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Cerebellar Granule Cell Migration and the Effects of Alcohol

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Abstract
In the developing brain the majority of neurons migrate from their birthplace to their final destination. This active movement is essential for the formation of cortical layers and nuclei. The impairment of migration does not affect the viability of neurons but often results in abnormal differentiation. The proper migration of neurons requires the orchestrated activities of multiple cellular and molecular events, such as pathway selection, the activation of specific receptors and channels, and the assembly and disassembly of cytoskeletal components. The migration of neurons is very vulnerable to exposure to environmental toxins, such as alcohol. In this article, we will focus on recent developments in the migration of cerebellar granule cells. First, we will describe when, where and how granule cells migrate through different cortical layers to reach their final destination. Second, we will present how internal programs control the sequential changes in granule cell migration. Third, we will review the roles of external guidance cues and transmembrane signals in granule cell migration. Finally, we will reveal mechanisms by which alcohol exposure impairs granule cell migration.

Introduction

The migration of postmitotic neurons from their sites of origin to their final destinations, where they make synaptic connections, is a fundamental cellular event essential for building large neuronal assemblies [1–3]. For example, the embryonic cerebrum displays 2 distinctive patterns of neuronal migration. Projecting neurons are primarily generated in the ventricular zone and then migrate to the developing cortical plate by means of radial migration [4–6]. In contrast, most GABAergic interneurons originate in the medial ganglionic eminence of the ventral telencephalon and follow tangential migratory routes through the intermediate zone and marginal zone to reach the cortical plate [7, 8]. In humans, distinct genetic mutations and environmental toxins can affect neuronal migration and result in abnormal cortical development [9–11]. Although it is still unclear how these cortical abnormalities develop, accumulated evidence has begun to indicate that the migration of neurons is controlled by the orchestrated activities of multiple molecular events [12–14]. These include the response to repulsive and attractive signals and the alterations of the second messenger signaling pathways [15, 16].

This review is based on studies of the migration of cerebellar granule cells. The prenatal and early postnatal cerebellum has been used as a model system for neuronal migration because the defined neuronal cytoarchitecture

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and the small number of neuronal types in the cerebellum provide an ideal system for determining cellular and molecular mechanisms of neuronal migration [17, 18]. Specifically, the migration of granule cells has been intensively examined, and it has become apparent that the mechanisms underlying granule cell migration are utilized during the migration of immature neurons in other brain regions [2, 3, 15–17]. For example, the role of neuron-glia interaction in neuronal migration was first discovered in migrating granule cells and Bergmann glial cells in the developing cerebellum [19]. Thereafter, it has become apparent that in the developing cerebrum, immature neurons also use the processes of radial glia as a scaffold for their migration towards the cortical plate [20]. Furthermore, the role of cell adhesion molecules in neuronal migration was determined first in granule cell migration [21–24]. Later, wide varieties of cell adhesion molecules, which play a critical role in neuronal migration, were identified in the developing brain, including the cerebrum [2, 14, 17]. Moreover, the control of neuronal migration by neurotransmitters was first discovered in granule cells as a regulation of their migration by glutamate [25]. Thereafter, the role of not only glutamate but also γ-aminobutyric acid was discovered in the migration of cortical neurons in the developing cerebrum [26–28].

During the past decade, the use of cerebellar slices from early postnatal mice in conjunction with confocal microscopy and fluorescent lipophilic carbocyanine dyes revealed how granule cells migrate through the different cortical layers of the developing cerebellum and determined how an identified granule cell attains its final destination [29–31]. Furthermore, the varieties of extracellular and intracellular signals that regulate granule cell migration were discovered [15, 16]. Most recently, the cellular and molecular mechanisms by which alcohol impairs granule cell migration in a mouse model of fetal alcohol syndrome (FAS) were determined [32].

In this article, we will first describe the cortical-layer-specific changes in granule cell migration in the early postnatal mouse cerebellum. Second, we will reveal the internal programs which control the sequential changes in granule cell migration in vitro. Third, we will cover the extracellular molecules and intracellular signals that regulate granule cell migration. Finally, we will discuss the latest developments in the study of how alcohol impairs granule cell migration.

Cortical-Layer-Specific Changes in Granule Cell Migration in the Early Postnatal Cerebellum

Granule cells alter the mode, tempo and direction of migration as they traverse different cortical layers of the cerebellum (as schematically presented in fig. 1a) [29–31]. In this section, we will describe granule cell migration layer by layer in the early postnatal mouse cerebellum.

The External Granular Layer

At the top of the external granular layer (EGL), granule cell precursors proliferate every 18–20 h [31]. After final mitosis, granule cells remain in the EGL for 24–48 h before initiating their radial migration across the molecular layer (ML) [31, 33]. During this latent period the cells tangentially migrate within the middle and bottom of the EGL [31]. In the middle of the EGL, coinciding with the extension of 2 uneven horizontal processes oriented parallel to the longitudinal axis of the folium, the cells start to migrate tangentially in the direction of the larger process [31]. Their morphology and speed of movement change systematically with their position within the EGL [31]. The rate of movement is fastest (~14.8 μm/h) in the middle of the EGL, when the cells have 2 short horizontal processes. As the 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 at the slowest rate (~4.1 μm/h), their somata round and then begin to extend couples of descending processes into the ML. After the stationary period, the 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 [31].

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 [19]. In this layer, the rates of granule cell movement depend critically on the age of the cerebellum [29]. The average rate of migration in the ML increases systematically from 9.6 μm/h in P7 to 18.0 μm/h in P13. Consequently, the cells traverse the developing ML within a relatively constant time period (10–11 h) despite the doubling in width of the layer during the second week of postnatal life [29, 30]. The movement of the cells in this layer is characterized by alternations of short stationary phases with movement in a forward or

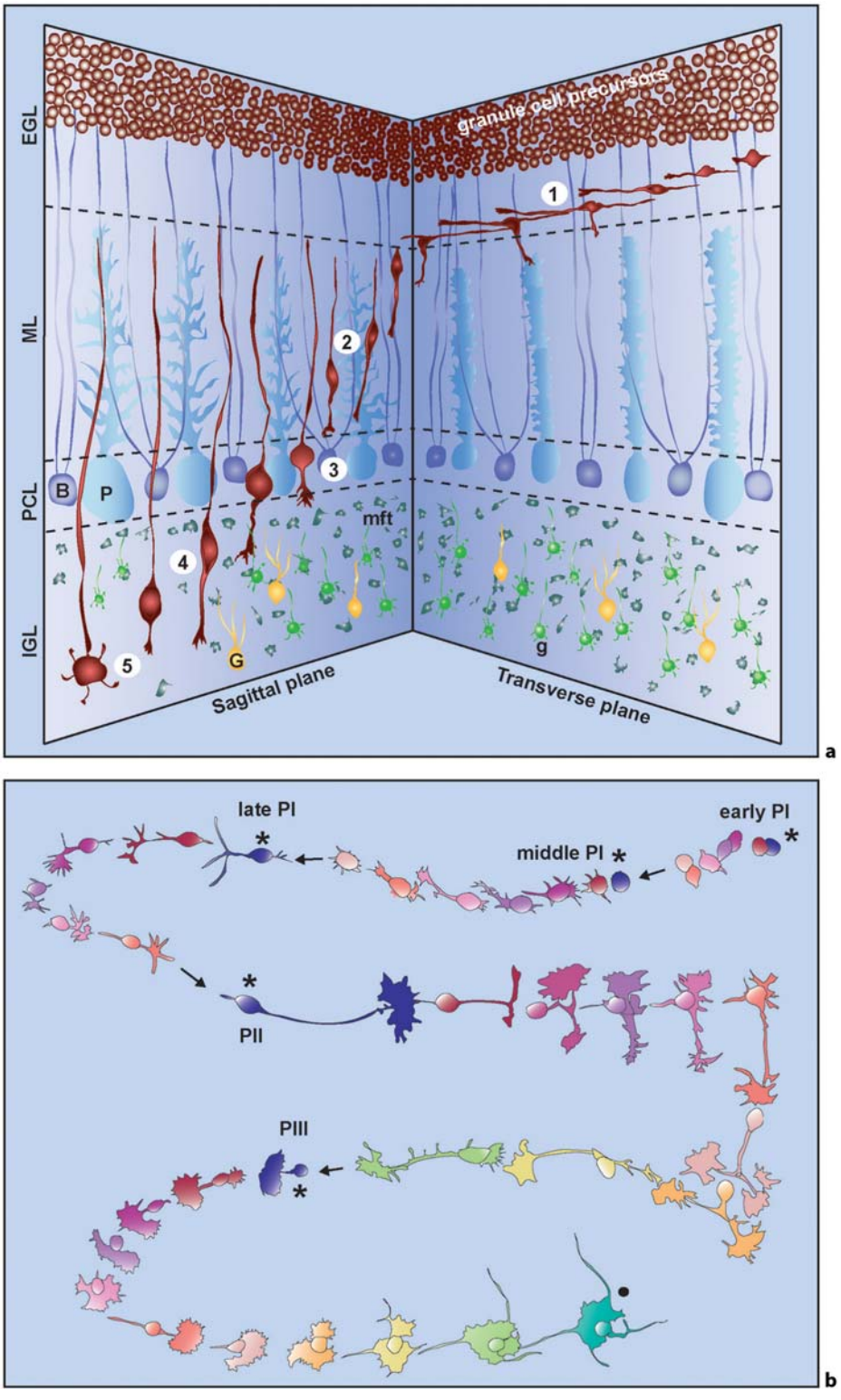


Fig. 1. Alterations of the mode and direction of granule cell migration in vivo and in vitro. **a** Three-dimensional representation of granule cell migration from the external granular layer (EGL) to the internal granular layer (IGL) in the early postnatal mouse cerebellum. 1 = Tangential migration in the middle and bottom of the EGL; 2 = Bergmann-glia-guided radial migration in the molecular layer (ML); 3 = stationary state in the Purkinje cell layer (PCL); 4 = glia-independent radial migration in the IGL; 5 = completion of migration in the middle or the bottom of the IGL; P = Purkinje cell; B = Bergmann glia; G = Golgi cell; g = postmigratory granule cell; mft = mossy fiber terminal. **b** Schematic representation of sequential changes in granule cell morphology and modes of turning in vitro. Isolated granule cells go through 3 characteristic changes in their migration and morphology without cell-cell contact. The first phase (PI) is a period of 0–20 h in vitro, when granule cells initiate their migration. 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. The third phase III (PIII) is a period of 40–60 h in vitro, when granule cells terminate their migration. Asterisks indicate first images of granule cells taken during each series of observation. A 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.

backward direction [29]. The net displacement of the cells depends on the duration and frequency of these phases as well as on the speed of movement [29].

The Purkinje Cell Layer

Upon entering the Purkinje cell layer (PCL), the granule cells detach from the processes of the Bergmann glial cells, and the shape of their cell bodies abruptly transforms from a vertically elongated spindle to a sphere [30]. The rounded somata significantly slow their movement and stop completely in the PCL [30]. The somata remain stationary in the PCL for an average of 115 min, with times ranging from 30 to 220 min [30]. 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 cells in the PCL begin to re-extend their somata and leading processes. During this transformation, the cells gradually accelerate the rate of migration and cross the border between the PCL and the internal granular layer (IGL) [30].

The IGL

In this layer, the granule cells migrate radially towards the bottom of the IGL without an association of glial cells at a rate comparable to that observed in the ML [30]. The long axis of their somata remains oriented perpendicularly to the PCL-IGL boundary line during this radial migration. Interestingly, once the tip of a leading process approaches the IGL-white-matter border, their somata become rounded again [30]. Thereafter, the cells gradually slow their migration and stop movement near the IGL-white-matter border [30]. In early postnatal mouse cerebella, the majority of granule cells complete their migration at the bottom stratum of the IGL.

In vitro studies with the use of real-time observation of granule cell migration in acute cerebellar slices revealed that in the P10 mouse cerebellum, the granule cells first move tangentially about 220 μm in the EGL and then migrate radially about 250 μm to reach their final position in the IGL [29, 30]. 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 [29–31]. Therefore, 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) [29–31].

Comparison of Cerebellar Granule Cell Migration in the Studies Using in vivo and in vitro Models

In vivo analysis of granule cell migration using ^{3}H-thymidine autoradiography revealed that in P10 mice, granule cell precursors occupy the top half of the EGL and actively proliferate [33–35]. After final mitosis, granule cells translocate to the bottom half of the EGL and stay there for approximately 20–30 h [36]. Thereafter, granule cells migrate through the ML and the PCL to the IGL. The entire population of the granule cells in the IGL arises postnatally in the EGL between P1 and P18, and 95% of the granule cells in the IGL are produced between P4 and P15 [36]. Based on the observation of changes in the position of ^{3}H-thymidine-labeled granule cells in the cerebellar cortical layers after an injection of ^{3}H-thymidine, Fujita et al. [36] estimated that the transit times of the granule cells migrating across the EGL and the ML of P10 mice are approximately 21 and 4 h, respectively. The estimated transient time of granule cells in the EGL and the ML in vitro studies is shorter than that obtained in the in vivo studies. This difference may result from the wide range (20–48 h) in the period of time required for granule cells to initiate their radial migration into the ML after final cell division [31, 33].

Recently, studies with the use of 5-bromo-2′-deoxyuridine (BrdU) showed the time course of the translocation of cerebellar granule cells in early postnatal mice after their final mitosis [31]. P10 mice were injected with BrdU and then sacrificed 2, 24 and 48 h later. Two hours after injection, BrdU-labeled cells were localized at the outermost 2 rows of the EGL, indicating that granule cell precursors proliferate within the top level of the EGL, while the middle and bottom levels of the EGL contain unlabeled postmitotic granule cells [31]. Within the following 24 h, the BrdU-labeled cells spread across the entire EGL, and some begin their descent to the ML [31]. Forty-eight hours after BrdU injection, approximately 50% of the BrdU-labeled granule cells left the EGL and translocated their soma into the ML, PCL or IGL. This suggests that within 48 h after final mitosis, many postmitotic granule cells begin to migrate radially towards the IGL, and some of them have already reached their final destinations in the IGL [31]. It has been shown that the postsynthetic phase and the mitotic phase of the granule cell precursor's cycle in 10-day-old mouse cerebella last 2 and 0.5 h, respectively [33]. Taken together, these results demonstrate that in the early postnatal cerebellum, granule cells are able to attain their final destination in the IGL approximately 46 h after their final mitosis.

Interestingly, the estimated time required for granule cells to reach their final migration in vivo is similar to that obtained from the studies using acute cerebellar slice preparations.

Although radial migration of granule cells along the Bergmann glial processes in the ML has been extensively analyzed using in vivo models, little is known about the early behavior of postmitotic granule cells within the EGL before they start their descent to the ML. A major impediment in the analysis of granule cell behavior in the EGL was the availability of a reliable assay system. For example, the use of ^{3}H-thymidine labeling alone could not reveal the extent, direction and rate of cell migration within a histologically homogeneous cell layer such as the EGL. The use of replication-incompetent retrovirus suggests that granule cells may migrate tangentially in both rostrocaudal and mediolateral planes before the onset of the radial migration [37]. The in vitro studies of granule cell behavior directly demonstrated that, at the various levels of the EGL, postmitotic granule cells exhibit a different morphology and rate of tangential movement before the onset of radial migration.

Intrinsic Programs for Controlling 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 cerebellum. Although such changes in the migration are likely to be induced by responses to local environmental cues, the alterations may also depend, at least in part, on an internal clock or intrinsic programs. This is because in the microexplant cultures of early postnatal mouse cerebella, granule cells sequentially go through 3 characteristic phases of migration and morphology without cell-cell contact, suggesting that inherent (intrinsic) mechanisms control the alterations of their migration and morphology [38]. The three sequential changes in the migration and morphology of granule cells in the cerebellar microexplant cultures are as follows:

The First Phase (a Period of 0–20 h in vitro)

At the early stage of the first phase (PI), the granule cells repeatedly change the shape of their somata from spherical to spindle and vice versa [38]. The cells frequently turn to the left or right without extending leading processes (as illustrated in fig. 1b). At the point at which the cells change their direction of movement, they stop movement, become round and then extend their somata 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 cells repeatedly extend and withdraw short leading processes and move at a fast rate only after the process fully extends [38]. The extension of a new leading process towards a different direction is an essential prerequisite for changing the direction of cell movement. At the end of PI, the 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 [38].

The Second Phase (a Period of 20–40 h in vitro)

At the early stage of the second phase (PII), the granule cells develop another mode of turning behavior as follows: (a) the tip of leading process bifurcates; (b) both branches extend in the opposite direction; (c) one of the branches collapses and retracts, and (d) the cell body follows the direction of extension of the remaining branch [38]. The cells exhibit this mode of turning behavior throughout PII. At the late stage of PII, the cells often become stationary for 2–3 h and retract their processes [38].

The Third Phase (a Period of 40–60 h in vitro)

At the early stage of the third phase (PIII) the granule cells start to exhibit characteristic changes in their leading process, which are the initial signs of the termination of their migration [38]. At the late stage of PIII, the cells gradually slow down their movement. At the end the cells become permanently stationary, extend a lamellipodium around the soma and emit several thin processes [38]. In the microexplant cultures of early postnatal mouse cerebella, the majority of granule cells terminate their migration 50–60 h after the initiation of their movement without external cues [38]. Interestingly, this 50- to 60-hour term of granule cell migration is comparable to the time required for granule cells to migrate from the EGL to the IGL in vivo [15, 16].

Several features of granule cell migration also systematically change as the elapsed time goes on after an initiation of cultures, indicating intrinsic programs for cell migration [38]. For example, in PI, the cells migrate at an average rate of 26.0 μm/h, which is determined by dividing the total traveling distance by the period of the observation, 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 [38]. The length of the

cycle of cell movement and stationary state is shortest (218 min). In PII, the cells extend a long and thick 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 rate of turning is lowest (0.3 turn/h). In PIII, the cells decrease the rate of the movement (25.2 μm/h) but slightly increase the turning rate (0.5 turn/h). The length of the cycle of cell movement further increases to 297 min. There is a statistical significance ($p < 0.01$) in the average rate of granule cell migration (1) between PI and PII and (2) between PII and PIII [38].

There is also other evidence suggesting that intrinsic programs control the changes in granule cell migration. For example, granule cells isolated from different postnatal stages also undergo the same patterns of changes in migratory behavior [38]. Furthermore, the majority of granule cells, which migrate on different concentrations and types of extracellular matrix molecules, exhibit the same patterns of changes during the migration in isolation as follows: (1) the rate of cell movement in PII is fastest; (2) the rate of turning behavior in PI is highest; (3) the length of cycle of cell movement systematically increases as time in vitro increases; (4) the length of the stationary state in PII is longest; (5) the number of processes in PI is largest, and (6) the length of the process in PIII is longest [38]. These results demonstrate the existence of intrinsic (inherent) programs for controlling granule cell migration in an age-dependent manner (or a developmental-stage-dependent manner).

Mechanisms and Molecules of Controlling Granule Cell Migration

To date, large numbers of molecules and genes have been discovered as potential regulators or modulators of neuronal cell migration. In this section, we will review signaling molecules and genes which play crucial roles in controlling granule cell migration.

Ca^{2+} Channels and Ca^{2+} Signaling

The activity of voltage-gated Ca^{2+} channels, especially the N-type Ca^{2+} channel, plays a role in granule cell migration in the early postnatal mouse cerebellum [39, 40]. The granule cells in the EGL start to express N-type Ca^{2+} channels prior to the initiation of migration [39]. The number of N-type Ca^{2+} channels expressed on the plasmalemmal surface of the cells rapidly increases during migration [39]. The blockade of N-type Ca^{2+} channel activity significantly slows down granule cell migration, suggesting that the activity of N-type Ca^{2+} channels is required for migration [39]. Interestingly, granule cells exhibit distinct frequencies of transient Ca^{2+} elevations as they migrate in different cortical layers and complete migration only after the loss of Ca^{2+} elevations [41]. There is a positive correlation between the rate of migration and the frequency of Ca^{2+} elevations [40, 41]. The reduction of the frequency of Ca^{2+} elevations halts granule cell migration prematurely, while increasing the frequency significantly delays the completion of migration [41]. The timing of the loss of Ca^{2+} elevations is intrinsically set in the granule cells and influenced by external cues [41]. The results suggest that Ca^{2+} signaling functions as a mediator for controlling granule cell migration.

N-Methyl-D-Aspartate Subtype of Glutamate Receptors

The presence of spontaneous activity of the NMDA (N-methyl-D-aspartate) receptors on the surface of migrating granule cells has been confirmed by patch-clamp analysis [42]. Migrating granule cells coexpress the NR1 and NR2A or NR2B subunits of NMDA receptors, whereas postmigratory granule cells in the IGL express the NR1 and NR2C types [43, 44]. Importantly, blocking NMDA receptor activity significantly decreases the rate of granule cell movement [25], whereas stimulating NMDA receptor activity significantly increases the rate [32]. Furthermore, the elevation of extracellular glutamate concentrations by inhibiting glutamate uptake by astrocytes increases the frequency of spontaneous NMDA-receptor-coupled channel activity and significantly accelerates the rate of granule cell migration [25]. Endogenous extracellular glutamate could control granule cell migration through activation of the NMDA receptors by nonsynaptic means because the cells do not form synapses before the completion of migration [25].

Brain-Derived Neurotrophic Factor

Granule cells express brain-derived neurotrophic factor (BDNF) and its high-affinity receptor (TrkB) [45, 46]. The level of BDNF expression increases during early postnatal development [45, 46]. Interestingly, in $BDNF^{-/-}$ mice granule cells exhibit impaired migration, and the application of BDNF stimulates movement [47].

Neurotrophin-3

Neurotrophin-3 (NT-3) mRNA expression begins with the emergence of the EGL in the prenatal cerebellum and significantly increases between P5 and P20 [48, 49]. Granule cells in the EGL express the NT-3 receptor (TrkC)

before the initiation of migration [50]. Chronic application of exogenous NT-3 results in a significant reduction of the thickness of the EGL without cell death, suggesting that NT-3 accelerates the exit of granule cells from the EGL [51].

Neuregulin

Granule cells express neuregulin (NRG), and glial cells express its receptor, erbB4 [52]. Blockade of the glial erbB4 receptors impairs granule cell migration along the glial fibers, indicating that NRG and erbB4 are essential for glia-associated migration of granule cells in the ML [52].

Stromal-Cell-Derived Factor 1α

Stromal-cell-derived factor 1α (SDF-1α) expression is present in the pial membrane, while granule cell precursors express its receptor CXCR4 [53, 54]. In the SDF-1α- or CXCR4-deficient mice, granule cell precursors prematurely migrate away from the EGL and locate ectopically outside the EGL [53, 55]. SDF-1α induces chemotactic responses in granule cell precursors [56]. These results suggest that SDF-1α and CXCR4 play a crucial role in retaining granule cell precursors in the EGL by chemoattracting toward the pia mater.

Ephrin-B2

Ephrin-B2 and its receptor, EphB2, are expressed in the EGL of the early postnatal mouse cerebellum [57]. Interestingly, the chemoattractant effect of SDF-1α to the granule cells is selectively inhibited by soluble EphB2 receptor through reverse signaling of ephrin-B2 [57]. These results suggest that when granule cells are ready to migrate out from the EGL, they lose responsiveness to SDF-1α [57].

Astrotactin

Astrotactin null mice show a decrease in granule cell binding to the glial cells and a reduction in the rate of granule cell migration [58]. This suggests that astrotactin functions in granule cell migration along glial processes in the ML.

Tenascin

Tenascin, an extracellular matrix molecule, influences the migration of granule cells along the Bergmann glial processes through different domains in the fibronectin type III homologous repeats [59].

Tissue Plasminogen Activator

Granule cells turn on the gene for tissue plasminogen activator (tPA) as they begin their migration into the ML [60]. The cells both secrete tPA and bind tPA to their cell surface. Interestingly, $tPA^{-/-}$ mice have >2-fold more migrating granule cells in the ML [61]. In vitro assays reveal that granule cells in $tPA^{-/-}$ mice migrate through the ML at about half the rate measured in $tPA^{+/+}$ mice [62]. This suggests that tPA gene expression is required for maintaining the maximal rate of granule cell migration.

Platelet-Activating Factor

Platelet-activating factor (PAF) has been implicated in the human neuronal migration disorder Miller-Dieker lissencephaly [63, 64]. In this disorder, the brain has a smooth cortical surface (lissencephaly) caused by a lack of complexity of the outer cortex, and the migration of cerebellar granule cells is disrupted [63–65]. The malformation of the brain results from a haploinsufficiency of the gene *LIS-1*, which encodes a 45-kDa subunit of the brain PAF acetylhydrolase, an enzyme that converts PAF into the inactive lyso-PAF by removing the acetyl group on the *sn2* position of the PAF molecule [66–68]. PAF receptor activation evokes changes in the cytoskeleton, which could lead to a disruption of neuronal migration [69]. Application of the nonhydrolyzable PAF receptor agonist yields a dose-dependent decrease in cerebellar granule cell migration [70].

Cyclin-Dependent Kinase 5

Cyclin-dependent kinase 5 (Cdk-5) is a unique member of the cyclin-dependent kinases [71]. Unlike other cyclin-dependent kinases, 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 [72]. In the cerebella of $cdk5^{-/-}$-$cdk5^{+/+}$ mice, significant numbers of granule cells are located in the ML, suggesting a failure to complete migration to the IGL [73]. The cells found within the ML of chimeric cerebella are nearly all Cdk5-deficient, while the cells within the IGL are a mixture of $cdk5^{-/-}$ and $cdk5^{+/+}$ cells [73]. These results suggest that Cdk-5 plays crucial roles in the execution of granule cell migration.

9-O-Acetyl GD3

9-O-acetyl GD3, a ganglioside, is expressed in the developing nervous system [74]. Interestingly, 9-O-acetyl GD3 is localized at the contact sites between migrating granule cells and Bergmann glial processes in the ML

[74]. Application of Jones monoclonal antibody, which recognizes 9-O-acetyl GD3, blocks granule cell migration in a dose-dependent manner [74]. These results suggest that 9-O-acetyl GD3 is involved in Bergmann-glia-associated migration of granule cells in the ML.

Somatostatin

Somatostatin (STT), a neuropeptide, has 2 bioactive products, somatostatin-14 (SST-14) and somatostatin-28 (SST-28), which bind to all 5 somatostatin receptors [75, 76]. Granule cells express all 5 types of SST receptor before an initiation of migration, while differentiated granule cells in the adult do not express the receptors [77]. High levels of SST-14 are present in Purkinje cells, Golgi cells and climbing fibers, and SST-28 is present in Golgi cells and mossy fiber terminals [78, 79]. Application of SST-14 or SST-28 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 [79]. In contrast, an STT antagonist, AC-178,335, 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 [79]. These results indicate that endogenous STT differentially controls the migration of granule cells in a cortical-layer-specific manner: STT accelerates the tangential migration of granule cells near their birthplace within the EGL but slows down radial movement near their final destination within the IGL.

In the above sections, we described the multiple molecules that have been identified as critical regulators for granule cell migration. Although these molecules affect granule cell migration through activating or inhibiting the wide varieties of downstream targets, there are 3 common signaling pathways [Ca^{2+} signaling, cyclic nucleotide signaling and mitogen-activated protein kinase (MAPK) signaling], which may play central roles in controlling granule cell migration. The role of the Ca^{2+} signaling pathway in granule cell migration is supported by the evidence that the activation of N-type Ca^{2+} channels and NMDA receptors increases the frequency of spontaneous elevations of intracellular Ca^{2+} levels in migrating granule cell somata by stimulating the Ca^{2+} influxes, leading to the acceleration of migration [25, 39, 40]. PAF and SDF-1α induce intracellular Ca^{2+} mobilization, which stimulates granule cell migration [80–82]. Furthermore, tPA indirectly modulates the Ca^{2+} signaling pathway by enhancing the activity of NMDA receptors [83]. Interestingly, SST regulates the frequency of Ca^{2+} transients in the somata of migrating granule cells in a cortical-layer-specific manner [79]. Application of SST increases the frequency of Ca^{2+} transients in the migrating granule cells near their birthplace but decreases the frequency near their final destination [79]. Collectively, these results suggest that the Ca^{2+} signaling pathway plays a key role in converting the extracellular guidance signals to intracellular signals, which are responsible for controlling granule cell migration.

Cyclic nucleotide signaling pathways, including cyclic AMP (cAMP) and cyclic GMP (cGMP), are also potential downstream targets for controlling granule cell migration. For example, the activation of NMDA receptors increases intracellular cGMP levels through the sequential stimulation of the following systems: nitric oxide synthase-nitric oxide-soluble guanylyl cyclase-cGMP [84]. tPA may increase the production of cGMP by stimulating the activity of NMDA receptors [83]. Furthermore, PAF increases intracellular concentrations of cAMP via the activation of adenylate cyclase (AC) [80]. In contrast, SST decreases intracellular cAMP levels by inhibiting AC activity via stimulating the Gi subunit of Gα-coupled receptors [85]. Recently, it was reported that the rate of granule cell migration is very sensitive to changes in the intracellular levels of cGMP and cAMP [32]. Therefore, it is possible that NMDA receptors, tPA, PAF and SST control granule cell migration by altering the cyclic nucleotide signaling pathways.

MAPK signaling is another potential target for extracellular- and intracellular-signal-induced alterations of granule cell migration. For example, BDNF and NT-3 stimulate MAPK through the activation of TrkB and TrkC receptors [86–88]. SDF-1α increases the phosphorylation of extracellular-signal-regulated kinases 1 and 2 (ERK1/2) via the activation of CXCR4 receptors [82]. Furthermore, Cdk5 regulates MAPK kinase 1/2 activity [89]. Importantly, the inhibition of MAPK activity by specific inhibitors significantly reduces the rate of granule cell migration [32]. Taken together, these results indicate that MAPK signaling pathways play a key role in the regulation of granule cell migration by BDNF, NT-3, SDF-1α and Cdk5.

In the following sections we will review the studies examining how alcohol exposure causes abnormal development of the brain and mechanisms by which alcohol exposure impairs granule cell migration.

Effects of Alcohol on Brain Development

Alcohol is presently the most common chemical teratogen causing malformation and mental deficiency in humans [90, 91]. Prolonged exposure to alcohol during gestation and lactation correlates with a pattern of abnormal development in newborns [90–92]. Jones and Smith [93] called this developmental disturbance 'fetal alcohol syndrome' (FAS). Maternal exposure to alcohol may not lead to full expression of FAS but rather may result in a variety of less pronounced dysmorphic, cognitive and behavioral effects, often termed 'fetal alcohol effects (FAE)' [90, 91]. The world incidence of FAS is estimated as approximately 1.9 per 1,000 live births, while the incidence of FAE is thought to be as high as 1 in 300 live births [94]. Overt FAS, a more clearly defined endpoint than FAE, is one of the most common causes of mental retardation worldwide. The clinical features of FAS are distinctive. A severe disturbance of growth is the hallmark of the disorder [90–92]. At birth the infants manifest a distinct pattern of growth retardation, with length affected more than weight. The growth deficiency persists postnatally, but weight gain becomes more disturbed than linear growth. Abnormalities of growth are not altered appreciably by attempts to change environmental variables such as nutritional factors, home setting and the like. The most serious feature of the syndrome is disturbance of the central nervous system [90–93, 95]. Microcephaly is present in nearly all cases, and this reflection of disturbed brain growth is accompanied by delayed neurologic development in approximately 90% of the cases [90, 91, 96]. Particular defects of speech and language development are also evident [92]. In addition to serious cognitive deficits, a far-reaching and pervasive state of disability is induced by behavioral disturbances, impaired communication skills and maladaptive social function, manifested by lack of reciprocal friendships, impulsive behavior, anxiety and dysphoria. Children with FAS also show neurological signs associated with cerebellar damage such as delayed motor development, problems with fine tasks and ataxia [97, 98].

The fundamental mechanisms whereby alcohol leads to disturbances of brain development have not been elucidated. Current evidence favors the concept that alcohol or a metabolic product is responsible for the teratogenic effects expressed in FAS [99, 100]. Alcohol rapidly crosses the placenta and the blood-brain barrier of the fetus. Data obtained from a variety of experimental models suggest that alcohol, or its metabolite, can adversely affect the developing brain through multiple mechanisms [99, 100]. These include direct effects such as transient impairment of uterine vessels and reduced fetal cerebral metabolic rate. Moreover, alcohol is also capable of adversely affecting DNA methylation, neurotransmitter receptors, signal transduction, trophic support of neurons and membrane fluidization [99–102].

Abnormal Neuronal Migration in the Brains of FAS Patients

Several aspects of the developmental program are involved in the alcohol-induced malformation of the brain [103]. Among them, the most striking abnormalities appear to involve the impairment of neuronal and glial migration [104, 105]. The most common abnormality is a leptomeningeal neuroglial heterotopia that assumes the form of a sheet of aberrant neuronal and glial cells covering portions of the cerebral, cerebellar and brain stem surfaces [90]. Aberrations of brain stem and cerebellar development, in large part related to faulty cell migration, have also been especially frequent, along with the migrational disturbances of schizencephaly and polymicrogyria [106]. Disordered midline prosencephalic formation, e.g. agenesis of the corpus callosum, septo-optic dysplasia and incomplete holoprosencephaly, has also been documented [107]. Multiple aspects of central nervous system development can be affected, including proliferation, migration, differentiation and synapse formation.

Animal Models for Studying FAS and FAE

Pre- and/or neonatal exposure to alcohol also induces long-term neuromorphologic, neurochemical and behavioral changes in experimental animals [108, 109]. Some of these changes are observed in humans and thus led to the identification of FAS. In mice and rats, the early postnatal period is equivalent to fetal development in humans, and alcohol exposure causes a reduction of their brain weight, especially in the cerebellum [108–110]. The alcohol-exposed rats are hypoactive during treatment and exhibit a reduction in cerebellar Purkinje cell numbers, especially in the early maturing lobules [111]. Furthermore, the numbers of granule cells in the IGL are significantly reduced in the alcohol-treated animals [112], suggesting that alcohol affects granule cell migration to the IGL. Due to the obvious limitations of human studies, animal models of FAS have been used to further docu-

ment the phenomenon of alcohol-induced defects in brain development and to study underlying mechanisms.

Previous Studies on the Effects of Alcohol on Neuronal Migration in the Brain other than the Cerebellum Using Animal Models

Prenatal exposure of ethanol induced morphological defects in the rat cerebral cortex, including heterotopia and disorganized cortical architecture, suggesting abnormal migration [113]. In the rat cerebrum, prenatal ethanol exposure delays the migration of early generated neurons to the deep cortical layers by 2 days and the migration of late generated neurons to the upper cortical layers by 4–6 days [105]. Moreover, ethanol exposure also reduces the rate of neuronal migration in the rat cerebrum [114]. It has been suggested that a likely mechanism underlying alcohol-induced defects in neuronal migration is disrupted cell adhesion. Neuronal cell adhesion molecule (nCAM) and L1 mediate neuronal migration through homophilic binding and consequent activation of intracellular signaling cascades central to cytoskeleton reorganization [115]. Ethanol inhibits L1-L1-mediated cell adhesion by physically blocking L1-L1 homophilic binding [116]. Furthermore, ethanol increases nCAM protein expression in cortical neurons [117]. Interestingly, ethanol-induced increases in nCAM are noted on the surface of migrating cortical neurons [114]. It has been shown that ethanol exposure disrupts transforming-growth-factor-β-mediated migration of cortical neurons [114].

Alcohol Inhibits Granule Cell Migration in the Developing Cerebellum

Recently, an important advancement was made in understanding the cellular and molecular mechanisms underlying ethanol-induced abnormal migration of cerebellar granule cells [32]. In the following sections, we examine how alcohol impairs granule cell migration in the developing cerebellum. To examine cellular and molecular mechanisms by which alcohol affects neuronal migration, the cerebellum of the early postnatal mouse is used as a model system, since the exposure to ethanol induces quantitative morphological changes in the rodent cerebellum [111, 112, 118]. Real-time observation of cell movement in cerebellar slices reveals that the administration of 100 mM of ethanol immediately slows the tangential migration of granule cells in the EGL of P10 mice [32]. Pharmacologically relevant concentrations of ethanol are based on blood-ethanol concentrations attained by alcohol consumption in humans [119]. Since ethanol readily crosses the placental and blood-brain barriers, and diffuses rapidly into all aqueous compartments of the body, these levels would readily be found in cases of alcohol consumption during pregnancy [120]. The effects of ethanol on the rate of granule cell migration in the cerebellar slices obtained from P10 mice are dose-dependent [32]. Although 2.5 mM of ethanol fails to alter the rate of cell movement, 10 mM of ethanol (equivalent to a blood ethanol level <50 mg/dl) significantly decreases the rate to 62% (EGL), 76% (ML) and 82% (IGL) of the control value. In the presence of 50 mM of ethanol, the rate is further reduced to 40% (EGL), 55% (ML) and 62% (IGL) of the controls. Finally, when 100 mM of ethanol is added, movement declines to 35% (EGL), 50% (ML) and 56% (IGL) of the control. It is noteworthy that the vulnerability of granule cells to ethanol exposure decreases as the cells migrate from the EGL to the IGL. The differential vulnerability suggests that the stage of differentiation (or maturation) is critical in producing the harmful effects of ethanol on granule cell migration.

Ethanol may alter the motility of granule cells directly, or indirectly by modifying the surrounding environment. For example, ethanol exposure affects the development and functions of glia, which in turn may alter granule cell motility [121]. To determine whether ethanol directly affects granule cell migration, microexplant cultures of P0–P3 mouse cerebella, in which isolated granule cells actively migrate in the absence of cell-cell contact, are used [38]. Application of ethanol at concentrations ranging from 25 to 200 mM appreciably slows the movement of isolated granule cells [32]. The average rate of cell movement is reduced to 85% (25 mM of ethanol), 71% (50 mM of ethanol), 63% (100 mM of ethanol) and 46% (200 mM of ethanol) of the control [32]. These results indicate that ethanol directly acts on granule cell migration in a dose-dependent manner.

Calcium Signalings Play a Role in Alcohol Action on Migration

How does alcohol slow the migration of granule cells? Although the effects of alcohol are initially believed to arise from alcohol-induced perturbations in the order of membrane lipids, the effects on membrane lipids are ac-

tually quite small at clinically relevant concentrations [122]. However, even low levels of alcohol can modulate the functions of voltage-gated and ligand-gated Ca^{2+} channels by binding to a hydrophobic pocket on the proteins [123]. This suggests that alcohol may affect the intracellular Ca^{2+} levels of migrating granule cells. This is intriguing because granule cell migration is highly sensitive to changes in intracellular Ca^{2+} levels and Ca^{2+} signaling [18, 40, 41]. Indeed, the use of a Ca^{2+} indicator dye reveals that application of ethanol significantly decreases the frequency of spontaneous Ca^{2+} transients in the granule cell somata in a dose-dependent manner [32], suggesting that one of the cellular mechanisms underlying alcohol action on granule cell migration is the alteration of Ca^{2+} signaling.

If alcohol slows granule cell migration by inhibiting Ca^{2+} signaling, enhancing Ca^{2+} release from internal Ca^{2+} stores or Ca^{2+} influxes across the plasma membrane may alter alcohol action on granule cell migration. To test this, (a) caffeine, which increases internal Ca^{2+} release through the ryanodine receptors, and (b) NMDA, which induces Ca^{2+} influxes through the NMDA-type glutamate receptors, are applied with ethanol. Intoxicating levels of alcohol are known to reduce the activity of these receptors [124, 125]. Application of 1 mM of caffeine or 30 μM of NMDA noticeably reduces the effects of 25–100 mM of ethanol on the rate of granule cell movement [32]. These results suggest that ethanol affects granule cell migration by altering multiple components of Ca^{2+} signaling.

Changes in Cyclic Nucleotide Signaling Pathways Affect Alcohol Action on Migration

Alcohol may also inhibit granule cell migration via altering cyclic nucleotide signaling pathways, such as those involving cAMP or cGMP. An intraperitoneal injection of ethanol (5 g/kg body weight) into P10 mice markedly increases cAMP levels but decreases cGMP levels in the cerebellum 1 h after injection [32]. Without ethanol, the stimulation of AC with forskolin, which is upstream of cAMP signaling, markedly reduces the rate of granule cell movement; however, the inhibition of protein kinase A (PKA) with PKI, which is downstream of cAMP signaling, markedly increases the migration rate [32]. Similarly, without ethanol, application of a competitive cAMP agonist, Sp-cAMPS, reduces the rate of granule cell migration, whereas a competitive cAMP antagonist, Rp-cAMPS, increases the rate. These results demonstrate that without ethanol, the stimulation of cAMP signaling slows down granule cell migration, whereas the inhibition of cAMP signaling accelerates migration. Importantly, the inhibition of PKA activity with PKI significantly reduces the effects of 25–50 mM of ethanol on the rate of granule cell migration but fails to change the action of 100 mM of ethanol on the migration [32]. Application of Rp-cAMPS completely reverses the effects of 25–100 mM of ethanol on the rate of granule cell migration [32]. Moreover, the inhibition of the activity of AC with 9CP-Ade significantly reduces the effects of 100 mM of ethanol [32]. In contrast, the stimulation of the AC activity with forskolin significantly enhances the effects of 25 mM of ethanol on the rate of granule cell migration. These results demonstrate that alcohol action on granule cell migration is highly sensitive to changes in the activity of cAMP signaling pathways: stimulating cAMP signaling amplifies alcohol action on granule cell migration, whereas inhibiting cAMP signaling reduces action. Because the application of alcohol increases cAMP levels, alcohol may slow granule cell migration by stimulating cAMP signaling pathways.

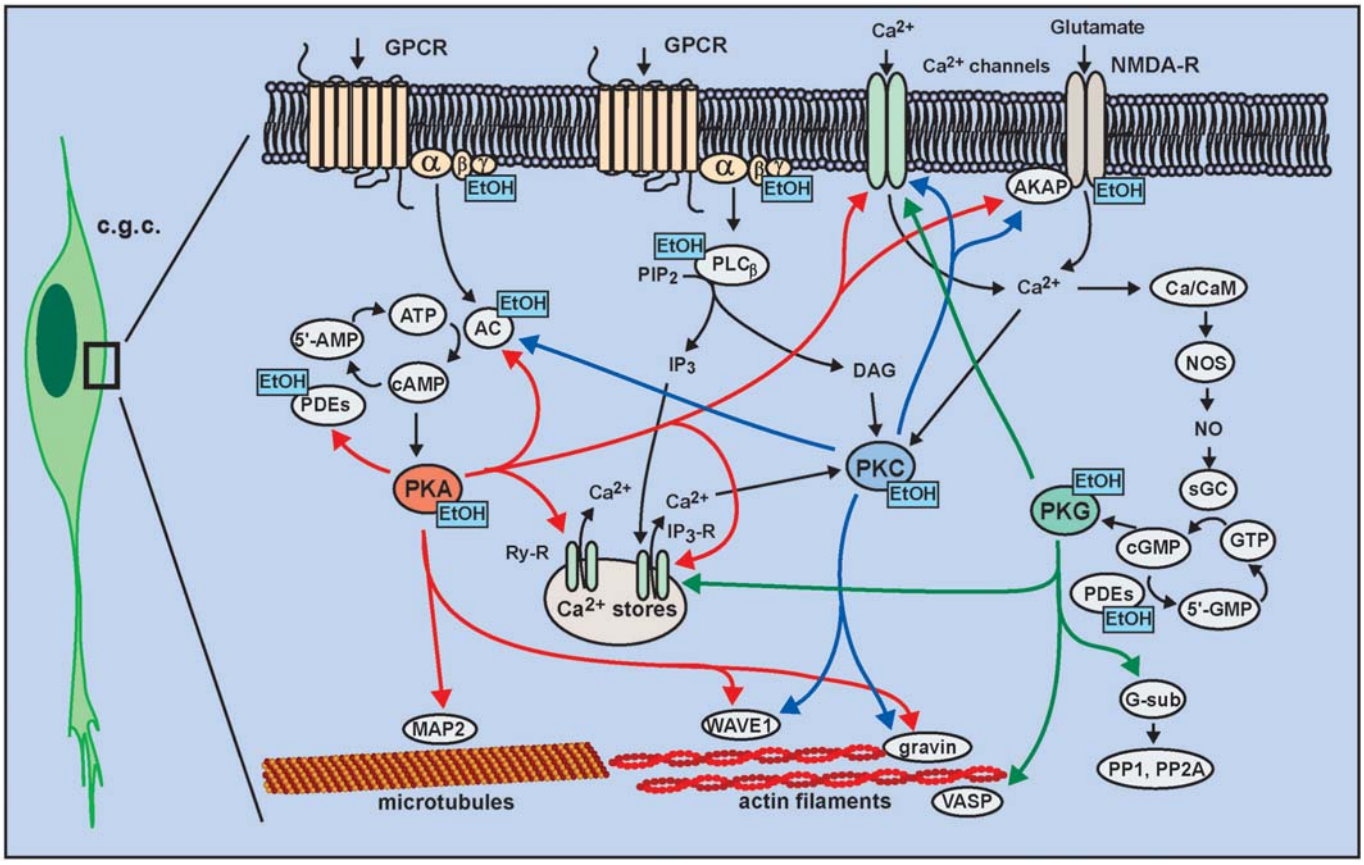
In the case of the cGMP signaling pathway, without ethanol, stimulating cGMP signaling with cGMP analogue, Br-cGMP, increases the rate of granule cell movement, whereas inhibiting cGMP signaling with cGMP antagonist, Rp-8-pCPT-cGMPS, decreases the rate [32]. Interestingly, application of Br-cGMP markedly reduces the effects of 25–100 mM of ethanol on granule cell migration [32]. In contrast, Rp-8-pCPT-cGMPS does not change ethanol action on granule cell migration [32]. These results demonstrate that cGMP signaling is also a target for the alcohol action on granule cell migration. Alcohol may slow granule cell migration by lowering the activity of cGMP signaling pathways.

If alcohol affects granule cell migration by altering the cAMP and cGMP signaling pathways, one mechanism controlling alcohol action might be the degradation of these cyclic nucleotides. Indeed, the alterations of the activity of cyclic nucleotide phosphodiesterases (PDE), which catalyze the hydrolysis of cAMP and/or cGMP, change alcohol action on granule cell migration [19]. For example, application of EHNA, a PDE2 inhibitor that blocks the cGMP-dependent cleavage of cAMP and cGMP, significantly reduces ethanol action on granule cell migration [32]. In contrast, 8-MM-IBMX, a PDE1 inhibitor that blocks the Ca^{2+}/calmodulin-dependent cleavage of cAMP and cGMP, does not significantly change the effects of ethanol on migration [32]. These results indicate that alcohol may affect granule cell migration by

a

stimulation / inhibition	The effects on rate of granule cell migratin	The effects on EtOH-induced impairment of granule cell migratin	stimulation / inhibition	The effects on rate of granule cell migratin	The effects on EtOH-induced impairment of granule cell migratin
NMDA-R by NMDA	Acceleration	Reduce the effects	GC by ODQ	No effect	Did not alter the effects
Rya-R by caffeine	No effect	Reduce the effects	cGMP by Br-cGMP	Acceleration	Reduce the effects
nAch-R by nicotine	No effect	Amplify the effects	cGMP by Rp-8-pCPT -cGMPS	Reduction	Did not alter the effects
Ca^{2+} by BAPTA	Reduction	Amplify the effects	PKA by PKI	Acceleration	Reduce the effects
PKC by calphostin C	Reduction	Amplify the effects	MAPK by U0126	Reduction	Did not alter the effects
CaMKII by KN93	No effect	Did not alter the effects	PI3K by LY294002	Reduction	Did not alter the effects
Rho by H-1152	Reduction	Amplify the effects	PP1 by tautomycin	Reduction	Did not alter the effects
AC by forskolin	Reduction	Amplify the effects	PP2B by deltamethrin	Reduction	Did not alter the effects
cAMP by Sp-cAMPS	Reduction	Did not alter the effects	PDE1 by 8-MM-IBMX	No effect	Did not alter the effects
cAMP by Rp-cAMPS	Acceleration	Reduce the effects	PDE2 by EHNA	No effect	Reduce the effects

b



altering the amplitude and duration of cyclic nucleotide signals by modifying the activity of a specific PDE family.

Ca^{2+}-Transient-Dependent and -Independent Mechanisms of the Reversal of Alcohol Action on Migration

Changes in the frequency of Ca^{2+} transients positively correlate with changes in the rate of granule cell migration [40, 41]. Do caffeine, NMDA, cAMP antagonists, and cGMP agonists reduce alcohol action on granule cell migration by increasing the frequency of Ca^{2+} transients? The use of a Ca^{2+} indicator dye reveals that without ethanol, the application of NMDA significantly increases the frequency of Ca^{2+} transients in migrating granule cells, whereas caffeine does not affect the frequency [32]. Importantly, application of NMDA or caffeine markedly reduces the effects of ethanol on the frequency of Ca^{2+} transients in granule cells [32]. In the case of cAMP signaling pathways, without ethanol, the stimulation of AC activity with forskolin significantly reduces the frequency of Ca^{2+} transients, while the inhibition of cAMP signaling with Rp-cAMPS significantly increases the frequency [32]. It is also noteworthy that Rp-cAMPS completely eliminates the effects of ethanol on the frequency of Ca^{2+} transients [32]. In contrast, neither stimulating cGMP signaling with Br-cGMP nor inhibiting the activity of PDE2 with EHNA changes the effects of ethanol on the frequency of Ca^{2+} transients [32]. These results indicate that Ca^{2+} signaling and cAMP signaling may reverse alcohol action on granule cell migration by increasing the frequency of Ca^{2+} transients in their somata, whereas cGMP signaling and cyclic nucleotide PDE may reduce alcohol action on migration without altering Ca^{2+} transients.

Fig. 2. Cellular mechanisms underlying alcohol-induced impairment of granule cell migration. **a** The roles of signaling molecules on granule cell migration and the effects of these molecules on alcohol-induced impairment of granule cell migration are summarized. **b** Schematic drawing showing the effects of ethanol on the cAMP-PKA, Ca^{2+}-PKC and cGMP-PKG signaling pathway and possible interactions between these signaling pathways in the migrating granule cells. AKAP = A-kinase anchoring proteins; c.g.c. = cerebellar granule cells; GPCR = G-protein-coupled receptors; NOS = nitric oxide synthase; NO = nitric oxide; sGC = soluble guanylyl cyclase.

Requirement of the Activities of Multiple Downstream Targets for Reversing Alcohol Action on Migration by Calcium and Cyclic Nucleotide Signaling

What downstream effectors are involved in the reversal of alcohol action on granule cell migration by altering Ca^{2+}, cAMP or cGMP signaling? Although these signals interact with large varieties of downstream targets, protein kinase C (PKC), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), calcineurin, protein phosphatase 1 (PP1), Rho GTPase, MAPK and phosphoinositide 3-kinase (PI_3K) are chosen as potential targets because these molecules are involved in controlling the motility of various types of cells [126–128]. The use of pharmacological tools reveals that the reversal of alcohol action on granule cell migration by controlling Ca^{2+}, cAMP/cGMP signaling pathways requires the activities of multiple but distinctive downstream effectors: (1) caffeine needs the activities of CaMKII, calcineurin, PP1, Rho GTPase, MAPK and PI_3K; (2) NMDA needs the activities of PKC, CaMKII, calcineurin, PP1, MAPK and PI_3K; (3) Rp-cAMP needs the activities of PKC, CaMKII, calcineurin, PP1, Rho GTPase and PI_3K, and (4) Br-cGMP needs the activities of PP1, Rho GTPase, MAPK and PI_3K [32].

The molecules, receptors and intracellular signaling pathways which are involved in the alcohol-induced impairment of granule cell migration are summarized in figure 2a, b.

Elimination of Alcohol Action on Migration in vivo by Controlling Calcium and Cyclic Nucleotide Signaling Pathways

The effects of alcohol on granule cell migration in vitro are significantly reduced by controlling Ca^{2+}, cAMP and cGMP signaling pathways [32]. Could controlling Ca^{2+}, cAMP and cGMP signaling pathways also reduce the effects of alcohol on granule cell migration in vivo? A single intraperitoneal injection of ethanol (5 g/kg body weight) into P10 mice raises blood ethanol levels to approximately 80 mM (equivalent to a blood ethanol level <400 mg/dl) within 1 h after injection, and prevents cerebellar granule cells from entering the ML and slows their radial migration in the ML, PCL and IGL [32]. Importantly, injections of caffeine (2 mg/kg body weight), Rp-cAMPS (0.4 mg/kg body weight) or Br-cGMP (0.4 mg/kg body weight) into the subarachnoid space between the skull and the surface of the P10 mouse cerebellum

with a single intraperitoneal injection of ethanol (5 g/kg body weight) completely reverses the effects of ethanol on granule cell migration in vivo [32]. In contrast, the administration of NMDA (>0.01 mg/kg body weight) into the subarachnoid space between the skull and the surface of the cerebellum often causes the death of injected pups possibly from neurotoxic effects of high doses of NMDA [32]. These results demonstrate that the effects of alcohol on granule cell migration in vivo can be reduced by controlling Ca^{2+}, cAMP and cGMP signaling pathways.

Future Works to Further Understand Neuronal Migration in FAS

Recent studies suggest the involvement of the Ca^{2+}-PKC, cAMP-PKA and cGMP-PKG signaling pathways in the alcohol-induced impairment of granule cell migration. These 3 signaling pathways interact with each other (schematically shown in fig. 2b). For example, the activity of adenylate cyclase 7 (AC7) is modified by PKC, and alcohol potentiates AC7 activity by altering the activity of PKCδ [129]. The stimulation of PKA and PKG enhances the activity of voltage-dependent N-type and L-type Ca^{2+} channels, which in turn affect the Ca^{2+} signaling pathway [130, 131]. Activation of the cAMP signaling pathways alters internal Ca^{2+} release through PKA-mediated phosphorylation of inositol 1,4,5-triphosphate receptors and ryanodine receptors [132, 133]. Moreover, the activity of PKA regulates NMDA receptor activity through an interaction with A-kinase anchoring protein 79 [134]. Therefore, it may be worth examining whether and how the interactions between the cAMP-PKA, Ca^{2+}-PKC and cGMP-PKG signaling pathways play roles in controlling alcohol action on granule cell migration.

Alcohol also directly and indirectly induces alterations in the activity of other signaling molecules (such as Rho GTPase, MAPK/ERK). There is a functional link between the activity of Rho GTPase and the activities of Ca^{2+} and PKA signaling. The changes in intracellular Ca^{2+} levels regulate Rho GTPase activity in various types of cells, although how the changes in Ca^{2+} levels modulate Rho GTPase activity is essentially unclear [135]. Furthermore, cAMP/PKA is involved in controlling cell migration and cytoskeletal organization via activation of Rho GTPase [136]. Interestingly, alcohol decreases the activity of MAPK/Erk in immature neurons [137]. The reduction of MAPK/Erk activity may be involved in the alcohol-induced impairment of granule cell migration. This is because the reduction of phosphorylated Erk1 and Erk2 by inhibition of MAPK kinase inhibits neurite outgrowth in cerebellar granule cells [138]. Moreover, PKC and PKA alter the phosphorylation of Erk [139]. Whether alcohol impairs granule cell migration via altering the activity of Rho GTPase and/or MAPK/Erk, remains to be examined.

Conclusion

In summary, granule cells alter the mode, tempo and direction of migration when they traverse through different cortical layers in the early postnatal brain. These alterations of granule cell migration are controlled by 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 STT. Furthermore, the internal (intrinsic) programs also play a role in controlling the sequential changes in granule cell migration. In the brains of FAS patients, the migration of immature neurons is severely disrupted. Recent studies suggest that Ca^{2+} signaling and cyclic nucleotide signaling are the central targets of alcohol action in neuronal migration. Importantly, experimental manipulations of these second-messenger pathways, through stimulating Ca^{2+} and cGMP signaling or inhibiting cAMP signaling, completely reverse alcohol action on neuronal migration in vitro as well as in vivo. The results suggest that the aberrant migration of immature neurons in the fetal brain caused by maternal alcohol consumption may be corrected by controlling the activity of these second-messenger pathways.

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