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Review

Recent advances in cerebellar granule cell migration

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Abstract. In the developing brain, postmitotic neurons exhibit dynamic changes in mode, direction, tempo and rate of migration as they traverse different cortical layers. Such changes in cell migration require orchestrated activities of multiple guidance cues and transmembrane signals. In this article, we first describe when, where and how cerebellar granule cells alter their migratory behavior during the entire course of their migration. We then present how internal (inherent) programs regulate the sequential changes in the migratory behavior of granule cells in vitro. Finally, we discuss the roles of external guidance cues and transmembrane signals in controlling granule cell migration.

Key words. Neuronal cell migration; cerebellar granule cell; intrinsic program; N-type Ca^{2+} channel; NMDA receptor; intracellular Ca^{2+} level; somatostatin.

Introduction

In the developing central nervous system, the majority of neurons migrate from their site of origin to their final destination, movement which is essential for the establishment of normal brain organization [1–5]. In the human, distinct genetic mutations and environmental toxins can affect neuronal migration, and result in abnormal cortical development [6–17]. Although the mechanisms by which these cortical abnormalities develop are still unclear [12], evidence has accumulated to indicate that the migration of neurons requires the orchestration of multiple molecular events, including the selection of a pathway, the formation of adhesive interactions, the activation of specific ion channels and receptors and the assembly and disassembly of cytoskeletal components [3, 4, 18, 19]. This review is based on studies of the migration of cerebellar granule cells. The prenatal and early postnatal cerebella have been used as a model system of neuronal cell migration, since the defined neuronal cytoarchitecture and the small number of neuronal types in the cerebellum provide an ideal system for determining cellular and molecular mechanisms of neuronal migration [18–23]. Specifically, the migration of cerebellar granule cells has been intensively examined, and cellular and molecular mechanisms underlying granule cell migration are known to be utilized during the migration of immature neurons in other brain regions [4, 24–27]. In this article, we will first describe recent studies on cortical layer-specific changes in the migratory behavior of granule cells in the early postnatal mouse cerebellum. We will then present internal (intrinsic) programs for controlling the alterations of granule cell behavior in vitro. Finally, we will demonstrate the roles of external guidance cues and signaling molecules in maintaining cell motility and in providing directional cues.

Cortical layer-specific alterations of granule cell migration in the early postnatal cerebellum

The combined use of a laser scanning confocal microscope and a fluorescent lipophilic carbocyanine dye (i.e., DiI) allows us to observe the migratory behavior of immature neurons in their natural cellular milieu [28–30].

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With this method, we found that granule cells alter their shape concomitantly with changes in the mode and rate of migration as they traverse different cortical layers [4, 19, 22, 28–30]. The cortical layer-specific changes in migratory behavior of cerebellar granule cells are schematically represented in figure 1. In this section, we will describe the translocation and transformation of cerebellar granule cells from their birthplace to their final destination.

The external granular layer

In the early postnatal cerebellum, granule cell precursors actively proliferate at the top of the external granular layer (EGL) [30–34]. After their final mitosis, the cerebellar granule cells remain in the EGL for 20–48 h before initiating their radial migration across the molecular layer (ML), but the significance of this latent period was not well understood. Recently, we found that coincident with the extension of two uneven horizontal processes oriented parallel to the longitudinal axis of the folium, the postmitotic granule cells start to migrate tangentially in the direction of the larger process [30]. Interestingly, their morphology and the speed of cell movement change systematically with their position within the EGL [30]. The rate of tangential cell movement is fastest (~14.8 μm/h) in the middle of the EGL, when the cells have two short horizontal processes. As the granule cells elongate their somata and extend longer horizontal processes at the bottom of the EGL, they move at a reduced rate (~12.6 μm/h). At the interface of the EGL and the ML where the cells migrate tangentially at the slowest rate (~4.1 μm/h), their somata round, and then begin to extend couples of the descending processes into the ML. After the stationary period, the granule cells abruptly extend a single vertical process and initiate the transition from tangential to radial migration, reshaping their rounded somata into a vertically elongated spindle [30].

The ML

In the ML, the granule cells have vertically elongated cell bodies, thin trailing processes, and more voluminous leading processes, and migrate radially along the Bergmann glial processes [4, 19, 28, 29]. Interestingly, the rates of

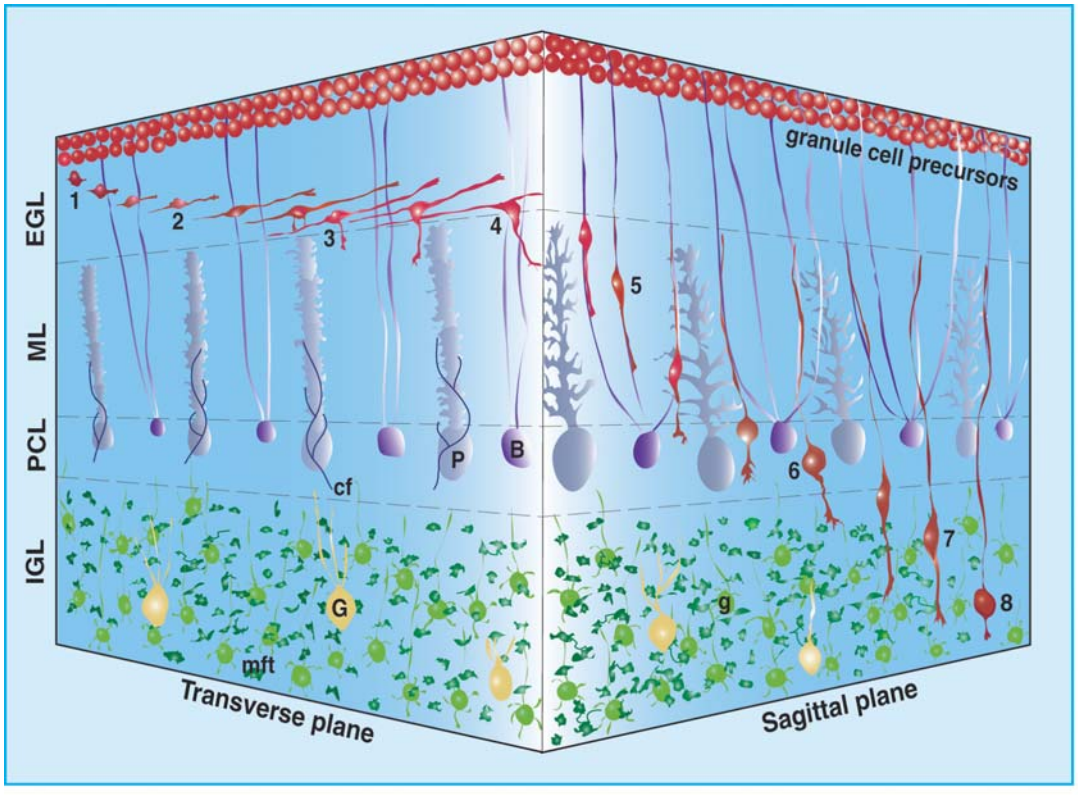

Figure 1. Three-dimensional representation of granule cell migration from the external granular layer (EGL) to the internal granular layer (IGL) in the early postnatal mouse cerebellum. 1, extension of two uneven horizontal processes near the top of the EGL; 2, tangential migration in the middle of the EGL; 3, development of vertical process near the border between the EGL and the molecular layer (ML); 4, initiation of radial migration at the EGL-ML border; 5, Bergmann glia-associated radial migration in the ML; 6, stationary state in the Purkinje cell layer (PCL); 7, glia-independent radial migration in the IGL; 8, completion of migration in the middle or the bottom of the IGL. Abbreviations: P, Purkinje cell; B, Bergmann glia; G, Golgi cell; g, postmigratory granule cell; cf, climbing fiber; mft, mossy fiber terminal.

granule cell movement in the ML depend critically on the age of the cerebellum. The average rate of cell migration in the ML increases systematically from 9.6 μm/h in postnatatal day (P)7 to 18.0 μm/h in P13 [28]. Consequently, the granule cells traverse the developing ML within a relatively constant time period despite the doubling in width of the ML during the second week of postnatal life [28]. Furthermore, granule cell movement in the ML is characterized by alternations of short stationary phases with movement in a forward or backward direction [28]. The net displacement of a cell depends on the duration and frequency of these phases as well as on the speed of movement.

The Purkinje cell layer

Once the granule cell soma enters the Purkinje cell layer (PCL), its shape transforms abruptly from a vertically elongated spindle to a sphere [29]. These rounded somata slow their movement significantly, and stop completely in the PCL [4, 29]. The rounded somata remain stationary in the PCL for an average of 115 min, with times ranging from 30 to 220 min [29]. However, highly motile lamellipodia and filopodia develop at the distal portion of the leading process, suggesting that the tips of leading processes actively search for potential guidance cues. After a prolonged stationary period, the granule cells in the PCL begin to re-extend their somata and leading processes. Furthermore, during this transformation, the granule cells gradually accelerate the rate of their migration and cross the border between the PCL and the internal granular layer (IGL) [29].

The IGL

The spindle-shaped granule cells migrate toward the bottom of the IGL at a rate comparable to that recorded for granule cells migrating along Bergmann glial fibers within the ML [4, 28, 29]. The long axis of the granule cell soma remains oriented perpendicular to the PCL-IGL boundary line during this radial migration. Interestingly, once the tip of a leading process approaches the IGL-white matter (WM) border, the granule cell soma becomes rounded [29]. The granule cell migration then slows and stops near the IGL-WM border. In the P10 mouse, the majority of granule cells complete their migration at the bottom stratum of the IGL [29].

In the P10 mouse cerebellum, the granule cells first move tangentially about 220 μm in the EGL [30], and then migrate radially about 250 μm to attain their final position in the IGL [29]. The average transit time of granule cells is 25.0 h in the EGL, 9.8 h in the ML, 5.2 h in the PCL, and 11.1 h to attain their final position in the IGL [29, 30]. Therefore, cerebellar granule cells move from the top of the EGL through the ML and the PCL to their final position in the IGL within about 2 days (average, 51 h) [23, 29, 30].

Intrinsic programs for controlling the sequential changes in granule cell migration

As described above, granule cells exhibit a distinct mode and tempo of migration in the different cortical layers of the early postnatal cerebellum. Although such changes in migratory behavior of granule cells are likely to be induced by responses to local environmental cues, the alterations of migratory behavior may also depend, at least in part, on an internal clock or intrinsic programs [35–43]. Recently, we found that isolated granule cells sequentially go through three characteristic phases of behavior and morphology without a cell-cell contact, indicating that inherent (intrinsic) mechanisms control the alterations of morphology and migratory behavior [44]. The isolated granule cells obtained from postnatal 0 to 2-day-old mouse cerebella migrate on the poly-L-lysine/laminin (20 μg/ml)-coated glass coverslip for up to ~60 h in vitro without contacting other neurons, neuronal processes or glial cells [44]. Three characteristic phases of sequential changes in granule cell behavior and morphology in vitro are as follows [44].

The first phase (PI, a period of 0–20 h in vitro)

At the early stage of PI, the granule cells with their spherical cell bodies migrate out from the explant, repeatedly change the shape of their somata from spherical to spindle and vice versa, and frequently turn to the left or right without extending leading processes (fig. 2A) [44]. At the point at which the granule cells change their direction of movement, they stop, become round, and then extend their cell bodies in the direction of the upcoming movement. Shortly after the extension, the cells resume their movement parallel to the direction of the longitudinal axis of the cell bodies. At the middle stage of PI, the granule cells repeatedly extend and withdraw short leading processes, and move at a fast rate only after the process extends fully (fig. 2B) [44]. Furthermore, the extension of a new leading process in a different direction is an essential prerequisite for changing the direction of cell movement. At the end of PI, the granule cells start to develop a new mode of turning behavior; first, the tip of the leading process turns in a new direction and then the cell body follows the changes (fig. 2C). Furthermore, the granule cells exhibit a dynamic cycle of cell advance and stationary phase every 3 h; the active cell migration lasts for approximately 2 h, and the stationary period is approximately 1 h in length.

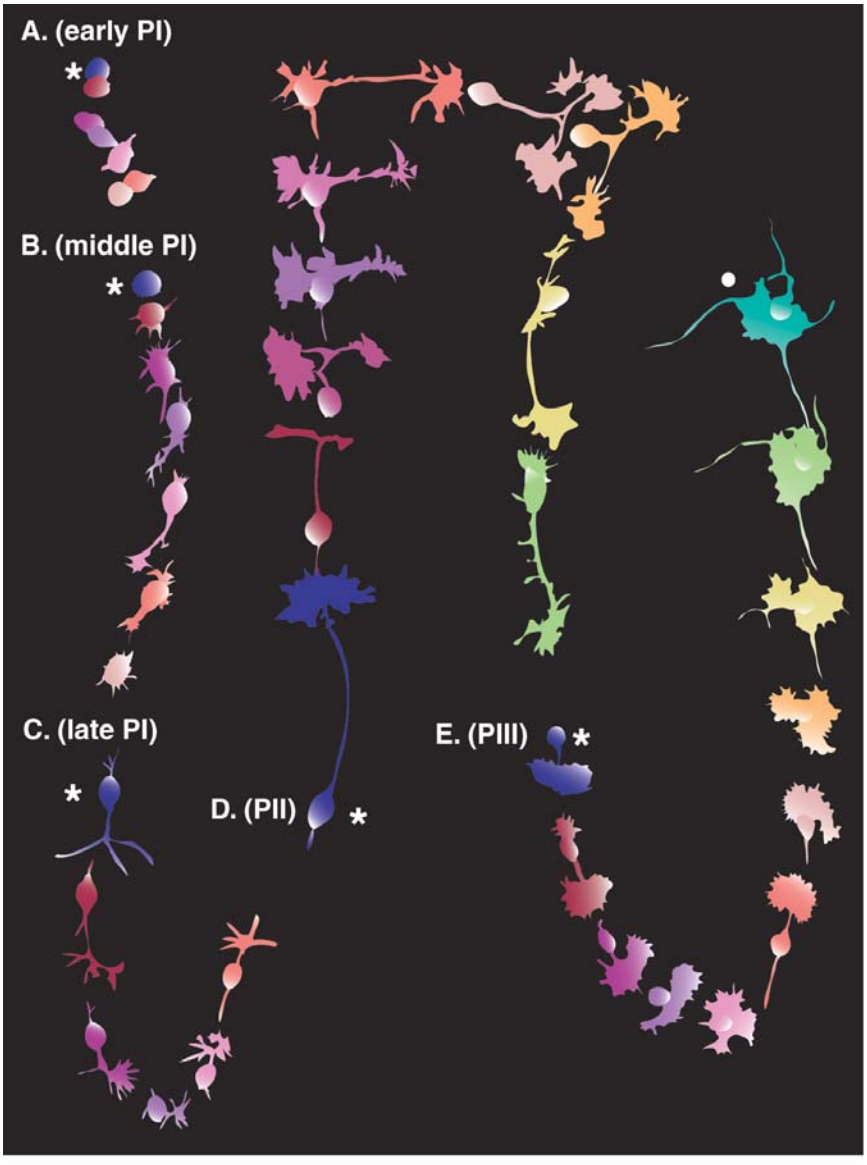

Figure 2. Schematic representation of autonomous changes in granule cell morphology and different modes of turning in vitro. Isolated granule cells go through three characteristic phases of behavior and morphology without cell-cell contact. Such alterations depend on elapsed time after an initiation of culture. (*A–C*) The first phase (PI) is a period of 0–20 h in vitro, when granule cells initiate their migration. (*D*) The second phase II (PII) is a period of 20–40 h in vitro, when granule cells have their long leading processes and move at the fastest rate. (*E*) The third phase III (PIII) is a period of 40–60 h in vitro, when granule cells terminate their migration. In the early stage of PI, undifferentiated granule cells, which do not have a leading process, alter the direction of movement by reorientation of the longitudinal axis of their somata (shown in *A*). In the middle stage of PI, granule cells withdraw their process, and then extend a new process toward the direction of upcoming movement (shown in *B*). In the late stage of PI, turning of the tip of the leading process to a new direction is followed by their somata (shown in *C*). In PII, the leading process bifurcates, and the nucleus and surrounding cytoplasm enter into one of the branches (shown in *D*). In PIII, granule cells become permanently stationary, extend a lamellipodium around the soma, and emit several thin processes (shown in *E*). White asterisks indicate first images of granule cells taken during each series of observation. A white circle indicates a postmigratory granule cell in the late stage of PIII. Pseudocolor images represent the images of the granule cells, which were taken approximately every 30 min.

The second phase (PII, a period of 20–40 h in vitro)
At the early stage of PII, the granule cells develop another mode of turning behavior as follows: (i) the tip of the leading process bifurcates, (ii) both branches extend in the opposite direction, (iii) one of the branches collapses and retracts, (iv) the cell body follows the direction of extension of the remaining branch (fig. 2D) [44]. Granule cells exhibit this mode of turning behavior throughout PII. The intrinsic changes in turning behavior may play a role in the alteration of direction of granule cell movement observed near the EGL-ML border in vivo. At the late stage of PII, the granule cells become stationary for 2–3 h and retract their processes, suggesting that the prolonged stationary state of granule cells in the PCL may be controlled, at least in part, by intrinsic signals.

The third phase (PIII, a period of 40–60 h in vitro)
At the early stage of PIII, the granule cells start to exhibit the initial signs of termination of migration, which is a morphological change of the leading process [44]. At the late stage of PIII, the granule cells slow their movement, and slightly increase the turning number. At the end, the cells become permanently stationary, extend a lamellipodium around the soma, and emit several thin processes (fig. 2E). The majority of granule cells terminate their migration 50–60 h after the initiation of their movement without external cues [44]. This 50 to 60-h term is comparable to the time required for granule cells to migrate from the EGL to the IGL in vivo [29, 30], suggesting that internal programs may be involved in the termination of granule cell migration in vivo.
The relationship between the migratory behavior of granule cells, their morphology and the elapsed time after an initiation of cultures also indicates intrinsic programs for cell migration [44]. In PI, the granule cells migrate at an average rate of 26.0 μm/h and exhibit the highest rate of turning behavior (1.3 turns/h), when the cells have multiple (3.7 processes/cell) and short (20.8 μm) processes. The length of the cycle of cell movement and stationary state is shortest (218 min). In PII, the granule cells extend a long and thick leading process-like process (55.6 μm), and exhibit an elongated cycle (244 min) of cell movement and stationary state. The rate of cell movement is fastest (33.1 μm/h), while the number of turnings is lowest (0.3 turns/h). In PIII, the granule cells slow their movement (25.2 μm/h), but slightly increase the turning number (0.5 turns/h). The length of the cycle of cell movement further increases to 297 min.
If there is a strong intrinsic program for controlling migratory behavior, isolated granule cells may exhibit similar characteristic phases of migration in different adhesive substrates and in different concentrations of laminin. Furthermore, granule cells isolated from different postnatal stages may also undergo the same patterns of changes in migratory behavior. To address these issues, we determined the migratory behavior of isolated granule cells in a fibronectin (20 μg/ml) and in a lower (4 μg/ml) or higher (100 μg/ml) concentration of laminin [44]. Moreover, we observed sequential changes of granule cell migration in the cultures prepared from postnatal 5-day-old mouse cerebella [44]. Although there are some differences in the rates of cell behavior among the four different experimental groups, the majority of isolated granule cells display the same patterns of behavioral and morphological changes during the three characteristic phases in isolation as follows: (i) the rate of cell movement in PII is fastest, (ii) the rate of turning behavior in PI is highest, (iii) the length of cycle of cell movement systematically increases as time in vitro increases, (iv) the length of the stationary state in PII is longest, (v) the number of processes is largest in PI, and (vi) the length of process in PIII is longest. These results demonstrate the existence of intrinsic (inherent) programs for controlling migratory behavior of granule cells in an age- or a developmental stage-dependent manner. These results suggest that such internal programs may play a crucial role in distinct modes of granule cell migration in different cortical layers of the developing cerebellum in vivo.
How do these intrinsic changes in migration in vitro compare to those observed in vivo? The comparison suggests distinctive roles of intrinsic signals and external guidance molecules in controlling granule cell migration in vivo [28–30, 44]. For example, although in PI (0–20 h in vitro) isolated granule cells most frequently turn left or right, the cells in the EGL in vivo migrate tangentially and do not alter the direction of cell movement until 20 h after the initiation of migration [30], suggesting that localized external cues or cell-cell contacts suppress the intrinsic turning activity of granule cells in the EGL. In PII (20–40 h in vitro), granule cells have two long processes and move at the fastest rate. Likewise, in the ML in vivo (20–30 h after the initiation of migration), granule cells have a long leading process and a trailing process and move radially at an increased rate [28, 29]. This similarity suggests that the changes in migratory behavior observed in the ML may be regulated, at least in part, by intrinsic programs. Furthermore, in PIII (40–60 h in vitro), granule cells terminate their migration without cell-cell contact and start to express the $\alpha 6$ subunit of $GABA_A$ receptors, which are expressed only when the cells arrive in the IGL in vivo [45, 46], suggesting that granule cells in PIII may be in a similar stage of differentiation as those in the IGL [44]. The time schedule for completion of migration in vitro is quite similar to that for granule cell migration in vivo [29, 30, 44]. This similarity indicates that an internal program (or clock) may be involved in determining the term of cell migration.
Granule cells exhibit distinct rates of cell movement while they migrate in different cortical layers in vivo [28–30].

Although these alterations of the rate in vivo are likely due to different modes of migration (glia associated or glia independent) and to differences in microenvironments [18, 21], sequential changes in the rate without cell-cell contact in vitro suggest that granule cells may intrinsically change their speed in vivo. The mechanisms underlying changes in the rate of cell movement in vitro and in vivo remain to be examined, but there is a possible scenario. In the in vitro studies, granule cells migrate on laminin, which promotes frequent and rapid migration among extracellular matrix molecules [47–49]. Importantly, the speed of cell movement depends on several variables related to receptor-ligand interactions, including ligand (such as laminin) levels, receptor (such as integrin) levels and receptor-ligand binding affinities [50]. For example, the laminin concentration promoting the maximum migration speed decreases reciprocally as integrin expression increases. On the other hand, increases in integrin-ligand affinity similarly result in maximum migration at reciprocally lower ligand concentrations [50]. Therefore, if granule cells alter the levels of receptors for extracellular matrix and cell adhesion molecules, the rate of cell movement may change even though their microenvironments are the same. Interestingly, granule cells sequentially express different complements of genes that encode for receptors for extracellular matrix and cell adhesion molecules along their migratory pathway in vivo [3, 51]. These lines of evidence, as a whole, suggest that changes in the speed of granule cell migration in vitro and in vivo may be explained, at least in part, by alteration in levels of receptors for extracellular matrix and cell adhesion molecules. However, exact levels of these receptors in vitro and in vivo remain to be determined.

The sequential expression of cytoskeletal components may also be involved in inherent changes in behavior and morphology, since granule cells are highly polarized in the direction of migration, and spatial and temporal changes in the cytoskeleton are required for alteration of orientation and cell movement [52–55]. The leading processes and trailing processes of granule cells exhibit a distinct orientation of cytoskeletal components [56], and express cytoskeletal components in the following order: (i) actin-containing microfilaments, (ii) microtubules, and (iii) neurofilaments [57]. These lines of evidence suggest that a genetically programmed order of expression of cytoskeletal components may be crucial for alterations of migratory behavior of granule cells and their morphology.

After establishing that cerebellar granule cells exhibit cortical layer-specific changes in migratory behavior *in vivo* and that sequential alterations of migratory behavior of granule cells in vitro depend, at least in part, on intrinsic programs, we discuss in the following sections how signaling molecules and external guidance cues participate in controlling the layer-specific changes in granule cell migration in the developing brain.

Intracellular signaling, transmembrane signaling, cell adhesion and external guidance cues in cortical layer-specific alterations of granule cell migration

To date, large numbers of molecules and genes have been discovered as potential regulators or modulators of neuronal cell migration [3–5]. Some of these are involved in modifying the migration of specific types of neuron, while others provide signals controlling the migratory behavior of many different types of neuron. To understand the cellular and molecular mechanisms underlying granule cell migration, we selected several signaling molecules and genes, which are known to play crucial roles in controlling granule cell migration. In this section, we will describe the distinct roles of the molecules and genes in cortical layer-specific changes in granule cell migration one by one.

Voltage-dependent Ca^{2+} channels

The combined use of acute slice preparations and pharmacological tools reveals the role of voltage-gated Ca^{2+} channels, especially the N-type Ca^{2+} channel, in granule cell migration [4, 58]. The postmitotic granule cells at the middle and the bottom of the EGL start to express N-type Ca^{2+} channels prior to the initiation of their migration [58]. The number of N-type Ca^{2+} channels on the plasmalemmal surface of granule cells rapidly increases during the translocation of the cell soma from the EGL to the IGL [58]. Importantly, blockade of N-type Ca^{2+} channel activity by a specific antagonist, ω-conotoxin GVIA, significantly reduces the rate of granule cell migration in the ML of the postnatal mouse cerebellum, while blocking other types of voltage-gated Ca^{2+} channels, such as L- or T-type Ca^{2+} channels, has no significant effect [58]. Likewise, blockade of the voltage-sensitive Na^{+} channels fails to alter the rate of granule cell migration [58]. These results suggest that the activity of N-type Ca^{2+} channels plays a critical role in controlling the rate of granule cell movement in the early postnatal cerebellum.

The activity of voltage-gated Ca^{2+} channels also directs the migration of immature neurons in the nematode *Caenorhabditis elegans* [59]. The *C. elegans unc-2* gene encodes a protein with a high degree of sequence similarity to the α1 subunits of vertebrate voltage-activated Ca^{2+} channels [60]. The UNC-2 amino acid sequence suggests that its closest vertebrate homologues are the non-L-type family of high-threshold Ca^{2+} channels, a group that includes the N-type and P/Q-type Ca^{2+} channels [59]. Interestingly, in mutants carrying loss-of-function alleles of the Ca^{2+} channel gene *unc-2*, the touch receptor neuron (AVM) and the interneuron (SDQR) fail to migrate an adequate distance and often migrate in the wrong direction, leading to misplacement of their cell bodies [59]. However, the AVM neurons in *unc-2* mutant animals ex-

tend axons in a wild-type pattern, suggesting that the UNC-2 Ca^{2+} channel specifically directs migrations of the neuronal cell body and is not required for axonal pathfinding [59].

N-methyl-D-aspartate subtype of glutamate receptors

The presence of spontaneous activity of the N-methyl-D-aspartate (NMDA) receptors on the surface of migrating cerebellar granule cells has been confirmed by patch-clamp analysis [61]. The frequency of spontaneous NMDA receptor-coupled channel activity is low in the middle and the bottom of the EGL, but high in the ML [61]. Migrating granule cells co-express the NR1 and NR2A or NR2B subunits of the NMDA receptor, whereas postmigratory cells in the IGL express the NR1 and NR2C types [62, 63]. Most importantly, blocking NMDA receptor activity with its antagonists significantly decreases the rate of granule cell movement in the ML [4, 24]. In contrast, the rate of cell migration is not substantially altered by blocking the activity of non-NMDA receptors (i.e., kainate and AMPA receptors), $GABA_A$ receptors and $GABA_B$ receptors [24]. The role of the NMDA receptor in granule cell migration is further supported by evidence that changes in Mg^{2+} or glycine concentration affect the rate of cell movement [24]. Because extracellular Mg^{2+} blocks NMDA receptor activity in a voltage-dependent manner [64] and application of glycine potentiates NMDA receptor activity [65], they are expected to influence cell migration. Indeed, the removal of Mg^{2+} from the medium significantly increases the rate of granule cell movement in the ML [24]. In contrast, the rate of cell movement is reduced in a high-Mg^{2+} medium. Likewise, the application of 10 μM glycine significantly increases the rate of cell movement in the ML. These results suggest that the activity of NMDA receptors controls the rate of granule cell movement [4, 24].

The question as to how the NMDA receptors expressed by migrating granule cells could be activated is intriguing, because the migrating granule cells do not form synapses before the completion of their translocation in the IGL [66]. One possibility is that endogenous extracellular glutamate may activate the immature form of the NMDA receptor by nonsynaptic means. Interestingly, the elevation of extracellular glutamate concentrations by inhibiting glutamate uptake by astrocytes increases the frequency of spontaneous NMDA receptor-coupled channel activity [61], and significantly accelerates the rate of granule cell movement in the ML [24]. These results suggest that endogenous glutamate may be an important signal for the activation of NMDA receptors and that the increase in extracellular glutamate could enhance the rate of cell migration until the concentration reaches a toxic level [67].

The NMDA receptors also play a role in controlling the migration of immature neurons in the embryonic cerebrum. For example, the activation of NMDA receptors stimulates the migration of neurons in the embryonic cerebral cortex [26], while the blockade of the NMDA receptors inhibits cortical cell migration [27]. Although the mutation of the NR1 subunit of NMDA receptors does not significantly affect the distribution of neurons in the cerebral cortex, implying normal rates and routes of migration, the cortical neurons in NMDA receptor mutant mice may have acquired compensatory mechanisms for cell migration [68].

Intracellular Ca^{2+} fluctuations

Transient elevations of intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) are essential for initiating and maintaining the movement of cells ranging in type from fibroblasts to immature glial cells [69–74]. For example, an increase in the rate of Schwann cell migration is correlated with a rise in $[Ca^{2+}]_i$ [74]. Likewise, migrating human neutrophils exhibit multiple increases and decreases in $[Ca^{2+}]_i$ [75]. The buffering of $[Ca^{2+}]_i$ or removal of extracellular Ca^{2+} blocks transient increases in $[Ca^{2+}]_i$ in neutrophils and, thus, reduces or inhibits cell migration [75]. Monitoring intracellular Ca^{2+} levels using Ca^{2+} indicator dyes demonstrates that migrating granule cells exhibit dynamic changes in $[Ca^{2+}]_i$ of the cell body [4, 76]. The average amplitude of $[Ca^{2+}]_i$ elevations is 14% of the baseline intensity of Fluo-3, and the average duration is 1.3 min [76]. Transient elevations of $[Ca^{2+}]_i$ occur 4–24 times/h, with average frequencies of 13/h. There is a clear positive correlation between the rate of cell movement and both the amplitude and frequency components of Ca^{2+} fluctuations. The correlation coefficient between the rate of cell movement and the amplitude of Ca^{2+} fluctuations is 0.74, and that of the rate of cell movement and the frequency of Ca^{2+} fluctuations is 0.83 [76]. Importantly, the reduction of Ca^{2+} influx by lowering extracellular Ca^{2+} concentrations or by blocking Ca^{2+} channels or NMDA receptors results in a decrease in the amplitude and frequency of spontaneous Ca^{2+} elevations [76]. This reduction is linearly related to the rate of cell movement. Taken together, these results indicate that the combination of the amplitude and frequency components of intracellular Ca^{2+} fluctuations provide an intracellular signal controlling granule cell migration.

The questions of how transient elevations of $[Ca^{2+}]_i$ control cell motility remain to be examined. One possibility is that fluctuations of $[Ca^{2+}]_i$ regulate the dynamic assembly and disassembly of cytoskeletal elements required for the operation of a force-generating mechanism involved in cell movement [56]. Furthermore, elevations of $[Ca^{2+}]_i$ in neutrophils lead to the disruption of specific sites of attachment to an adhesive substratum [75]. Changes in $[Ca^{2+}]_i$ in migrating neurons may modulate the repetitive

formation and elimination of binding sites between migrating neurons and their migratory substrates. Intracellular calcium may control conformational changes of cell adhesion molecules, such as integrins, which are expressed on the plasma membrane of migrating neurons [48, 77–79].

Brain-derived neurotrophic factor

Cerebellar granule cells express brain-derived neurotrophic factor (BDNF) and its high-affinity receptor (TrkB) [80–82]. The level of BDNF expression increases during early postnatal development [80, 81]. In vitro, *Bdnf*$^{-/-}$ granule cells initiate migration poorly and exogenous BDNF induces both wild-type and *Bdnf*$^{-/-}$ cells to migrate [83]. In vivo, a lack of BDNF results in impaired migration of granule cells [83].

Contradictorily, BDNF has been reported to significantly increase neurite length and the speed of growth cone migration of granule cells, but has no effect on the speed of granule cell body movement [84].

Neurotrophin-3

In the prenatal cerebellum neurotrophin-3 (NT-3) mRNA expression begins with the emergence of the EGL [85]. During postnatal development, there is a large transient increase in NT-3 mRNA expression in the cerebellum from P5 to P20 [86]. Both granule cell precursors in the EGL and differentiated granule cells in the IGL express its high-affinity receptor (TrkC) [80, 82, 86, 87]. Although mice with a single targeted gene deletion for *trkC* show no large-scale cerebellar abnormality [88], chronic application of exogenous NT-3 results in a significant reduction of the thickness of the EGL, but does not affect cell death, suggesting that NT-3 accelerates the exit of postmitotic granule cells from the EGL [89].

Neuregulin

In the developing cerebellum, granule cells express neuregulin (NRG), and radial glial cells express its receptor, erbB4 [90]. When the glial erbB4 receptors are blocked, the granule cells fail to induce radial glia formation, and their migration along radial glial fibers is impaired [90]. Moreover, soluble NRG is as effective as neuron-glia contact in the induction of radial glia formation. These results suggest that NRG and erbB4 play a role in the migration of cerebellar granule cells along radial glial fibers in the developing ML [90].

Stromal cell-derived factor 1α

In the prenatal and early postnatal cerebellum, stromal cell-derived factor 1α (SADF-1α) expression is restricted to the pial membrane, while granule cell precursors and immature granule cells express its receptor CXCR4 [91, 92]. Importantly, the deletion of SDF-1α or CXCR4 leads to the premature migration of granule cell precursors away from the proliferative zone of the EGL [91, 93]. Small numbers of granule cell precursors are found ectopically outside the EGL of SDF-1α- or CXCR4-deficient mice [91, 93]. Furthermore, SDF-1α induces chemotactic responses in granule cell precursors and enhances granule cell proliferative responses to Sonic hedgehog in vitro [94]. These results, as a whole, suggest that SDF-1α and CXCR4 play a crucial role in retaining granule cell precursors and granule cells in the EGL. In particular, SDF-1α would prevent inward migration of CXCR4-positive granule cells by chemoattracting them toward the pia mater.

Ephrin-B2

Although the expression of SDF-1α or CXCR4 persists during the early postnatal cerebellum, postmitotic granule cells initiate their migration toward the IGL. This raises a question: how do granule cells break away to migrate inward? Recently, Flanagan and his colleagues found possible molecular mechanisms that answer this question [95]. Ephrin-B2 is expressed in the EGL at P3 [95]. Its receptor EphB2 is also found in the EGL, with weak expression at P0 and strong expression at P3 [95]. Therefore, ephrin-B2 and EphB2 receptor are strongly expressed in the EGL prior to an initiation of inward migration of postmitotic granule cells. Most importantly, the chemoattractant effect of SDF-1α to the granule cells is selectively inhibited by soluble EphB2 receptor through reverse signaling of ephrin-B2 [95]. These results suggest that when granule cells are ready to migrate, they would lose responsiveness to SDF-1α. Such changes in responsiveness to SDF-1α could be mediated at least in part by EphB2 receptor and ephrin-B2.

Astrotactin

Astrotactin has been isolated as a neuronal ligand for migration on glial fibers [3, 5, 21, 48, 49]. The cerebella of astrotactin null mice are approximately 10% smaller than wild type [96]. In vivo and in vitro cerebellar granule cell assays show a decrease in neuron-glial binding and a reduction in the rate of granule cell migration in astrotactin null mice, suggesting that astrotactin functions in granule cell migration along glial processes in the ML [48, 96].

Tenascin

The extracellular matrix molecule tenascin has been implicated in neuron-glia recognition in the developing central and peripheral nervous system [97–100]. Schachner and colleagues demonstrated that tenascin influences neu-

rite outgrowth and the migration of cerebellar granule cells by different domains in the fibronectin type III homologous repeats [101].

Tissue plasminogen activator

In the developing cerebellum, granule cells turn on the gene for tissue plasminogen activator (tPA) as they begin their migration into the ML [102]. Granule cells both secrete tPA, an extracellular serine protease that converts the proenzyme plasminogen into the active protease plasmin, and bind tPA to their cell surface. Interestingly, mice lacking the tPA gene ($tPA^{-/-}$) have greater than two-fold more migrating granule cells in the ML during a period of active cell migration [103, 104]. A real-time analysis of cell movement in vitro reveals that granule cells in $tPA^{+/+}$ mice migrate at an average rate of 7.3 μm/h compared with rates of 3.7 μm/h in $tPA^{-/-}$ mice [105]. Thus, granule cells in $tPA^{-/-}$ mice migrate through the ML at about half the rate measured for migration of granule cells in $tPA^{+/+}$ mice [105]. These findings suggest that tPA gene expression is required for maintaining the maximal rate of granule cell migration during cerebellar development.

Platelet-activating factor

Platelet-activating-factor (PAF) has been implicated in the human neuronal migration disorder Miller-Dieker lissencephaly [106, 107]. In this disorder, the brain has a smooth cortical surface (lissencephaly) caused by a lack of outer cortex complexity [17, 106, 107]. The brains of these patients manifest a disruption of the migration of cerebellar granule cells [106, 108]. This brain malformation results from a haploinsufficiency of the gene *LIS-1* [109–111], which encodes a 45-kDa subunit of a brain PAF acetylhydrolase, an enzyme that converts PFA to the inactive lyso-PAF by removing the acetyl group on the *sn2* position of the PFA molecule [112, 113]. PAF receptor activation evokes changes in the neuronal cytoskeleton leading to growth cone collapse and neurite withdrawal, suggesting that similar PAF receptor-mediated changes in the cytoskeleton of immature neurons could lead to a disruption of neuronal migration [114]. Bix and Clark [115] found that the application of the nonhydrolyzable PAF receptor agonist methyl carbamyl PAF (mc-PAF) yields a dose-dependent decrease in cerebellar granule cell migration. This effect can be blocked by PAF receptor antagonists [115]. Although mc-PAF minimally inhibits neurite outgrowth, its primary effect is on the movement of granule cell somata [115]. Taken together, these results suggest that the stimulation of neuronal PAF receptors could be one critical stem for the regulation of cerebellar granule cells as well as immature cortical neurons.

Cyclin-dependent kinase 5

Cyclin-dependent kinase 5 (Cdk-5) is one unique member of the cyclin-dependent kinases [116, 117]. Unlike other cyclin-dependent kinases, which are known to control cell cycle in eukaryotes, Cdk-5 expression and kinase activity are not high during cell division. The appearance of active Cdk-5 is correlated with the cessation of neurogenesis and the beginning of differentiation of neuronal cells in the developing brain [118]. To elucidate the role of Cdk-5 during postnatal cerebellar development, Ohshima and colleagues generated a series of $cdk5^{-/-}$-$cdk5^{+/+}$ chimeric mice, because $cdk5^{-/-}$ mice die around birth and much cerebellar development occurs postnatally [119–121]. In the $cdk5^{-/-}$-$cdk5^{+/+}$ chimeric cerebella of 2- to 3-month-old mice, significant numbers of granule cells are located in the ML, suggesting a failure to complete migration from the EGL to the IGL [121]. In fact, the granule cells found within the ML of chimeric cerebella are nearly all Cdk5 deficient [121]. In contrast, the granule cells within the IGL are a mixture of $cdk5^{-/-}$ and $cdk5^{+/+}$ cells [121]. These results indicate that the block in cell migration that leaves many granule cells stranded within the ML is intrinsic to $cdk5^{-/-}$ cells, suggesting that Cdk-5 may play crucial roles in the execution of cerebellar granule cell migration.

9-O-acetyl GD3

9-O-acetyl GD3 is a ganglioside, and is expressed in the developing nervous system during periods of neuronal migration [122]. Electron microscopic analysis reveals that 9-O-acetyl GD3 is localized at the contact sites between migrating granule cells and Bergmann glial processes in the EGL and ML [122]. Furthermore, the application of Jones monoclonal antibody, which recognizes 9-O-acetyl GD3, blocks the migration of cerebellar granule cells in a dose-dependent manner [122]. These results suggest that 9-O-acetyl GD3 is involved in glia-associated migration of cerebellar granule cells in the developing ML.

Somatostatin

Somatostatin, a neuropeptide, has two bioactive products, somatostatin-14 (SST-14) and somatostatin-28 (SST-28), which is a congener of SST-14 extended at the N-terminus [123–126]. Five somatostatin receptors (SSTRs) have been cloned and named SSTR1–5 according to their order of identification [127–131]. Both SST-14 and SST-28 bind to all five SSTRs. The SSTR2 gene displays a cryptic intron at the 3′ end of the coding segment, which gives rise to two spliced variants, a long (SSTR2A) form and a short (SSTR2B) form [132, 133]. The activation of somatostatin receptors elicits their cellular responses through G-protein-linked modulation of multiple second-messenger systems including adenylyl cyclase, Ca^{2+} and

K^+ channels, Na^+/H^+ antiporter, guanylate cyclase, phospholipase C, phospholipase A2, MAP kinase, and protein tyrosine phosphatase [134–145]. SST and its analog octreotide act as chemoattractants for primitive ($CD34^+$) hematopoietic cells [146]. In contrast, SST attenuates thrombin-stimulated migration of fibroblasts [147].

SST is expected to play a critical role in neurogenesis or neural differentiation, because numerous brain regions, including the cerebral cortex, cerebellum, hippocampus, brain stem and spinal cord, exhibit high levels of somatostatin and its receptor expression early in development, followed by a decrease to adult levels [148–153]. However, little is known about the function of somatostatin in the developing brain, although in an adult brain, somatostatin functions as a neurohormone, a neurotransmitter and a neuromodulator [154–158]. Recently, we found that endogenous somatostatin regulates the migratory behavior of cerebellar granule cells in a cortical layer-specific manner [46]. Postmitotic granule cells express all five types of SSTR before an initiation of migration, while differentiated granule cells in the adult do not express the receptors [159, 160]. High levels of somatostatin are present along the migratory route of granule cells and in their final destination [46, 161, 162]. For example, during periods of granule cell migration, SST-14 is present in Purkinje cells, Golgi cells and climbing fibers [163, 164], and SST-28 is present in Golgi cells and mossy fiber terminals [46]. To determine whether somatostatin controls the migratory behavior of granule cells, we used cerebellar slice preparations obtained from P10 mice [28–30]. Most importantly, the addition of 1 μM of SST-14 or SST-28 to the medium significantly *increases* the rate of granule cell movement in the EGL, slightly *decreases* the rate in the ML and significantly *decreases* the rate in the IGL [46]. In contrast, the addition of a somatostatin antagonist, AC-178,335 (10 μM) [165] to the medium significantly *decreases* the rate of granule cell migration in the EGL, slightly *increases* the rate in the ML and significantly *increases* the rate in the IGL [46]. Taken together, these results demonstrate that endogenous somatostatin differentially controls the migration of granule cells in the EGL, the ML and the IGL. Somatostatin accelerates the tangential movement of granule cells near the birthplace within the EGL, but significantly slows down radial movement near their final destination within the IGL.

Next, to determine whether somatostatin acts directly on migrating granule cells or other cells, which then indirectly influence granule cell migration, we used the microexplant cultures of P0–P2 mouse cerebella [44, 76]. The application of 1 μM SST-14 or SST-28 significantly *increases* the rate of cell movement at 1 day in vitro, while SST-14 or SST-28 substantially *decrease* the rate at 2 days in vitro [46]. These results suggest that somatostatin acts directly on migrating granule cells in a stage-specific manner. Furthermore, we monitored changes in $[Ca^{2+}]_i$ before and after the application of somatostatin. SST-14 (1 μM) increases the size and frequency of Ca^{2+} fluctuations of granule cells at 1 day in vitro, whereas it eliminates the spike-like Ca^{2+} transients at 2 days in vitro [46]. An increase in the rate of cell movement follows the enlargement of Ca^{2+} fluctuations at 1 day in vitro, while the elimination of Ca^{2+} fluctuations at 2 day in vitro decreases the rate [46]. These results suggest that the differential effects of somatostatin at 1 day in vitro and 2 days in vitro on $[Ca^{2+}]_i$ might explain, at least in part, how somatostatin switches its effect on granule cell migration from acceleration at the early phase of migration to slowdown at a late phase of migration.

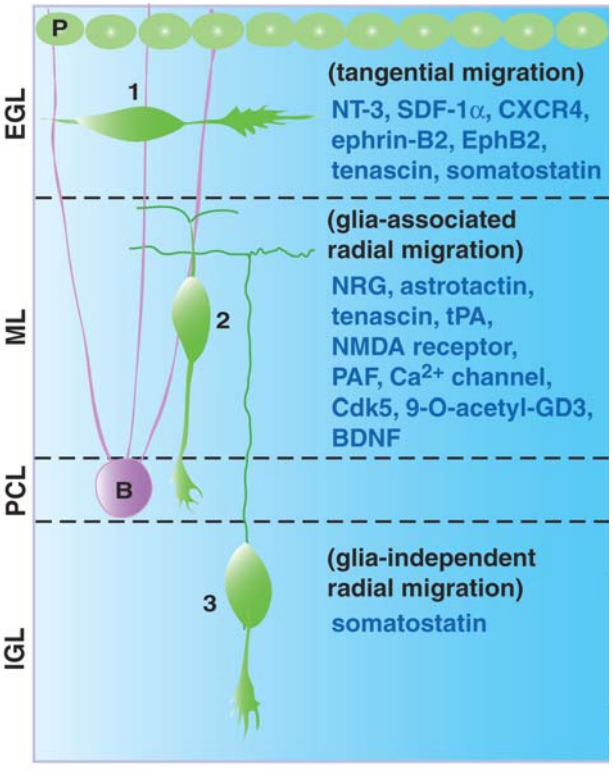

Figure 3. Transmembrane signals, cell adhesion molecules and external guidance cues involved in the control of the cortical layer-specific changes in migratory behavior of cerebellar granule cells in vivo. 1, tangentially migrating granule cells in the EGL; 2, radially migrating granule cells along the process of the Bergmann glial fiber; 3, radially migrating granule cells in the IGL. Abbreviations: P, granule cell precursor; B, Bergmann glia.

The transmembrane signals, cell adhesion molecules and external guidance cues involved in the control of the cortical layer-specific changes in granule cell migration are summarized in figure 3.

Concluding remarks and prospects

Position-specific changes in migratory behavior of cerebellar granule cells require the orchestrated activity of

multiple molecules and signaling systems, including Ca^{2+} channels, NMDA receptors, intracellular Ca^{2+} fluctuations, BDNF, NRG, SDF-1α, ephrin-B2, EphB2 receptor, astrotactin, tenascin, tPA, PAF, cdk5, 9-O-acetyl GD3 and somatostatin. However, little is known about how these molecules interact with each other during the translocation of granule cells and which signaling cascades are activated by these molecules. These questions should be answered in the future. Furthermore, the isolation of undiscovered extracellular guidance molecules responsible for layer-specific changes in neuronal migration remains a continuing challenge. In particular, molecular mechanisms underlying the changes in the direction of granule cell movement from tangential to radial near the EGL-ML border remain to be elucidated. Moreover, the question of which local environmental cues induce the completion of granule cell movement in the IGL will be addressed in the future. Although this review focuses on the signaling mechanisms and external guidance cues related to the control of cerebellar granule cell migration, we are confident that the information obtained from granule cell migration can apply to neuronal migration in other brain areas with some modifications.

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1 Sidman R. L. and Rakic P. (1973) Neuronal migration, with special reference to developing human brain: a review. Brain Res. **62:** 1–35
2 Rakic P. (1990) Principles of neuronal cell migration. Experientia **46:** 882–891
3 Hatten M. E. and Heintz N. (1995) Mechanisms of neural patterning and specification in the developing cerebellum. Annu. Rev. Neurosci. **18:** 385–408
4 Komuro H. and Rakic P. (1998) Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular Ca^{2+} fluctuations. J. Neurobiol. **37:** 110–130
5 Hatten M. E. (1999) Central nervous system neuronal migration. Annu. Rev. Neurosci. **22:** 511–539
6 Miller M. W. (1986) Effects of alcohol on generation and migration of cerebral cortex neurons. Science **233:** 1308–1311
7 Chevassus-au-Louis N. and Represa A. (1999) The right neuron at the wrong place: biology of heterotopic neurons in cortical neuronal migration disorders, with special reference to associated pathologies. Cell. Mol. Life Sci. **55:** 1206–1215
8 Ross M. E., Allen K. M., Strivastava A. K., Featherstone T., Gleeson J. G., Hirsch B. et al. (1997) Linkage and physical mapping of X-linked lissencephaly/SBH (XLIS): a gene causing neuronal migration defects in human brain. Hum. Mol. Genet. **6:** 555–562
9 Allen K. M. and Walsh C. A. (1999) Genes that regulate neuronal migration in the cerebral cortex. Epilepsy Res. **36:** 143–154
10 Walsh C. A. (2000) Genetics of neuronal migration in the cerebral cortex. MRDD Res. Rev. **6:** 34–40
11 Gressens P., Baes M., Leroux P., Lombet A., Van Veldhoven P., Janssen A. et al. (2000) Neuronal migration disorder in Zellweger mice is secondary to glutamate receptor dysfunction. Ann. Neurol. **48:** 336–343
12 Flint A. C. and Kriegstein A. R. (1997) Mechanisms underlying neuronal migration disorders and epilepsy. Curr. Opin. Neurol. **10:** 92–97
13 Copp A. J. and Harding B. N. (1999) Neuronal migration disorders in humans and in mouse models – an overview. Epilepsy Res. **36:** 133–141
14 Lammens M. (2000) Neuronal migration disorders in man. Eur. J. Morphol. **38:** 327–333
15 Uher B. F. and Golden J. A. (2000) Neuronal migration defects of the cerebral cortex: a destination debacle. Clin. Genet. **58:** 16–24
16 Gleeson J. G. (2001) Neuronal migration disorders. MRDD Res. Rev. **7:** 167–171
17 Barth P. G. (1987) Disorders of neuronal migration. Can. J. Neurol. Sci. **14:** 1–16
18 Rakic P., Cameron R. S. and Komuro H. (1994) Recognition, adhesion, transmembrane signaling and cell motility in guided neuronal migration. Curr. Opin. Neurobiol. **4:** 63–69
19 Rakic P. and Komuro H. (1995) The role of receptor/channel activity in neuronal cell migration. J. Neurobiol. **26:** 299–315
20 Rakic P. (1971) Neuron-glia relationship during granule cell migration in developing cerebellar cortex: a golgi and electron microscopic study in *Macacus rhesus*. J. Comp. Neurol. **141:** 283–312
21 Hatten M. E. and Mason C. A. (1990) Mechanisms of glial-guided neuronal migration in vitro and in vivo. Experientia **46:** 907–916
22 Komuro H. and Rakic P. (1999) In vitro analysis of signal mechanisms involved in neuronal migration. In: The Neuron in Tissue Culture, pp. 57–69, Haynes L. W. (ed.), Wiley, New York
23 Komuro H. and Yacubova E. (2001) Migration of cerebellar granule cell. Cell Technol. **20:** 513–519
24 Komuro H. and Rakic P. (1993) Modulation of neuronal migration by NMDA receptors. Science **260:** 95–97
25 Ryder E. F. and Cepko C. L. (1994) Migration patterns of clonally related granule cells and their progenitors in the developing chick cerebellum. Neuron **12:** 1011–1029
26 Behar T. N., Scott C. A., Greene C. L., Wen X., Smith S., Maric D. et al. (1999) Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. J. Neurosci. **19:** 4449–4461
27 Hirai H., Yoshioka H., Kihara M., Hasegawa K., Sakamoto T., Sawada T. et al. (1999) Inhibiting neuronal migration by blocking NMDA receptors in the embryonic rat cerebral cortex: a tissue culture study. Dev. Brain Res. **114:** 63–67
28 Komuro H. and Rakic P. (1995) Dynamics of granule cell migration: a confocal microscopic study in acute cerebellar slice preparations. J. Neurosci. **15:** 1110–1120
29 Komuro H. and Rakic P. (1998) Distinct modes of neuronal migration in different domains of developing cerebellar cortex. J. Neurosci. **18:** 1478–1490.
30 Komuro H., Yacubova E., Yacubova E. and Rakic P. (2001) Mode and tempo of tangential cell migration in the cerebellar external granular layer. J. Neurosci. **21:** 527–540
31 Miale I. L. and Sidman R. L. (1961) An autoradiographic analysis of histogenesis in the mouse cerebellum. Exp. Neurol. **4:** 277–296
32 Fujita S., Shimada M. and Nakamura T. (1966) H^3-thymidine autoradiographic studies on the cell proliferation and differentiation in the external and the internal granular layers of the mouse cerebellum. J. Comp. Neurol. **128:** 191–208
33 Fujita S. (1967) Quantitative analysis of cell proliferation and differentiation in the cortex of the postnatal mouse cerebellum. J. Cell. Biol. **32:** 277–287
34 Altman J. (1972) Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. J. Comp. Neurol. **145:** 353–398

35 Morest D. K. (1970) A study of neurogenesis in the forebrain of opossum pouch young. Z. Anat. Entwicklungsgesch. **130:** 265–305

36 Trenkner E. and Sidman R. L. (1977) Histogenesis of mouse cerebellum in microwell cultures. J. Cell Biol. **75:** 915–940

37 Trenkner E., Smith D. and Segil N. (1984) Is cerebellar granule cell migration regulated by an internal clock? J. Neurosci. **4:** 2850–2855

38 Liesi P. (1985) Do neurons in the vertebrate CNS migrate on laminin? EMBO J. **4:** 1163–1170

39 Nakatsuji N. and Nagata I. (1989) Paradoxical perpendicular contact guidance displayed by mouse cerebellar granule cell neurons in vitro. Development **106:** 441–447

40 Nagata I. and Nakatsuji N. (1990) Granule cell behavior on laminin in cerebellar microexplant cultures. Dev. Brain Res. **52:** 63–73

41 Liang S. and Crutcher K. A. (1992) Neuronal migration on laminin in vitro. Dev. Brain Res. **66:** 127–132

42 Liang S. and Crutcher K. A. (1993) Movement of embryonic chick sympathetic neurons on laminin in vitro is preceded by neurite extension. J. Neurosci. Res. **36:** 607–620

43 Powell S. K., Rivas R., Rodriguez-Boulan E. and Hatten M. E. (1997) Development of polarity in cerebellar granule neurons. J. Neurobiol. **32:** 223–236

44 Yacubova E. and Komuro H. (2002) Intrinsic program for migration of cerebellar granule cells in vitro. J. Neurosci. **22:** 5966–5981

45 Mellor J. R., Merlo D., Jones A., Wisden W. and Randall A. D. (1998) Mouse cerebellar granule cell differentiation: electrical activity regulates the $GABA_A$ receptor $\alpha 6$ subunit gene. J. Neurosci. **18:** 2822–2833

46 Yacubova E. and Komuro H. (2002) Stage specific control of neuronal migration by somatostatin. Nature **415:** 77–81

47 Liesi P. (1990) Extracellular matrix and neuronal movement. Experientia **46:** 900–907

48 Fishell G. and Hatten M. E. (1991) Astrotactin provides a receptor system for CNS neuronal migration. Development **113:** 755–765

49 Fishman R. B. and Hatten M. E. (1993) Multiple receptor systems promote CNS neuronal migration. J. Neurosci. **13:** 3485–3495

50 Palecek S. P., Loftus J. C., Ginsberg M. H., Lauffenbuger D. A. and Horwitz A. F. (1997) Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature **385:** 537–540

51 Kuhar S. G., Feng L., Vidan S., Ross M. E., Hatten M. E. and Heintz, N. (1993) Changing patterns of gene expression define four stages of cerebellar granule neuron differentiation. Development **117:** 97–104

52 Gregory W. A., Edmondson J. C., Hatten M. E. and Mason C. A. (1988) Cytology and neuron-glial apposition of migrating cerebellar granule cells in vitro. J. Neurosci. **8:** 1728–1738

53 Lin C-H., Thompson C. A. and Forscher P. (1994) Cytoskeletal reorganization underlying growth cone motility. Curr. Opin. Neurobiol. **4:** 640–647

54 Ono K., Nakatsuji N. and Nagata I. (1994) Migration behavior of granule cell neurons in cerebellar cultures. II. An electron microscopic study. Dev. Growth Differ. **36:** 29–38

55 Rivas R. J. and Hatten M. E. (1995) Motility and cytoskeletal organization of migrating cerebellar granule neurons. J. Neurosci. **15:** 981–989

56 Rakic P., Knyihar-Csillik E. and Csillik B. (1996) Polarity of microtuble assemblies and during neuronal cell migration. Proc. Natl. Acad. Sci. USA **93:** 9218–9222

57 Cambray-Deakin M. A., Morgan A. and Burgoyne R. D. (1987) Sequential appearance of cytoskeletal components during the early stages of neurite outgrowth from cerebellar granule cell in vitro. Dev. Brain Res. **37:** 197–207.

58 Komuro H. and Rakic P. (1992) Selective role of N-type calcium channels in neuronal migration. Science **257:** 806–809

59 Tam T., Mathews E., Snutch T. P. and Schafer W. R. (2000) Voltage-gated calcium channels direct neuronal migration in *Caenorhabditis elegans*. Dev. Biol. **226:** 104–117

60 Schafer W. R. and Kenyon C. J. (1995) A calcium channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans*. Nature **375:** 73–78

61 Rossi D. and Slater T. N. (1993) The developmental onset of NMDA receptor channel activity during neuronal migration. Neuropharmacology **32:** 1239–1248

62 Farrant M., Feldmeyer D., Takahashi T. and Cull-Candy S. G. (1994) NMDA-receptor channel diversity in the developing cerebellum. Nature **368:** 335–339

63 Monyer H., Burnashev N., Lauria D. J., Sakman B. and Seeburg P. H. (1994) Development of regional expression in the rat brain and functional properties of four NMDA receptors. Neuron **12:** 529–540

64 Nowark L., Bregestovski P, Ascher P., Herbet A. and Prochiantz A. (1984) Magnesium gates glutamate-activated channels in mouse central neurons. Nature **307:** 462–465

65 Johnson J. W. and Ascher P. (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature **325:** 529–531

66 Rakic P. and Sidman R. L. (1973) Sequence of developmental abnormalities leading to granule cell deficit in cerebellar cortex of *weaver* mutant mice. J. Comp. Neurol. **152:** 103–132

67 Marret S., Gressens P. and Evrard P. (1996) Arrest of neuronal migration by excitatory amino acid in hamster developing brain. Proc. Natl. Acad. Sci. USA **93:** 15463–15468

68 Messersmith E. K., Feller M. B., Zhang H. and Shatz C. J. (1997) Migration of neocortical neurons in the absence of functional NMDA receptors. Mol. Cell Neurosci. **5:** 347–357

69 Newgreen D. F. and Gooday D. (1985) Control of the onset of migration of neural crest cells in avian embryos. Cell Tissue Res. **239:** 329–336

70 Sawyer D. W., Sullivan J. A. and Mandell G. L. (1985) Intracellular free calcium localization in neutrophils during phagocytosis. Science **230:** 663–666

71 Jaconi M. E. E., Theler J. M., Schlegel W., Appel R. D., Wright S. D. and Lew P. D. (1991) Multiple elevation of cytosolic-free Ca^{2+} in human neutrophils: initiation by adherence receptors of the integrin family. J. Cell Biol. **112:** 1249–1257

72 Moran D. (1991) Voltage-dependent-L-type Ca^{2+} channels participate in regulating neural crest migration and differentiation. Am. J. Anat. **192:** 14–22

73 Brundage R. A., Fogarty K. E., Tuft R. A. and Fay F. S. (1993) Chemotaxis of newt eosinophils: calcium regulation of chemotactic response. Am. J. Physiol. **265:** C1527-C1543

74 Anton E., Hadjiargyrou M., Patterson P. H. and Matthew W. D. (1995) CD9 plays a role in Schwann cell migration in vitro. J. Neurosci. **15:** 584–595

75 Marks P. W. and Maxfield F. R. (1990) Transient increases in cytosolic calcium appear to be required for the migration of adherent human neutrophils. J. Cell Biol. **110:** 43–52

76 Komuro H. and Rakic P. (1996) Intracellular Ca^{2+} fluctuations modulate the rate of neuronal migration. Neuron **17:** 275–285

77 Hynes R. O. (1992) Integrins: versatility, modulation, and signaling in cell adhesion. Cell **69:** 11–25

78 Clark E. A. and Brugge J. S. (1995) Integrins and signal transduction pathways: the road taken. Science **268:** 233–239

79 Lawson M. A. and Maxfield F. R. (1995) Ca^{2+}- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. Nature **377:** 75–79

80 Rocamora N., Garcia L. F., Palacios J. M. and Mengod G. (1993) Differential expression of brain-derived neuro-

trophic factor, neurotrophin-3, and low-affinity nerve growth factor receptor during the postnatal development of the rat cerebellar system. Brain Res. Mol. Brain Res. **17:** 1–8
81 Wetmore C., Ernfors P., Persson H. and Olson L. (1990) Localization of brain-derived neurotrophic factor mRNA to neurons in the brain by in situ hybridization. Exp. Neurol. **109:** 141–152
82 Segal R., Pomeroy S. and Stiles C. (1995) Axonal growth and fasciculation linked to differential expression of BDNF and NT3 receptors in developing cerebellar granule cells. J. Neurosci. **15:** 4970–4981
83 Borghesani P. R., Michel Peyrin J., Klein R., Rubin J., Carter A. R., Schwartz P. M. et al. (2002) BDNF stimulates migration of cerebellar granule cells. Development **129:** 1435–1442
84 Tanaka S., Sekino Y. and Shirao T. (2000) The effects of neurotrophin-3 and brain-derived neurotrophic factor on cerebellar granule cell movement and neurite extension in vitro. Neuroscience **97:** 727–734
85 Ernfors P., Merlio J. P. and Persson H. (1992) Cells expressing mRNA for neurotrophins and their receptors during embryonic rat development. Eur. J. Neurosci. **4:** 1140–1158
86 Neveu I. and Arenas B. (1996) Neurotrophins promote the survival and development of neurons in the cerebellum of hypothyroid rats in vivo. J. Cell Biol. **133:** 631–646
87 Lindholm D., Castren E., Tsoulfas P., Kolbeck R., Berzaghi MdP., Leingartner A. et al. (1993) Neurotrophin-3 induced triiodothyronine in cerebellar granule cells promotes Purkinje cell differentiation. J. Cell Biol. **122:** 443–450
88 Klein R. (1994) Role of neurotrophins in mouse neuronal development. FASEB J. **8:** 738–744
89 Doughty M. L., Lohof A., Campana A., Delhaye-Bouchaud N. and Mariani J. (1998) Neurotrophin-3 promotes cerebellar granule cell exit from the EGL. Eur. J. Neurosci. **10:** 3007–3011
90 Rio C., Rieff H. I., Qi P., Khurana T. S. and Corfas G. (1997) Neuregulin and erbB receptors play a critical role in neuronal migration. Neuron **19:** 39–50
91 Zou Y. R., Kottmann A, H., Kuroda M., Taniuchi I. and Littman D. R. (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature **393:** 595–599
92 McGrath K., Koniski A. D., Maltby K. M., McGann J. K. and Palis J. (1999) Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. Dev. Biol. **213:** 442–456
93 Ma Q., Jones D., Borghesani P. R., Segal R. A., Nagasawa T., Kishimoto T. et al. (1998) Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc. Natl. Acad. Sci. USA **95:** 9448–9453
94 Klein R. S., Rubin J. B., Gibson H. D., DeHaan E. N., Alvarez-Hernandez X., Segal R. A. et al. (2001) SDF-1α induces chemotaxis and enhances Sonic hedgehog-induced proliferation of cerebellar granule cells. Development **128:** 1971–1981
95 Lu Q., Sun E. E., Klein R. S. and Flanagan J. G. (2001) Ephrin-B reverse signaling is mediated by a novel PDZ-RGS protein and selectively inhibits G protein-coupled chemoattraction. Cell **105:** 69–79
96 Adams N. C., Tomoda T., Cooper M., Dietz G. and Hatten M. E. (2002) Mice that lack astrotactin have slowed neuronal migration. Development **129:** 965–972
97 Grumet M., Hoffman S., Crossin K. L. and Edelman G. M. (1985) Cytotactin, an extracellular matrix protein of neural and nonneural tissues that mediates glia-neuron interaction. Proc. Natl. Acad. Sci. USA **82:** 8075–8079
98 Kruse J., Kelihauer G., Faissner A., Timple R. and Schachner M. (1985) The J1 glycoprotein – a novel nervous system cell adhesion molecules of the L2/HNK-1 family. Nature **316:** 146–148
99 Wehrle B. and Chiquet M. (1990) Tenascin is accumulated along developing peripheral nerves and allows neurite outgrowth in vitro. Development **110:** 401–415
100 Lochter A., Vaughan L., Kaplony A., Prochiantz A., Schachner M. and Faissner A. (1985) J1/tenascin in substrate-bound and soluble form displays contrary effects on neurite outgrowth. J. Cell Biol. **113:** 1159–1171
101 Husmann K., Faissner A. and Schachner M. (1992) Tenascin promotes cerebellar granule cell migration and neurite outgrowth by different domains in the fibronectin type III repeats. J. Cell Biol. **116:** 1475–1486
102 Friedman G. C. and Seeds N. W. (1995) Tissue plasminogen activator mRNA expression in granule neurons coincides with their migration in the developing cerebellum. J. Comp. Neurol. **360:** 658–670
103 Krystosek A. and Seeds A. W. (1981) Plasminogen activator secretion by granule neurons in cultures of developing cerebellum. Proc. Natl. Acad. Sci. USA **78:** 7810–7814
104 Verrall S. and Seeds N. W. (1989) Characterization of ^{125}I-tissue plasminogen activator binding to cerebellar granule neurons. J. Cell Biol. **109:** 265–271
105 Seeds N. W., Basham M. E. and Haffke S. P. (1999) Neuronal migration is retarded in mice lacking the tissue plasminogen activator gene. Proc. Natl. Acad. Sci. USA **96:** 14118–14123
106 Stewart R. M., Richman D. P. and Caviness V. S. J. (1975) Lissencephaly and pachygyria: an architectonic and topographic analysis. Acta. Neuropathol. **31:** 1–12
107 Jellinger K. and Rett A. (1976) Agyria-pachygyria (lissencephaly syndrome). Neuropediatrics 7: 6691
108 Miller J. (1963) Lissencephaly in two siblings. Neurology **13:** 841–850
109 Ledbetter S. A., Kuwano A., Dobyns W. B. and Ledbetter D. H. (1992) Microdeletions of chromosome 17p13 as a cause of isolated lissencephaly. Am. J. Hum. Genet. **50:** 182–189
110 Mizuguchi M., Takashima S., Kakita A., Yamada M. and Ikeda K. (1995) Lissencephaly gene product-localization in the central nervous system and loss of immunoreactivity in Miller-Dieker syndrome. Am. J. Pathol. **147:** 1142–1151
111 Reiner O., Albrecht U., Gordon M., Chianese K. A., Wong C., Gal-Gerber O. et al. (1995) Lissencephaly gene (*LIS1*) expression in the CNS suggests a role in neuronal migration. J. Neurosci. **15:** 3730–3738
112 Hattori M., Arai H. and Inoue K. (1993) Purification and characterization of bovine brain platelet-activating factor acetylhydrolase. J. Biol. Chem. **268:** 18748–18753
113 Hattori M., Adachi H., Tsujimoto M., Arai H. and Inoue K. (1994) Catalytic subunit of bovine brain platelet-activating factor acetylhydrolase is a novel type of serine esterase. J. Biol. Chem. **269:** 23150–23155
114 Clark G. D., McNeil R. S., Bix G. J. and Swann J. W. (1995) Platelet-activating factor produces neuronal growth cone collapse. NeuroReport **6:** 2569–2575
115 Bix G. J. and Clark G. D. (1998) Platelet-activating factor receptor stimulation disrupts neuronal migration in vitro. J. Neurosci. **18:** 307–318
116 Meyerson M., Enders G. H., Wu C.L., Su L. K., Gorka C., Nelson C. et al. (1992) A family of human cdc2-related protein kinases. EMBO J. **11:** 2909–2917
117 Sherr C. J. (1993) Mammalian G1 cyclins. Cell **73:** 1059–1065
118 Tsai L-H., Takahashi T., Caviness V. S. J. and Harlow E. (1993) Activity and expression pattern of cyclin-dependent kinase-5 in the embryonic mouse central nervous system. Development **119:** 1029–1040
119 Ohshima T., Ward J. M., Huh C-G., Longenecker G., Pant H. C., Brady R. O. et al. (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis,

neuronal pathology and perinatal death. Proc. Natl. Acad. Sci. USA **93:** 11173–11178
120 Ohshima T., Gilmore E. C., Herrup K., Brady R. O. and Kulkarni A. B. (1997) Ontogeny of abnormal brain development in Cdk5(–/–) mice. Dev. Brain Dysfunct. **10:** 271–281
121 Ohshima T., Gilmore E.C., Longenecker G., Jacobowitz D. M., Brady R. O., Herrup K. et al. (1999) Migration defects of *cdk5*$^{-/-}$ neurons in the developing cerebellum is cell autonomous. J. Neurosci. **19:** 6017–6026
122 Santiago M. F., Berredo-Pinho M., Costa M. R., Gandra M., Cavalcante L.A. and Mendez-Otero R. (2001) Expression and function of ganglioside 9-O-acetyl GD3 in postmitotic granule cell development. Mol. Cell Neurosci. **17:** 488–499
123 Schindler M., Humphrey P. P. A. and Emson P. C. (1996) Somatostatin receptors in the central nervous system. Prog. Neurobiol. **50:** 9–47
124 Patel Y. C. (1997) Molecular pharmacology of somatostatin receptor subtypes. J. Endocrinol. Invest. **20:** 348–367
125 Benoit R., Bohlen P., Ling N., Briskin A., Esch F., Brazeau P. et al. (1982) Presence of somatostatin-28-(1–12) in hypothalamus and pancreas. Proc. Natl. Acad. Sci. USA **79:** 917–921
126 Pradayrol L., Jornvall H., Mutt V. and Ribet A. (1980) N-terminally extended somatostatin: the primary structure of somatostatin 28. FEBS Lett. **109:** 55–58
127 Bruno J. F., Xu Y., Song J. and Berelowitz M. (1992) Molecular cloning and functional expression of a brain specific somatostatin receptor. Proc. Natl. Acad. Sci. USA. **89:** 11151–11155
128 O'Carroll A. M., Lolait S. J., Konig M. and Mahan L. C. (1992) Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin 28. Mol. Pharmacol. **42:** 939–946
129 Yamada Y., Post S. R., Wang K., Tager H. S., Bell G. I. and Seino S. (1992) Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract and kidney. Proc. Natl. Acad. Sci. USA. **89:** 251–255
130 Yasuda K., Domiano S., Breder C. D., Law S., Saper C. B., Reisine T. et al. (1992) Cloning of a novel somatostatin receptor SSTR3, coupled to adenylyl cyclase. J. Biol. Chem. **267:** 20422–20428
131 Rocheville M., Lange D. C., Kumar U., Patel S. C., Patel R. C. and Patel Y. C. (2000) Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science **288:** 154–157
132 Vanetti M., Kouba M., Wang X., Vogt G. and Hollt V. (1992) Cloning and expression of a novel mouse somatostatin receptor (SSTR2B). FEBS Lett. **311:** 290–294
133 Patel Y. C., Greenwood M. T., Kent G., Panetta R. and Srikant C. B. (1993) Multiple gene transcripts of the somatostatin receptor SSTR2: tissue selective distribution and cAMP regulation. Biochem. Biophys. Res. Commun. **192:** 288–294
134 Florio T. and Schettini G. (1996) Multiple intracellular effectors modulate physiological functions of the cloned somatostatin receptors. J. Mol. Endocrinol. **17:** 89–100
135 Patel Y. C., Greenwood M. T., Panetta R., Demchyshyn L., Niznik H. and Srikant C. B. (1995) The somatostatin receptor family: a mini review. Life Sci. **57:** 1249–1265
136 Reisine T. and Bell G. I. (1995) Molecular properties of somatostatin receptors. Neuroscience **67:** 777–790
137 Chen C. (1997) G_{O2} and G_{i3} proteins mediate the action of somatostatin on membrane Ca^{2+} and K^+ currents in ovine pituitary somatotrophs. Clin. Exp. Pharmacol. Physiol. **24:** 639–645
138 Chen C. (1998) G_{i-3} protein mediates the increase in voltage-gated K^+ currents by somatostatin on cultured ovine somatotrophs. Am. J. Physiol. **275:** E278–E284
139 Connor M., Yeo A. and Henderson G. (1997) Neuropeptide Y Y_2 receptor and somatostatin sst_2 receptor coupling to mobilization of intracellular calcium in SH-SY5Y human neuroblastoma cells. Br. J. Pharmacol. **120:** 455–463
140 Degtiar V. E., Wittig B., Schultz G. and Kalkbrenner F. (1996). A specific G_O heterotrimer couples somatostatin receptors to voltage-gated calcium channels in RINm5F cells. FEBS Lett. **380:** 137–141
141 Reardon D. B., Dent P., Wood S. L., Kong T. and Sturgill T. W. (1997) Activation in vitro of somatostatin receptor subtypes 2, 3, or 4 stimulates protein tyrosine phosphatase activity in membranes from transfected ras-transformed NIH 3T3 cells: coexpression with catalytically inactive SHP-2 blocks responsiveness. Mol. Endocrinol. **11:** 1062–1069
142 Rodriguez-Sanchez M. N., Puebla L., Lopez-Sanudo S., Rodriguez-Martin E., Martin-Espinosa A., Rodriguez-Pena M. S. et al. (1997) Dopamine enhances somatostatin receptor-mediated inhibition of adenylate cyclase in rat striatum and hippocampus. J. Neurosci. Res. **48:** 238–248
143 Takano K., Yasufuku-Takano J., Kozasa T., Nakajima S. and Nakajima Y. (1997) Different G proteins mediate somatostatin-induced inward rectifier K^+ currents in murine brain and endocrine cells. J. Physiol. **502:** 559–567
144 Takano K., Yasufuku-Takano J., Teramoto A. and Fujita T. (1997) G_{i3} mediates somatostatin-induced activation of an inwardly rectifying K^+ current in human growth hormone-secreting adenoma cells. Endocrinology **138:** 2405–2409
145 Traina G. and Bagnoli P. (1999) Mechanisms mediating somatostatin-induced reduction of cytosolic free calcium in PC12 cells. Neurosci. Lett. **265:** 123–126
146 Oomen S. P., Hennik P. B. van, Antonissen C., Lichtenauer-Kaligis E. G., Hofland L. J., Lamberts S. W. J. et al. (2002) Somatostatin is a selective chemoattractant for primitive ($CD34^+$) hematopoietic progenitor cells. Exp. Hematol. **30:** 116–125
147 Buchan A. M. J., Lin C-Y., Choi J. and Barber D. L. (2002) Somatostatin, acting at receptor subtype 1, inhibits Rho activity, the assembly of actin stress fibers, and cell migration. J. Biol. Chem. **277:** 28431–28438
148 Shinoda H., Marini A. M. and Schwartz J. P. (1992) Developmental expression of the proenkephalin and prosomatostatin genes in cultured cortical and cerebellar astrocytes. Dev. Brain. Res. **67:** 205–210
149 Maubert E., Slama A., Ciofi P., Viollet C., Tramu G., Dupouy J. P. et al. (1994) Developmental patterns of somatostatin-receptors and somatostatin-immunoreactivity during early neurogenesis in the rat. Neuroscience **62:** 317–325
150 Thoss V. S., Perez J., Duc D. and Hoyer D. (1995) Embryonic and postnatal mRNA distribution of five somatostatin receptor subtypes in the rat brain. Neuropharmacolgy **34:** 1673–1688
151 Thoss V. S., Duc D. and Hoyer D. (1996) Somatostatin receptors in the developing rat brain. Eur. J. Pharmacol. **297:** 145–155
152 Carpentier V., Vaudry H., Mallet E., Tayot J., Laquerriere A. and Leroux P. (1997) Ontogeny of somatostatin binding sites in respiratory nuclei of the human brainstem. J. Comp. Neurol. **381:** 461–472
153 Marty S. and Onteniente B. (1997) The expression pattern of somatostatin and calretinin by postnatal hippocampal interneurons is regulated by activity-dependent and -independent determinants. Neuroscience **80:** 79–88
154 Thal L. J., Laing K., Horowitz S. G. and Makman M. H. (1986) Dopamine stimulates rat cortical somatostatin release. Brain Res. **372:** 205–209
155 Gray D. B., Zelazny D., Manthay D. and Pilar G. (1990) Endogenous modulation of Ach release by somatostatin and differential role of calcium channels. J. Neurosci. **10:** 2687–2698

156 Hathway G. J., Emson P. C., Humphrey P. P. A. and Kendrick K. M. (1998) Somatostatin potently stimulates in vivo striatal dopamine and γ-aminobutyric acid release by a glutamate-dependent action. J. Neurochem. **70:** 1740–1749
157 Lanneau C., Viollet C., Faivre-Bauman A., Loudes C., Kordon C., Epelbaum J. et al. (1998) Somatostatin receptor subtypes sst1 and sst2 elicit opposite effects on the response to glutamate of mouse hypothalamic neurons: an electrophysiological and single cell RT-PCR study. Eur. J. Neurosci. **10:** 204–212
158 Pittaluga A., Feligioni M., Ghersi C., Gemignani A. and Raiteri M. (2001) Potentiation of NMDA receptor function through somatostatin release: a possible mechanism for the cognition-enhancing activity of $GABA_B$ receptor antagonists. Neuropharmacology **41:** 301–310
159 Gonzalez B., Leroux P., Bodenant C., Braquet P. and Vaudry H. (1990) Pharmacological characterization of somatostatin receptors in the rat cerebellum during development. J. Neurochem. **55:** 729–737
160 Viollet C., Bodenant C., Prunotto C., Roosterman D., Schaefer J., Meyerhof W. et al. (1997) Differential expression of multiple somatostatin receptors in rat cerebellum during development. J. Neurochem. **68:** 2263- 2272
161 Inagaki S., Shiosaka S., Takatsuki K., Iida H., Sakanaka M., Senba E., et al. (1982) Ontogeny of somatostatin-containing neuron system of the rat cerebellum including its fiber connections: an experimental and immunohistochemical analysis. Dev. Brain Res. **3:** 509–527
162 Naus C. C. G. (1990) Developmental appearance of somatostatin in the rat cerebellum: in situ hybridization and immunohistochemistry. Brain Res. Bull. **24:** 583–592
163 Villar M. J., Hokfelt T. and Brown J. C. (1989) Somatostatin expression in the cerebellar cortex during postnatal development. Anat. Embryol. **179:** 257–267
164 Inagaki S., Shiosaka S., Sekitani M., Nohuchi K., Shimada S. and Takagi H. (1989) In situ hybridization analysis of the somatostatin-containing neuron system in developing cerebellum of rats. Mol. Brain Res. **6:** 289–295
165 Baumbach W. R., Carrick T. A., Pausch M. H., Bingham B., Carmignac D., Robinson I. C. et al. (1998) A linear hexapeptide somatostatin antagonist blocks somatostatin activity in vitro and influences growth hormone release in rats. Mol. Pharmacol. **54:** 864–873