with small interference RNAs (siRNA), and by overexpressing a dominant negative form of TRPC3. Fluorescent imaging of $[Ca^{2+}]_i$ showed that the $[Ca^{2+}]_i$ elevation at the growth cone triggered by BDNF was dramatically reduced after perturbing the TRPC channels. It has been established that the ion channels formed by the TRP superfamily of proteins act as the sensors for temperature, osmolarity, mechanical stress and taste. This finding has further expanded the function of TRP channels in sensory processes to include the chemosensory response of the nerve growth cone towards extracellular guidance factors.

### 1.5 Identification of netrin-4 receptors

Netrin-4 is a novel member of the netrin family of axon guidance molecules. It has been shown to promote neurite extension [46]. However, the receptors mediating the netrin-4 effect in neurons remain unclear. Using cell surface binding assays, Zhang ChengGang and colleagues at the Beijing Institute of Radiation Medicine, Chinese National Human Genome Center, investigated the binding of netrin-4 with two known receptors for netrin-1, Deleted in Colorectal Cancer (DCC) and UNC5 homolog 1 (UNC5H1) [47]. They observed that netrin-4 bound to DCC and UNC5H1 with the laminin VI domain (Laminin N-terminal domain, LNT). Both the LNT and the heparin-binding domain (Netrin C-terminal domain, NCT), but not the EGF- like domain (EGFL) of netrin-4, also bound to an unknown receptor on the surface of cultured cortical neurons, with the binding activity of the LNT being about 3-fold higher than that of the NCT. This binding partner of netrin-4 remains unclear, and which receptor mediates the growth-promoting effect of netrin-4 in cortical neurons needs to be further clarified.

### 1.6 Generation of a transgenic mouse line for axon guidance research

The animal models in which specific neural circuits are genetically labeled are very useful for tracing the circuit development and examining the underlying mechanisms. Zhao ChunJie and colleagues at the Southeast University developed a transgenic mouse line in which Tau-LacZ is driven by the promoter of one Wnt receptor, *Frizzled10* [48]. The nociceptive circuits are specifically labeled by the Tau-LacZ transgene in developing spinal cord and DRG in this mouse line. In the spinal cord, commissural axons crossing the floor plate are specifically labeled. This mouse line may

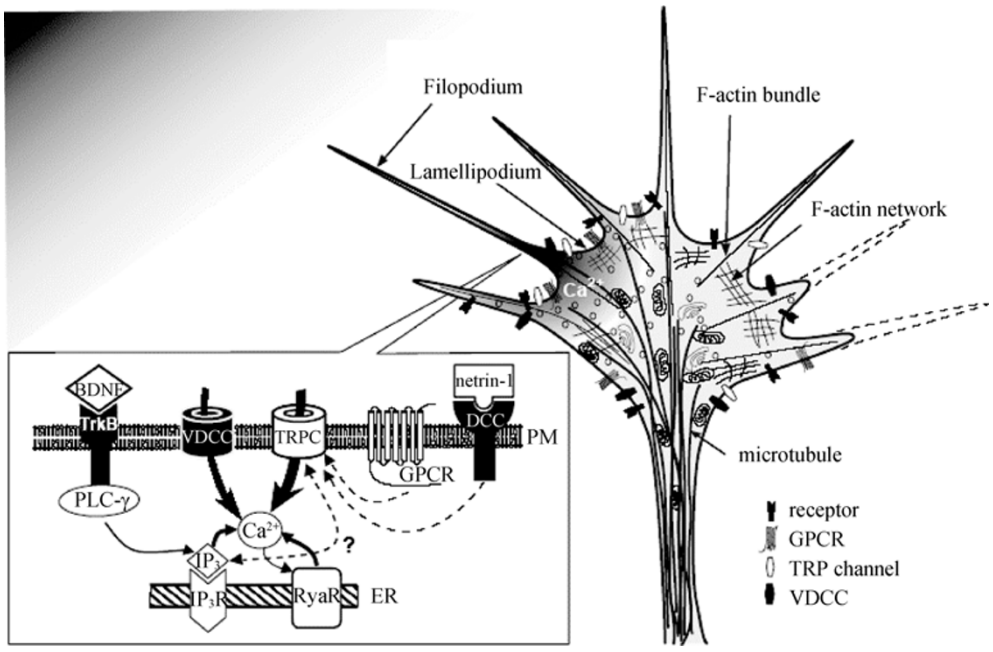


**Figure 1** TRPC channels mediate the activation of the growth cone $Ca^{2+}$ signal by chemotropic guidance factors. An extracellular gradient of guidance cue (from the left-up corner) triggers the opening of TRPC channels at the growth cone surface mainly through the PLC-IP3 signaling pathway. This $Ca^{2+}$ signal may be amplified by $Ca^{2+}$-induced $Ca^{2+}$ release from ER via the ryanodine receptor channels. Dashed lines on the right side of the growth cone indicate retracting filopodium. PM, Plasma membrane; ER, endoplamic reticulum; PLC, phospholipase C; IP3, inositol-1, 4,5-trisphosphate; RyaR, ryanodine receptors; VDCC, voltage-dependent calcium channel.

provide a useful genetic tool for studying the guidance mechanism underlying the development of spinal nociceptive circuits and midline crossing of commissural axons.

### 1.7 Computational modeling of nerve growth and pathfinding

Zheng XiaoXiang and coauthors at the Zhejiang University developed an open-L-systems-based framework for modeling and simulating axon guidance [49]. The L-systems are the parallel rewriting systems that successively and recursively enable the replacement parts of a simple object [50]. It has been used to simulate the development of a branching structure such as a tree [51] and neuronal dendritic arborizations [52,53]. As the extension of the L-systems, the open L-systems contain a computational module to simulate the communication between the object and its environment. In the modeling, three kinds of growth cone behavior are considered, including advance/retraction, turning and branch formation/elimination, and three kinds of environment-growth cone interactions are involved, including contact attraction, contact repulsion and chemoattraction. A computer program was developed to reduce the parameter number required from the user in the simulation. This is the first attempt of computational modeling of the axon pathfinding by Chinese researchers. This modeling system laid a foundation for the theoretical research concerning the axon guidance mechanisms, possibly by incorporating more computational modules that describe specific signal transduction events.

Other discoveries regarding axon guidance made by Chinese researchers include the identification and characterization of splice variants of ephrin-A3 and ephrin-A5 by Nancy IP and colleagues at the HUSK [54], the clone and function study of semaphorin 6C and 6D by He FuChu and colleagues [55] at the Beijing Institute of Radiation Medicine, Chinese National Human Genome Center, the discovery of a novel zebrafish gene slit-like 2 by Xue JinLun, Yao JiHua and their colleagues at the Fudan Univerisity [56], the dissection of the high-resolution X-ray crystal structure of the srGAP1 SH3 domain (a crucial intracellular signaling module in the slit-Robo pathway) by Rao ZiHe [57] at the Tsinghua Univeristy, and the finding of semaphorin-3F as a potential attractant to growth cones of cultured cerebellar granule neurons by Yuan XiaoBing and colleagues at the ION [58].

## 2 Guidance of neuronal migration

### 2.1 $Ca^{2+}$ mediates the repulsive guidance of neuronal migration

In the developing brain, neurons migrate in close association with each other or along the fibers of glial processes, i.e., the radial migration of newborn pyramidal neurons along radial glial filaments in the developing cerebral cortex. Feng LinYin and colleagues at the ION examined the migration of cerebellar granule neurons along the surface of co-cultured astroglial cells [4]. Using a micropipette, they delivered a gradient of the guidance factor in front of migrating neurons and monitored their responses. They discovered that a frontal gradient of the repulsive guidance factor slit-2 was able to stop and even reverse the neuron migration along the astroglial fiber in a $Ca^{2+}$-dependent manner. Fluorescent ratiometric analysis of the $[Ca^{2+}]_i$ showed that the soma of migrating neurons existed an asymmetric distribution of $Ca^{2+}$ with higher $[Ca^{2+}]_i$ in the rear end, but no apparent asymmetry in $[Ca^{2+}]_i$ was observed in stationary neurons. A frontal slit-2 gradient elevated the $[Ca^{2+}]_i$ and led to the reversal of the pre-existing asymmetry of the $[Ca^{2+}]_i$ distribution in the soma of migrating neurons, a process closely related with the reversal of the neuronal migration. These results suggest that a frontal gradient of slit-2 controls the stop and reversal of neuronal migration by triggering the $[Ca^{2+}]_i$ elevation and changing the $[Ca^{2+}]_i$ polarity in migrating neurons. Although it had been shown that $[Ca^{2+}]_i$ fluctuation regulates the speed of neuronal migration, this is the first demonstration that the $Ca^{2+}$ signal commands the directionality of neuronal migration.

### 2.2 $Ca^{2+}$ wave coordinates changes in motility of the growth cone and soma

The aforementioned study was further extended by Poo MuMing, Yuan XiaoBing and their colleagues at the ION using low density culture of the dissociated cerebellar granule neurons, which exhibit robust spontaneous migration on the culture substrate laminin [3]. In this simplified culture preparation, behavior of different parts of the migrating neurons was monitored and the signaling mechanism of neuronal response to the locally delivered guidance cues was tackled because of the lack of the cell-cell interaction. Consistent with the early study, in isolated migrating neurons application of a slit-2 gradient in front of the leading process led to the collapse of the leading growth cone, followed by the reversal of soma migration. This directional change was not observed upon application of slit-2 in front of the soma or trailing neurite, indicating that the leading growth cone directly influences the sensation of extracellular guidance cues. Consistently the slit-2 receptor Robo2 is preferentially located at the growth cone. Fluorescent imaging showed that the reversal of neuronal migration triggered by the frontal gradient of slit-2 was preceded by a propagating $Ca^{2+}$ wave from the growth cone to the soma. Blocking this $Ca^{2+}$ wave abolished the slit-2-induced reversal of soma migration, and the reversal required the activity of the small GTPase RhoA, which was downregulated by either slit-2 or $[Ca^{2+}]_i$ elevation itself. In migrating neurons,

active RhoA proteins accumulated to the front of the soma, and this asymmetry was reversed during the slit2-induced reversal of neural migration. Frontal application of ryanodine, which induces $[Ca^{2+}]_i$ elevation at the soma, was sufficient to induce the reversal of neuronal migration and the redistribution of RhoA without causing the growth cone collapse. Thus, long-range $Ca^{2+}$ signaling coordinated slit-2-induced changes in motility at two distant parts of migrating neurons by regulating RhoA distribution. This work described for the first time a long-range signal from the growth cone to the soma in the guidance of neuronal migration.

### 2.3 Chemotropic guidance of cortical radial migration

The mammalian cerebral cortex has the typical laminar structure, which is essential for neurons in each layer to establish the specific input and output connections with other brain regions. The formation of the laminar structure depends on the stereotyped radial migration of newborn pyramidal neurons, which are originated from the ventricle wall but move outwardly towards the superficial layer of cortex under the guidance of radial glial fibers [59,60]. However, the mechanism behind this stereotyped radial migration is yet well understood. A potential guidance mechanism that has long been overlooked in this field is whether the radial migration of newborn neurons is guided by the gradient of diffusible factors (Figure 2) or simply driven by a mass action of newly generated neurons at the ventricular zone [61]. Yuan XiaoBing and colleagues [62] at the ION found that the radial migration of newborn cortical neurons from deep cortical layers toward the surface is guided by the descending gradient of an extracellular guidance molecule semaphorin-3A. They observed that receptors of semaphorin-3A are expressed in migrating neurons. By using *in utero* electroporation, they monitored the migration of a subpopulation of cortical neurons in the native environment and examined the effect of perturbing semaphorin-3A signaling. They observed that the conditional knockout or knockdown of co-receptors for semaphorin-3A in newborn cortical neurons impeded their radial migration accompanied with a loss of their orientation during migration. The requirement of semaphorin-3A as a chemoattractant for radial migration was further supported by time-lapse imaging of the motility of migrating neurons upon the interference of the concentration gradient of endogenous semaphorin-3A in cultured cortical slices. This is the first demonstration by *in vivo* experiments that radial migration of cortical neuron is guided by the gradient of extracellular guidance factors (Figure 2). It may help to clarify how the highly ordered laminar structure of cortex is formed.

### 2.4 PKCδ regulates cortical radial migration by stabilizing the Cdk5 activator p35

Cdk5 and its neuronal activator p35 are essential for the radial migration of newborn neurons in the developing cortex. How p35 is regulated by intracellular and extracellular signaling cascades during the cortical radial migration remains largely unknown. Yuan XiaoBing and colleagues at the ION found that PKCδ, a member of novel PKCs, is a contributing factor in the regulation of p35 by preventing its

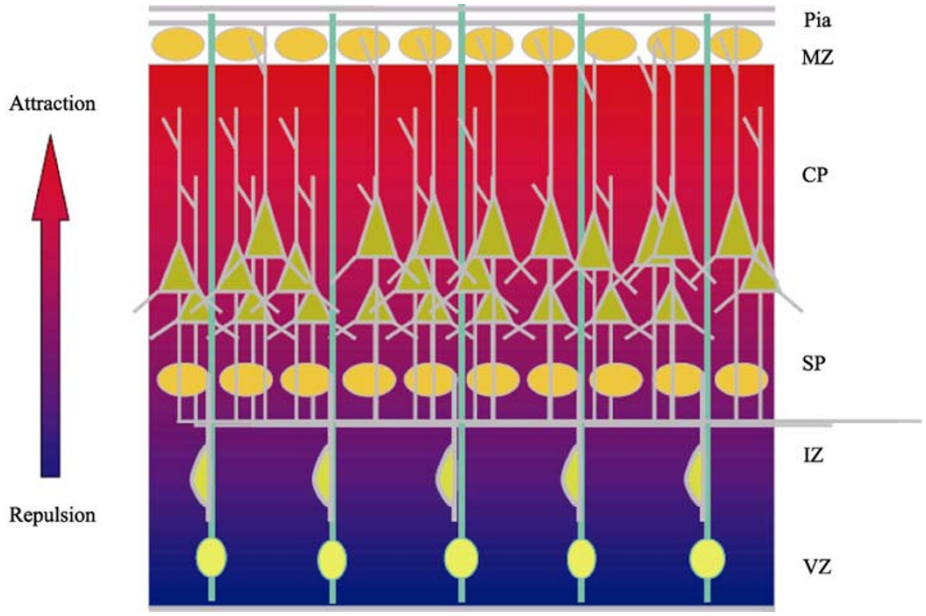


**Figure 2** A hypothesis of chemotropic guidance of cortical radial migration. The inside-out migration of newborn neurons (yellow) along the radial glial fibers (white) may be guided by the attractants (red) from the upper layers of CP and the repellents (blue) from VZ. MZ, Marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone; VZ, ventricular zone.

degradation through reversible phosphorylation on p35 [63]. PKCδ attenuates the degradation of p35 but not its mutant derivative, which could not be phosphorylated by PKCδ. Knockdown of PKCδ by *in utero* electroporation of specific siRNA severely impaired the radial migration of cortical neurons without affecting the proliferation or the survival of cortical progenitors and newborn neurons. This migration defect was similar to that caused by down-regulation of p35 and could be prevented by co-transfection with the wild-type but not the mutant p35. In cultured cortical neurons, PKCδ could be activated by the pro-migratory factor brain-derived neurotrophic factor (BDNF) through the PLC signaling pathway and is required for the activation of Cdk5 by BDNF. Moreover, both PKCδ and p35 were required for the pro-migratory effect of BDNF on cultured newborn neurons. This study has identified the first upstream regulator of p35 during the cortical radial migration.

### 2.5 SUN-domain proteins and KASH-domain proteins mediate centrosome-nucleus coupling in mammalian neurons.

Neuronal migration is associated with the coordinated advance of the centrosome with the nucleus. Although the dynein motor protein complex is known for its roles in nucleus-centrosome coupling during interkinetic nuclear migration (INM) and neuronal migration, the molecules connecting the dynein motor complex to the nuclear envelope (NE) remain unclear. Xu RenEr and Han Min and their colleagues at the Institute of Developmental Biology and Molecular Medicine, School of Life Science, Fudan University discovered that SUN-domain proteins SUN1 and SUN2, mammalian homologs of the *C. elegans* UNC-84 that has been shown to recruit KASH-domain proteins to the NE for nuclear migration and anchorage in *C. elegans*, *Drosophila*, and *Zebrafish* retina [64–66], are also crucial in the nucleokinesis process during neurogenesis and neuronal migration in the mouse brain using similar mechanisms [67]. They found that SUN1 and SUN2 redundantly formed complexes with the KASH-domain proteins Syne-2/Nesprin-2 to mediate the centrosome-nucleus coupling during both INM and radial neuronal migration in the developing cerebral cortex, and Syne-2 was connected to the centrosome through interactions with both dynein and kinesin motor complexes. These results demonstrate a conserved molecular mechanism underlying the motility of the nucleus during neuronal migration.

### 2.6 ErbB4 expression in tangentially migrating interneurons

Most GABAergic inhibitory interneurons in the forebrain are generated from embryonic ganglionic eminence and undergo the long-distance tangential migration into other brain regions, including the cerebral cortex, hippocampus, and olfactory bulb [60]. The guidance mechanism underlying this tangential migration remains largely unknown. Neuregulins (NRGs 1–4) have been identified as a family of EGF domain-containing molecules that promote cell proliferation, migration and differentiation in different tissues [68,69]. The effects of NRG have been shown to be mediated by the epidermal growth factor (EGF) receptor family member ErbB2 and ErbB4 [68,69]. Yau HauJie and colleagues at the Institute of Neuroscience, National Yang-Ming University (Taiwan, China), investigated the developmental ontogeny of the expression of ErbB4 in the dorsal telencephalon using immunohistochemistry [70]. They observed that the NRG receptor ErbB4 is preferentially expressed by interneurons migrating tangentially from the ventral to the dorsal rat telencephalon. By contrast, no ErbB4 expression was detected in Tbr1-positive glutamatergic projection neurons during the development. It is the first report concerning the critical function of NRG/ErbB4 signaling in the regulation of the tangential migration of newborn interneurons. Later studies have shown that NRG serves as an attractive guidance factor in the tangential migration of interneurons [71,72].

Other published work regarding the neuronal migration by Chinese researchers includes the identification of DCC as the essential guidance receptor for the tangential migration of noradrenergic neurons in the locus coeruleus of the mouse brain by Ding YuQiang and colleagues at the ION [73], the discovery of Shh as a repulsive factor for the migration of enteric neural crest cells by Tam KwongHang and colleagues at the University of Hong Kong (HKU) [74], and the demonstration by Xiao ZhiCheng and colleagues at the HKU of the modulation of fucosylation and sialylation of cell surface proteins and/or lipids by the adhesion molecule L1 to promote survival, neurite outgrowth, and migration in neurons [75].

## 3 Concluding remarks

In the past decade China has witnessed rapid progress in the basic research of axon guidance and neuronal migration. Further investigation will be made in the field of signal transduction mechanisms underlying the guidance of neurite extension and neuronal migration, the basic molecular machinery of the neuronal migration, and genetic mechanisms regulating the neural circuit development. These studies will help us to understand the wiring of brain circuits, and to design new diagnostic and therapeutic approaches for a wide variety of neurological disorders caused by the aberrant axon pathfinding and neuronal migration during development.

*We thank Dr. Poo Mu-Ming for careful editing of the manuscript, and Cui Kai and Chen Gang for drawing figures. This work was supported by the Innovation Project of the Chinese Academy of Sciences* (*Grant No. KSCX2-YW-R-103*)*, National Natural Science Foundation of China* (*Grant*

*No. 30625023), and the National Basic Research Program of China* (*Grant Nos. 2006CB806600 and 2006CB943903*).


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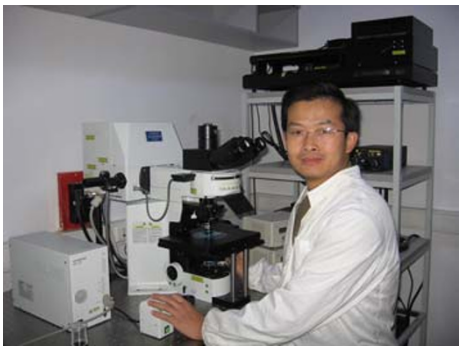
## Research Interests



YUAN XiaoBing

The central nervous system is composed of highly ordered cell architecture and precise neuronal connections, which are essential for the sophisticated functions of the brain. The development of complex neural circuits encompasses a series of critical steps, including the production of neurons from progenitor cells (neurogenesis), the determination of discrete neuronal phenotypes (neuronal differentiation), the migration of young neurons from their birth place into appropriate positions, the pathfinding of growing axons and dendrites, and the final formation of specific synaptic connections. The goal of our research is to understand how the functional neural circuits in the neocortex and cerebellum are generated. Current studies are focused on the molecular mechanisms underlying the appropriate neuronal migration and axon/dendrite pathfinding. Through combined approaches including the *in vivo* gene transfer using electroporation, *in vitro* growth cone turning assay, fluorescence imaging in cultured brain slice, genetics and pharmacology methods, we are addressing the following questions:

1. Which factors/receptors guide the radial migration of newborn neurons and determine their final lamination in the rat neocortex, and what are the underlying signaling mechanisms?

2. How is the radial scaffold in developing cortex maintained and how do newborn neurons initiate radial migration?

3. What factors control the cerebellar granule cells to migrate from the external germinal layer into the internal granule cell layer during postnatal development?

4. How is the polarity of neuronal migration reversed upon application of a frontal gradient of the repulsive factor Slit?

5.How are neurons propelled to migrate? What is the driving force for neuronal migration?