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The Role of Calcium and Cyclic Nucleotide Signaling in Cerebellar Granule Cell Migration under Normal and Pathological Conditions

Yutaro Komuro,[1] Ludovic Galas,[2,3] Alexis Lebon,[2,3,4,5] Emilie Raoult,[2,3,4,5] Jennifer K. Fahrion,[1] Amanda Tilot,[1] Tasturo Kumada,[6] Nobuhiko Ohno,[7] David Vaudry,[2,3,4,5] Hitoshi Komuro[1]

[1] Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195

[2] INSERM, PRIMACEN, Cell Imaging Platform of Normandy, 76821 Mont-Saint-Aignan, France

[3] University of Rouen, Institute for Research and Innovation in Biomedicine (IRIB), Rouen, France

[4] INSERM, U982, DC2N, 76821 Mont-Saint-Aignan, France

[5] International Associated laboratory Samuel de Champlain, INSERM-INRS, France, Canada

[6] Department of Occupational Therapy, Tokoha University, Hamamatsu 431-2102, Japan

[7] Department of Anatomy and Molecular Histology, Interdisciplinary Graduate school of Medicine and Engineering, University of Yamanashi, Yamanashi 409-3898, Japan

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ABSTRACT: **In the developing brain, immature neurons migrate from their sites of origin to their final destination, where they reside for the rest of their lives. This active movement of immature neurons is essential for the formation of normal neuronal cytoarchitecture and proper differentiation. Deficits in migration result in the abnormal development of the brain, leading to a variety of neurological disorders. A myriad of extracellular guidance molecules and intracellular effector molecules is involved in controlling the migration of immature neurons in a cell type, cortical layer and birth-date-specific manner. To date, little is known about how extracellular guidance molecules transfer their information to the intracellular effector molecules, which regulate the migration of immature neurons. In this article, to fill the gap between extracellular guidance molecules and intracellular effector molecules, using the migration of cerebellar granule cells as a model system of neuronal cell migration, we explore the role of second messenger signaling (specifically Ca^{2+} and cyclic nucleotide signaling) in the regulation of neuronal cell migration. We will, first, describe the cortical layer-specific changes in granule cell migration. Second, we will discuss the roles of Ca^{2+} and cyclic nucleotide signaling in controlling granule cell migration. Third, we will present recent studies showing the roles of Ca^{2+} and cyclic nucleotide signaling in the deficits in granule cell migration in mouse models of fetal alcohol spectrum disorders and fetal Minamata disease.**

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Keywords: **granule cell migration; Ca^{2+} signaling; cyclic nucleotide signaling; fetal alcohol spectrum disorders; fetal Minamata disease.**

Correspondence to: H. Komuro (hitoshikomuro@gmail.com).

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INTRODUCTION

During the long journey from the birthplace to the final destination, the direction, speed, and mode of migration of immature neurons are controlled by a myriad of extracellular guidance molecules expressed at the right place and right time (Rakic et al., 1994; Rakic and Komuro, 1995; Yacubova and Komuro, 2002a, 2003; Botia et al., 2007; Falluel-Morel et al., 2011; Li et al., 2012; Fahrion et al., 2013; Raoult et al., 2011, 2014). The last two decades have yielded several critical advances in our understanding of the role of extracellular guidance molecules in controlling neuronal cell migration (Sarkisian et al., 2008; Cameron et al., 2007, 2009; Valiente and Marín, 2010; Komuro et al., 2012; Zhu and Murakami, 2012; Evsyukova et al., 2013), but little is known about the sequential intracellular events triggered by the initial response to the extracellular guidance molecules that lead to the assembly and disassembly of cytoskeletal elements and alteration of migration. In this review, to fill the gaps between the extracellular guidance molecules and the intracellular effector molecules, which regulate cytoskeletal dynamics, we will explore the role of Ca^{2+} and cyclic nucleotide signaling in the migration of cerebellar granule cells. This is because these signaling pathways are known to be intracellular downstream targets for many extracellular guidance molecules (Nishiyama et al., 2003; Ooashi et al., 2005; Murray et al., 2009; Nicol et al., 2011; Forbes et al., 2012). Furthermore, granule cell migration has provided an ideal system for determining cellular mechanisms underlying neuronal cell migration (Komuro et al., 2013). It has become apparent that cellular mechanisms underlying the regulation of granule cell migration are also utilized during the migration of immature neurons in other brain regions (Komuro and Rakic, 1998b, 1999; Komuro and Yacubova, 2003).

In the last part of this review, we will discuss the role of Ca^{2+} and cyclic nucleotide signaling in deficits in granule cell migration caused by exposure to toxic substances, such as alcohol and methylmercury (MeHg). Exposure to alcohol or MeHg during embryonic and early postnatal development often results in the abnormal development of babies, which are known as fetal alcohol spectrum disorders (FASD) (Riley et al., 2011; Paintner et al., 2012; Coriale et al., 2013) and fetal Minamata disease (FMD) (Harada, 1978; Nakai and Satoh, 2002; Counter and Buchanan, 2004). Patients with FASD and FMD exhibit abnormal brain development, including morphological and neurological abnormalities (Harada, 1964; Synder, 1971; Marcus, 1987; Eto, 1997; Riley and McGee, 2005; Welch-Carre, 2005). Although exposure to alcohol or MeHg could affect many aspects of brain development, such as proliferation, differentiation, and cell death, it has long been assumed that exposure to alcohol and MeHg inhibits the migration of immature neurons (Miller, 1986, 1993; Kunimoto and Suzuki, 1997; Kakita, 2002). In this review, we will present recent studies showing (1) how exposure to alcohol and MeHg causes deficits in granule cell migration, and (2) the role of Ca^{2+} and cyclic nucleotide signaling in alcohol- and MeHg-induced deficits in granule cell migration using mouse models of FASD and FMD.

CORTICAL LAYER-SPECIFIC ALTERATIONS OF GRANULE CELL MIGRATION IN THE DEVELOPING CEREBELLUM

Granule cells alter the direction, mode and speed of their migration as they traverse different cerebellar cortical layers as schematically presented in Fig. 1. In this section, we will depict the migration of granule cells from their birthplace (the external granular layer: EGL) to their final destination (the internal granular layer: IGL).

Tangential Migration in the EGL

After clonal expansion in the EGL, granule cell precursors begin to produce postmitotic granule cells during the first and second postnatal weeks (Miale and Sidman, 1961). Shortly after the final mitosis, postmitotic granule cells in the EGL start to migrate tangentially in the direction of their larger process, coincident with the extension of two uneven horizontal processes oriented parallel to the longitudinal axis of the folium. Interestingly, the morphology and migration speed of granule cells change systematically with their position within the EGL (Komuro et al., 2001). For example, the migration speed is fastest in the middle of the EGL, when granule cells have two short horizontal processes. As granule cells elongate their somata and extend longer horizontal processes at the bottom of the EGL, they move at a reduced speed. At the EGL-molecular layer (ML) border where granule cells migrate tangentially at the slowest speed, their somata become round, and then begin to extend pairs of descending processes into the ML. At the EGL-ML border, granule cells retain two elongated horizontal processes while their nuclei and surrounding cytoplasm start to enter into the short vertical process descending into the ML. After the completion of the translocation of the nucleus and

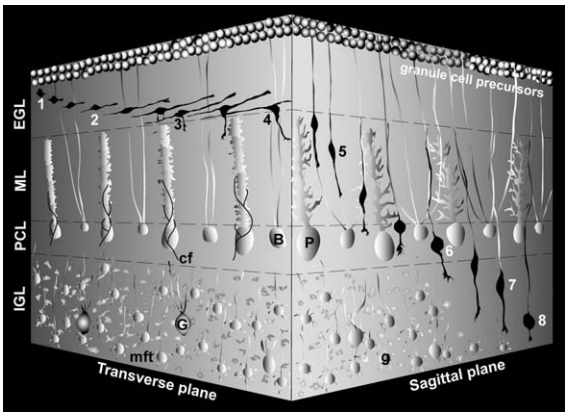


Figure 1 A three-dimensional representation of granule cell migration from their birthplace, the EGL, to their final destination, the IGL, in the developing cerebellum. 1, Extension of two uneven horizontal processes near the top of the EGL; 2, Tangential migration in the middle of the EGL; 3, Development of vertical processes near the border between the EGL and the 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 PCL; 7, Glia-independent radial migration in the IGL; 8, Completion of migration in the bottom of the IGL. EGL, external granular layer; B, Bergmann glia; G, Golgi cell; cf, climbing fiber; g, postmigratory granule cell; IGL, internal granular layer; mft, mossy fiber terminal; ML, molecular layer; P, Purkinje cell; PCL, Purkinje cell layer.

surrounding cytoplasm from the horizontally extended process to the vertical process, granule cell somata start to move towards the bottom of the ML.

Bergmann Glia-Associated Radial Migration in the ML

In the ML, migrating granule cells have a vertically elongated cell body, a thin trailing process, and a more voluminous leading process. The granule cell soma, trailing process, and leading process are closely attached to the surface of Bergmann glial fibers, suggesting that granule cells move along Bergmann glial fibers during the entire translocation of the soma across the ML (Rakic, 1971). Granule cell movement in the ML is characterized by alternations of short stationary phases with movement in a forward direction (Komuro and Rakic, 1995). The net displacement of granule cells depends on the duration and frequency of these phases as well as on the speed of movement.

Stationary State in the PCL

At the bottom of the ML, the vertically elongated granule cell somata move towards the Purkinje cell layer (PCL), while the length of their leading process gradually decreases. Upon entering the PCL, the shape of granule cell somata transforms from a vertically elongated spindle to a sphere (Komuro and Rakic, 1998a). These rounded somata significantly slow their movement, which stop completely in the PCL. The rounded somata remain stationary in the PCL for approximately 2 hr. After a prolonged stationary period, granule cells in the PCL begin to re-extend their somata and leading processes. During this transformation, granule cells gradually accelerate their migration speed and cross the PCL-IGL border.

Glia-Independent Radial Migration in the IGL

In the IGL, the spindle-shaped granule cells migrate towards the bottom of the IGL at a speed comparable to that recorded for granule cells migrating along Bergmann glial fibers within the ML (Komuro and Rakic, 1998a). The long axis of the granule cell soma remains oriented perpendicular to the PCL-IGL boundary line during this radial migration. Granule cells migrate radially towards the deep strata of the IGL in the absence of guidance cues provided by Bergmann glial cells.

Completion of Migration near the IGL-WM Border

Once the tip of the leading process approaches the IGL-white matter (WM) border, the granule cell soma becomes rounded. Granule cells then slow their migration and stop their movement near the IGL-WM border (Komuro and Rakic, 1998a). In the P10 mouse cerebellum, granule cells move from the top of the EGL through the ML and the PCL to their final position in the bottom of the IGL within about 2 days after the initiation of their tangential migration in the middle of the EGL (Komuro and Yacubova, 2003).

CONTROL OF GRANULE CELL MIGRATION BY CALCIUM AND CYCLIC NUCLEOTIDE SIGNALING

To alter the direction, mode, tempo and speed of migration of immature neurons at the right place and right time, Ca^{2+} and cyclic nucleotide signaling plays a key role in converting the information from locally expressed guidance molecules to intracellular effector molecules, which control migration by regulating the dynamics of cytoskeletal components (Zheng and Poo, 2007; Nicol et al., 2011; Forbes et al., 2012;

Sheng et al., 2013). In the following sections, we will review the role of Ca^{2+} and cyclic nucleotide signaling in the control of granule cell migration.

Role of Ca^{2+} Signaling in the Control of Granule Cell Migration

The First Study Reporting the Role of Ca^{2+} Signaling in Granule Cell Migration. In 1992, real-time recordings of cell movement in slice preparations demonstrated that the inhibition of N-type Ca^{2+} channel activity by its specific antagonist, ϖ-conotoxin GVIA, decelerates granule cell migration in the ML of P10 mouse cerebellum (Komuro and Rakic, 1992). The following experiments testing the effects of the alterations of extracellular Ca^{2+} concentrations on migration confirm the idea that Ca^{2+} influxes via N-type Ca^{2+} channel play a role in granule cell migration. For example, lowering extracellular Ca^{2+} concentrations, which can result in reductions of Ca^{2+} influxes, decelerates granule cell migration in the ML, whereas increasing extracellular Ca^{2+} concentrations, which can result in increases of Ca^{2+} influxes, accelerates migration in the ML. These results suggest that Ca^{2+} influxes, especially through the N-type Ca^{2+} channels, regulate the speed of granule cell migration.

Ca^{2+} Influxes Through NMDA Receptor Affect Granule Cell Migration. The discovery of the role of Ca^{2+} influxes through N-type Ca^{2+} channels in granule cell migration led to studies examining the role of *N*-methyl-D-aspartate (NMDA) glutamate receptors, which also induce Ca^{2+} influxes, in migration. Migrating granule cells express the NR1 and NR2A or NR2B subunits of NMDA receptors, whereas postmigratory granule cells in the IGL express the NR1 and NR2C subunits (Farrant et al., 1994; Monyer et al., 1994). Migrating granule cells exhibit spontaneous activation of NMDA receptors (Rossi and Slater, 1993). The frequency of NMDA receptor activation is low in the EGL, and gradually increases as granule cells move towards the IGL (Rossi and Slater, 1993). Real-time recordings of cell movement in cerebellar slices reveal that the inhibition of NMDA receptor activity with its antagonists (D-AP5 or MK-801) decreases the speed of granule cell migration in the ML of P10 mouse cerebellum (Komuro and Rakic, 1993). The elevation of extracellular glutamate concentrations by inhibiting glutamate uptake by astrocytes increases the frequency of spontaneous activation of NMDA receptors and accelerates granule cell migration in the ML. These results suggest that the activation of NMDA receptors by endogenous extracellular glutamate accelerates granule cell migration by increasing Ca^{2+} influxes.

Migrating Granule Cells Exhibit Spontaneous Elevations of Intracellular Ca^{2+} Levels in their Somata. Since activation of N-type Ca^{2+} channels and NMDA receptors can induce Ca^{2+} influxes into cells, it is possible that migrating granule cells exhibit spontaneous elevations in intracellular Ca^{2+} levels. Time-lapse recordings of intracellular Ca^{2+} levels reveal that in microexplant cultures of the early postnatal mouse cerebellum, migrating granule cells exhibit spontaneous Ca^{2+} elevations (Ca^{2+} spikes) with average frequencies of 13 times/hr (Komuro and Rakic, 1996). There is a positive correlation between the migration speed and both the amplitude and frequency components of Ca^{2+} spikes, suggesting that both the amplitude and frequency components of Ca^{2+} spikes are one of the intracellular signals controlling the speed of granule cell migration (Komuro and Rakic, 1996).

Causal Relationships Between Ca^{2+} Spikes and Migration Speed. The role of Ca^{2+} spikes in granule cell migration is supported by the following studies. Inhibiting N-type Ca^{2+} channels with ϖ-conotoxin GVIA, inhibiting NMDA receptors with D-AP5, or lowering extracellular Ca^{2+} concentrations reduces the amplitude and frequency of Ca^{2+} spikes, (Komuro and Rakic, 1996). Importantly, these reductions in the amplitude and frequency of Ca^{2+} spikes of granule cells are linearly related to changes in the speed of granule cell migration. Furthermore, the reduction of Ca^{2+} release from intracellular Ca^{2+} stores by thapsigargin decreases the amplitude of Ca^{2+} spikes and slows granule cell movement without significant effects on the frequency of Ca^{2+} spikes (Komuro and Rakic, 1996). The causal relationship between the changes in Ca^{2+} spikes and migration speed is confirmed by experimentally induced-ectopic Ca^{2+} spikes (Komuro and Rakic, 1996). For example, pulse-like application of 30 m*M* KCl induces large elevations in intracellular Ca^{2+} levels and concomitant increases in the forward movement of granule cells. Pulse-like application of KCl also increases the distance traveled by granule cells during the accelerated forward movement. These results indicate that the combination of amplitude and frequency of Ca^{2+} spikes provides an intracellular signal for controlling granule cell migration.

Granule Cells Exhibit Distinct Patterns of Ca^{2+} Spikes along their Migratory Pathway. If Ca^{2+} spikes regulate the migration of granule cells, the cells would

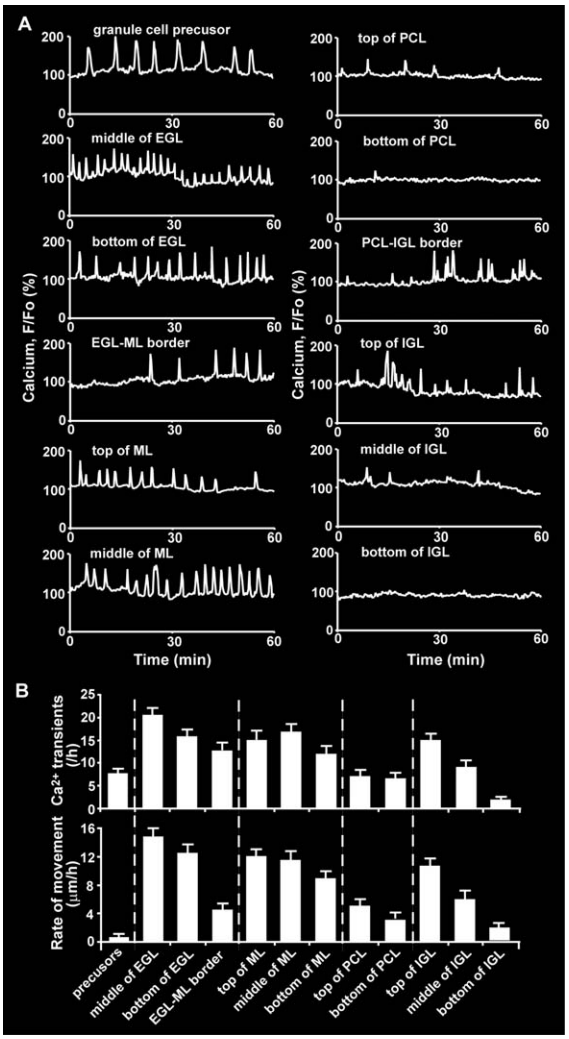


Figure 2 Alterations of the Ca^{2+} transients of granule cells from the initiation to the completion of migration. A. Sequential changes in the Ca^{2+} transients of granule cells along the migratory pathway. B. Co-relation between the changes in the Ca^{2+} transient frequency (top) and migration rate (bottom) of granule cells along the migratory pathway. Bar, S.D.

exhibit different amplitudes and frequencies of Ca^{2+} spikes when they migrate through different terrains. Indeed, distinct patterns of Ca^{2+} spikes are observed in granule cells as they migrate through different cortical layers (Fig. 2) (Kumada and Komuro, 2004; Komuro and Kumada, 2005).

At the EGL. Concomitant with the initiation of tangential migration at the middle of the EGL, granule cells increase the frequency of Ca^{2+} spikes. The Ca^{2+} spikes gradually decrease in number at the bottom of the EGL and the EGL-ML border, and the rhythm becomes irregular, containing short, silent periods.

At the ML. Once granule cells enter the ML, the cells slightly increase the frequency of Ca^{2+} spikes. At the bottom of the ML, the frequency of Ca^{2+} spike gradually decreases and the amplitudes become variable.

At the PCL. Upon entering the PCL, granule cells reduce the frequency of Ca^{2+} spikes with long, silent periods, and also decrease the amplitude of Ca^{2+} spikes.

At the IGL. At the top of the IGL granule cells increase the Ca^{2+} spike frequency, although the rhythms are irregular and the amplitudes are variable. As the granule cells traverse the middle of the IGL, the frequency of Ca^{2+} spikes gradually decreases and the amplitude becomes smaller. At the bottom of the IGL, the Ca^{2+} spikes disappear, or significantly decrease in frequency.

Granule cells reduce the Ca^{2+} spike frequency and the migration speed at each boundary between cerebellar cortical layers (Fig. 2). During the migration from the EGL to the IGL, the Ca^{2+} spike frequency in granule cells positively correlates with the migration speed (correlation coefficient, 0.85), suggesting that Ca^{2+} spike frequency is one of the factors which control the cortical layer-specific alterations of granule cell migration.

Loss of Ca^{2+} Spikes Triggers the Completion of Migration. Disappearance or significant reduction of Ca^{2+} spikes at the bottom of the IGL suggests the role of Ca^{2+} signaling in the termination of migration. The use of simultaneous recordings of cell movement and intracellular Ca^{2+} levels provides the answer to this question. At the bottom of the IGL granule cells initially migrate with variable amplitudes of Ca^{2+} spikes, but completely lose Ca^{2+} spikes before becoming permanently stationary, suggesting that the loss of Ca^{2+} spikes may be prerequisite for completing granule cell migration (Kumada and Komuro, 2004: Komuro and Kumada, 2005). Furthermore, this possibility is examined by altering the Ca^{2+} spike frequency at the top and bottom of the IGL. At the top of the IGL, granule cells migrate at an average speed of 11.3 μm/hr with average Ca^{2+} spikes of 15.1 /hr, and are expected to move for an additional 8 to 13 hrs before completing the migration. In contrast, at the bottom of the IGL, granule cells migrate at a significantly reduced speed with fewer numbers of Ca^{2+} spikes, and are expected to cease migration within 3 to 5 hrs. Chelating the intracellular Ca^{2+} levels with BAPTA-AM, reducing Ca^{2+} influx by lowering extracellular Ca^{2+} concentrations, blocking the voltage-dependent Ca^{2+} channels with $CdCl_2$, inhibiting the NMDA receptors with D-AP5, or decreasing internal Ca^{2+} release with thapsigargin results in a significant reduction of the Ca^{2+} spike

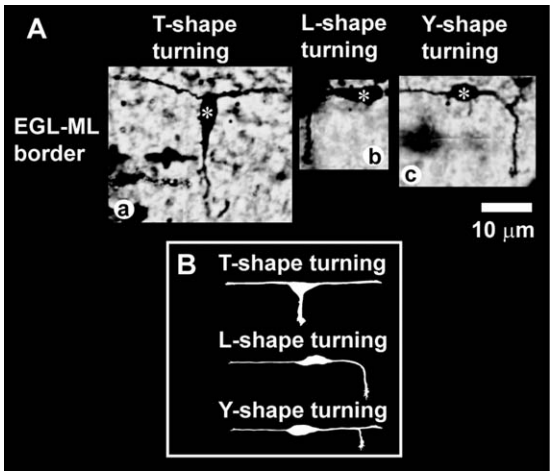


Figure 3 T-, L-, and Y-shape turning of cerebellar granule cells in the developing cerebellum. A. Micrographs showing three different modes of granule cell turning at the EGL-ML border of the P10 mouse cerebellum. a, T-shape turning of granule cells. b, L-shape turning of granule cells. c, Y-shape turning of granule cells. B. Schematic representation of T-, L-, and Y-shape turning of granule cells at the EGL-ML border.

frequency and the speed of migration regardless of the position of granule cells in the IGL. In contrast, stimulating internal Ca^{2+} release through the ryanodine receptors with caffeine or the inositol 1,4,5-trisphosphate receptors (IP3 receptors) with thimerosal significantly increases the Ca^{2+} spike frequency and accelerates granule cell movement at the bottom of the IGL, but not at the top of the IGL. These results indicate that the reduction of the Ca^{2+} spike frequency is always accompanied by the slowdown of granule cell movement, implying that the loss of Ca^{2+} transients may provide an intracellular signal for triggering molecular cascades leading to the completion of migration (Kumada and Komuro, 2004). The comparison of the effects of caffeine and thimerosal on the migration between the top and bottom of the IGL suggests that the basic amount of spontaneous Ca^{2+} release from internal stores are reduced in granule cells at the bottom of the IGL. Such reduction of internal Ca^{2+} release may be responsible, at least in part, for the slowdown (or termination) of granule cell migration at the bottom of the IGL.

Intrinsic Programs Determine the Timing of Ca^{2+} Spike Loss. The loss of Ca^{2+} spikes may be induced by external stop signals, but intrinsic programs may also be responsible. This is because in microexplant cultures of the early postnatal cerebellum, isolated granule cells complete their migration without external stop signals within 2 to 3 days after being placed in culture (Yacubova and Komuro, 2002b). In the cultures, isolated granule cells also exhibit spontaneous Ca^{2+} spikes in their somata (Kumada and Komuro, 2004). The Ca^{2+} spike frequency depends on the elapsed time after plating and positively correlates with the speed of granule cell migration (correlation coefficient, 0.81). Importantly, the Ca^{2+} spikes disappear or have significantly reduced occurrences when isolated granule cells stop migrating at 50 to 60 hr *in vitro* (Kumada and Komuro, 2004). The loss of Ca^{2+} spikes always precedes the completion of migration. The experimentally induced reduction of the Ca^{2+} spike frequency with the use of pharmacological tools is always accompanied by the slowdown of movement regardless of the elapsed time after plating. On the other hand, the prevention of the Ca^{2+} spike loss by stimulating internal Ca^{2+} release significantly increases the speed of granule cell movement at the final phase of migration (50–60 hr *in vitro*), leading to a delay in the completion of migration. These results suggest that intrinsic programs set the timing of the Ca^{2+} spike loss in isolated granule cells at approximately 50 to 60 hr *in vitro*, and trigger the completion of migration. This 50 to 60 hr term set by the internal clock is comparable to the time required for granule cells to complete their migration from their birthplace in the EGL to their final destination in the IGL (Komuro and Yacubova, 2003), suggesting that internal programs are involved in controlling the completion of granule cell migration in the developing cerebellum by inhibiting the occurrence of the Ca^{2+} spikes.

Turning of Granule Cells in the Developing Cerebellum is Controlled by Ca^{2+} Signaling. Granule cells alter the direction of migration from tangential to radial at the EGL-ML border of the developing cerebellum prior to entering the ML (Fig. 1). Granule cell turning at the EGL-ML border is classified into three distinct modes (T-, L-, and Y-shape turning) (Fig. 3). T-shape turning is characterized by the extension of vertical processes into the ML from the soma of tangentially-oriented granule cells, which have two horizontally-extended axon-like processes. The cell body follows the direction of extension of the newly-developed vertical processes. L-shape turning is characterized by the turning of the tip of the horizontally-extended leading processes towards the ML. The cell body follows the direction of the turning of the leading process, and enters the ML. Y-shape turning is characterized by the bifurcation of the tip of the horizontally-oriented leading process. One of the branches extends horizontally, while the other extends vertically into the ML. The cell body follows

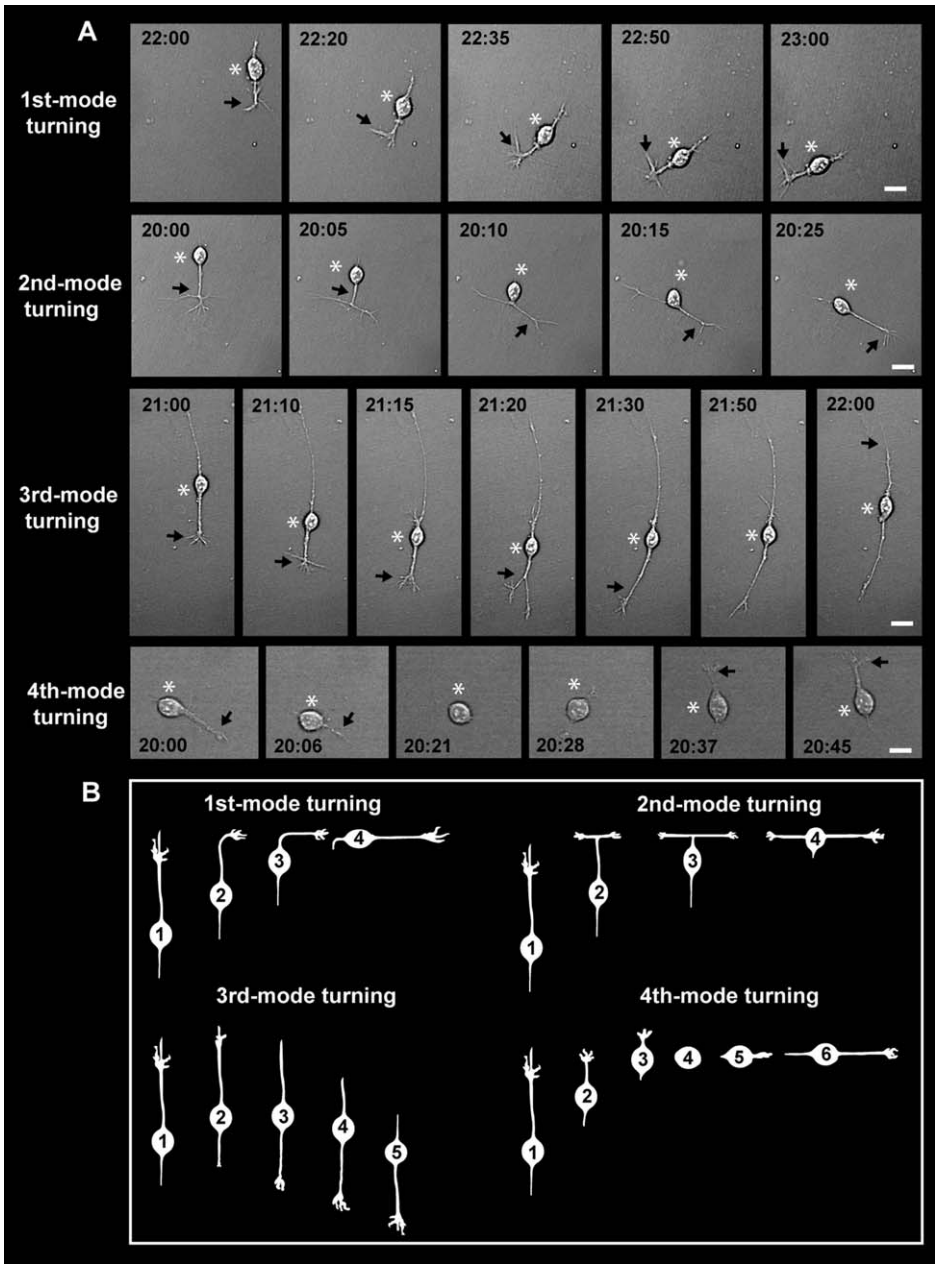


Figure 4 Four distinct modes of granule cell turning *in vitro*. A. The first mode of turning: the tip of the leading process turns to the left side and then, the cell body follows the changes. The second mode of turning: the tip of the leading process bifurcates, and both branches extend in the opposite direction. Subsequently, one of the branches collapses and retracts, and the cell body follows the direction of extension of the remaining branch. The third mode of turning: the leading process of the granule cells transforms to the trailing process and the trailing process becomes the leading process. Thereafter, the cells start to migrate in the reversed direction. The fourth mode of turning: the granule cell completely withdraws the leading process, and extends a new leading process. Subsequently, the cell migrates toward the direction of the extension of the new leading process. Scale bar: 10 μm. B. Schematic representation of four distinct modes of granule cell turning *in vitro*. The numbers in granule cell somata indicate the order in each mode of turning.

the direction of extension of the vertical process. There is preference in the occurrence of each mode of turning (Kumada et al., 2009). For example, in the P10 mouse cerebellum, the occurrence of each mode of turning decreases in the following order: L-shape turning > T-shape turning > Y-shape turning mode.

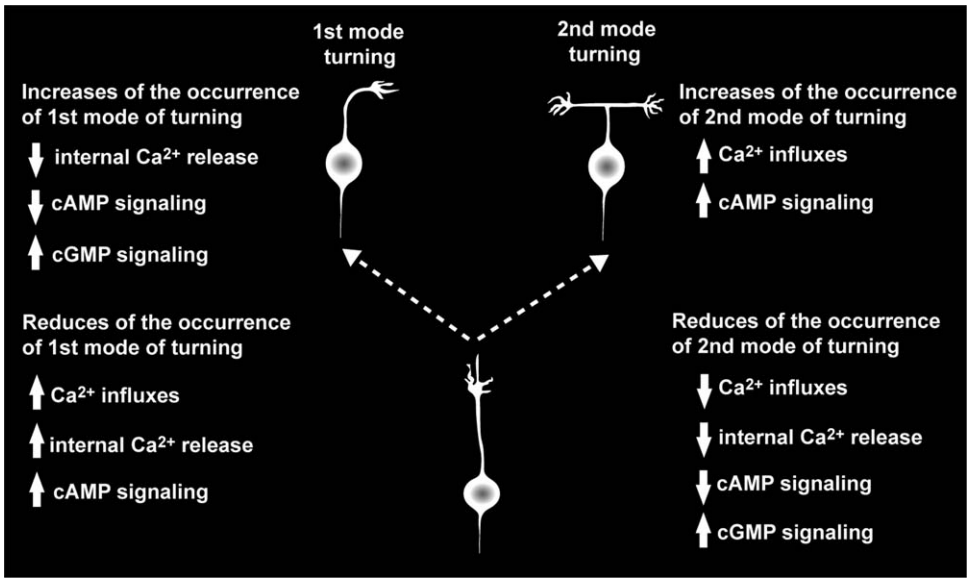


Figure 5 The summary of the effects of altering the Ca^{2+} and cyclic nucleotide signaling pathways on the first and second modes of granule cell turning *in vitro*.

Interestingly, manipulation of Ca^{2+} signaling alters the number and modes of granule cell turning at the EGL-ML border (Kumada et al., 2009). Injection of caffeine or NMDA into the subarachnoid space between the skull and the surface of the P10 mouse cerebellum increases the number of granule cells exhibiting turning towards the ML at the EGL-ML border, compared with the results obtained from saline injection. Furthermore, the injection of caffeine or NMDA increases the occurrence of the T- and Y-shape turning of granule cells, but decreases the occurrence of the L-shape turning. These results indicate that stimulating the Ca^{2+} influx or internal Ca^{2+} release accelerates granule cell turning at the EGL-ML border, and alters the mode of the turning.

Ca^{2+} Signaling Regulates Autonomous Turning of Granule Cells In Vitro. *In vitro* studies provide direct evidence for the role of Ca^{2+} signaling in granule cell turning (Kumada et al., 2009). For example, in microexplant cultures of early postnatal mouse cerebellum, isolated granule cells exhibit four distinct modes of autonomous turning over time (Fig. 4). The first mode of turning is characterized by the turning of the tip of the leading process to a new direction, which is followed by the cell body. The second mode is initiated by the bifurcation of the tip of the leading process. Both branches extend in the opposite direction, and then one of the branches collapses and retracts. The cell body follows the direction of extension of the remaining branch. The third mode is a reversal of the cell polarity. The leading process transforms to the trailing process, the trailing process become the leading process, and then cells start to migrate in the direction of extension of the new leading process. The fourth mode is initiated by withdrawing the leading process, which is followed by extending the new leading process towards the direction of upcoming movement. Individual granule cells exhibit multiple modes of autonomous turning over time, but there is preference in the occurrence of each mode of turning. During the period of 20 to 35 hr after *in vitro*, the occurrence of each mode of turning decreases in the following order: the second mode > the first mode > the fourth mode > the third mode. Alteration of Ca^{2+} signaling results in changes in the number and modes of granule cell turning *in vitro* as schematically presented in Fig. 5 (Kumada et al., 2009). Stimulating Ca^{2+} influx increases the turning frequency while inhibiting Ca^{2+} influx decreases the turning frequency. Furthermore, stimulating Ca^{2+} influx increases the occurrence of the second mode of turning, but decreases the occurrence of the first mode. Inhibiting Ca^{2+} influx decreases the occurrence of the second mode of turning. Stimulating internal Ca^{2+} release decreases the occurrence of the first mode of turning. In contrast, inhibiting internal Ca^{2+} release increases the occurrence of the first mode of turning, but decreases the occurrence of the second mode. Collectively, these results indicate that Ca^{2+} signaling differentially controls the first and second mode of autonomous turning of granule cells. The role of Ca^{2+} signaling in the occurrence of the third and fourth mode of granule cell turning remains to be

determined, because the low rates of the occurrence of these third and fourth modes have prevented the analysis of the effects of an alteration of Ca^{2+} signaling pathways.

Possible Relationship Between the Modes of Granule Cell Turning In Vivo and In Vitro. Ca^{2+} signaling controls granule cell turning both *in vivo* and *in vitro*. The similarities in the sequential steps of granule cell turning and the responses to the alterations of Ca^{2+} signaling suggest that the first mode of turning *in vitro* and the L-shape turning *in vivo* are controlled by the same group of Ca^{2+} signaling pathways, whereas the second mode of turning *in vitro* and the Y-shape turning of granule cells *in vivo* are controlled by the other group of Ca^{2+} signaling pathways (Kumada et al., 2009).

Possible Cellular Mechanisms Underlying the Control of the Migration of Cerebellar Granule Cells and other Neurons by Ca^{2+} Signaling. To date, little is known about how Ca^{2+} signaling regulates the migration of cerebellar granule cells and other neurons, but recent studies have started to elucidate the possible mechanisms. Recently, it has been reported that in culture, the tip of the leading process of isolated granule cells actively pulls the soma forward during migration through a myosin II-dependent forward F-actin flow along the leading process (He et al., 2010). Such F-actin flow is required for the forward translocation of the soma in migrating granule cells (He et al., 2010). Interestingly, it has also been reported that in gonadotropin-releasing hormone-expressing neurons, Ca^{2+} release through IP3 receptors selectively stimulates F-actin flow in the leading process away from the nucleus during migration (Huchins et al., 2013). These results suggest that Ca^{2+} spikes induced by Ca^{2+} release through IP3 receptors control the migration of neurons via regulating the leading process F-actin flow, which is essential for somal forward movement towards the tip of the leading process.

The loss of Ca^{2+} transients induces the termination of granule cell migration at their final destination, the IGL (Kumada and Komuro, 2004). Recent studies demonstrate how migrating cortical interneurons lose Ca^{2+} transients after entering their final destination, the developing cortex (Bortone and Polleux, 2009). On the way to the developing cortex, migrating cortical interneurons exhibit spontaneous Ca^{2+} transients, which are mediated by voltage-gated L-type Ca^{2+} channels after the depolarization of the membrane potential by the activation of $GABA_A$ receptors and AMPA/NMDA receptors. Upon entering the developing cortex, migrating cortical interneurons start to express high levels of K^+/Cl^- cotransporter (KCC2), which switches the response of $GABA_A$ receptor activation from the depolarization of the membrane potential to the hyperpolarization of the membrane potential (Bortone and Polleux, 2009). In the developing cortex, ambient GABA causes the hyperpolarization of the membrane potential of migrating cortical interneurons via activation of $GABA_A$ receptors, which blocks the opening of L-type Ca^{2+} channels, leading to the reduction of the Ca^{2+} transient frequency or the loss of Ca^{2+} transients. The significant reduction (or loss) of the recurrence of spontaneous Ca^{2+} spikes results in the termination of the migration of cortical interneurons in the developing cortex (Bortone and Polleux, 2009).

Recent studies show that Ca^{2+} transients due to voltage-gated L-type Ca^{2+} channels do not directly govern the migration speed of immature interneurons in the olfactory bulb (Darcy and Isaacson, 2009). For example, the application of nimodipin (L-type Ca^{2+} channel antagonist) inhibits spontaneous Ca^{2+} transients of immature interneurons in the olfactory bulb, but does not affect the speed of migration (Darcy and Isaacson, 2009). Interestingly, it has been reported that the manipulation of Ca^{2+} signaling alters the migration speed of immature interneurons in the rostral migratory stream and the subventricular zone prior to entering the olfactory bulb (Bolteus and Bordey, 2004). These results suggest that immature interneurons may shift the cellular mechanisms underlying the control of the speed of migration from Ca^{2+} signaling-dependent to Ca^{2+} signaling-independent after entering the olfactory bulb. The role of Ca^{2+} signaling in the control of neuronal migration may vary among different cell types, different stages of migration, and different microenvironments.

Role of Cyclic Nucleotide (cAMP and cGMP) Signaling in the Control of Granule Cell Migration

Reciprocal Control of Granule Cell Migration by cAMP and cGMP Signaling. Real-time recordings of cell movement in microexplant cultures of the early postnatal mouse cerebellum reveal that cAMP and cGMP signaling control granule cell migration in opposing ways (Kumada et al., 2006). In the case of cAMP signaling, stimulating adenylyl cyclase (AC) with forskolin, which is upstream of cAMP signaling, decelerates the migration of isolated granule cells. In contrast, inhibiting protein kinase A (PKA) with PKI, which is downstream of cAMP signaling, accelerates the migration. The application of Sp-cAMPS

(a competitive cAMP agonist) decelerates the migration, whereas the application of Rp-cAMPS (a competitive cAMP antagonist) accelerates the migration. In the case of cGMP signaling, stimulation with Br-cGMP (a cGMP analogue) accelerates the migration of isolated granule cells, whereas inhibition with Rp-8-pCPT-cGMPS (a cGMP antagonist) decelerates the migration. These results suggest that cAMP signaling acts as a "brake" on granule cell movement, while cGMP signaling acts as an "accelerator".

cAMP Signaling Regulates Granule Cell Migration via Altering Ca^{2+} Signaling. cAMP and cGMP signaling may control granule cell migration by altering Ca^{2+} signaling because both cAMP and cGMP pathway interact with Ca^{2+} signaling. As expected, stimulating cAMP signaling with forskolin reduces the Ca^{2+} spike frequency in granule cells, whereas inhibiting cAMP levels with Rp-cAMPS increases the frequency (Kumada et al., 2006). However, stimulating cGMP signaling with Br-cGMP does not alter the Ca^{2+} spike frequency. These results suggest that cAMP signaling controls granule cell migration, at least in part, via altering Ca^{2+} signaling, whereas cGMP signaling controls migration without affecting Ca^{2+}signaling.

***cAMP Signaling, but not cGMP Signaling, Controls the Frequency of Granule Cell Turning both* In Vitro *and* In Vivo.** Cyclic nucleotide signaling also affects granule cell turning *in vitro* and *in vivo* (Kumada et al., 2009). In microexplant cultures of the early postnatal cerebellum, inhibiting cAMP production with 9CP-Ade (an adenylyl cyclase inhibitor) increases the frequency of granule cell turning. Likewise, inhibiting cAMP signaling with Rp-cAMPS or inhibiting the activity of PKA with PKI increases the frequency of granule cell turning, while stimulating cAMP signaling with forskolin or Sp-cAMPS does not affect the frequency. On the other hand, stimulating cGMP signaling with Br-cGMP, or inhibiting cGMP signaling with Rp-8-pCPT-cGMPS or ODQ (a guanylyl cyclase inhibitor) does not alter the frequency of granule cell turning. Furthermore, *in vivo* studies reveal that the injection of Rp-cAMPS into the subarachnoid space between the skull and the surface of the cerebellum of P10 mice increases the number of granule cells turning towards the ML at the EGL-ML border, while the injection of Br-cGMP fails to alter the number of turning granule cells. These results indicate that inhibiting cAMP signaling increases the occurrence of granule cell turning, whereas cGMP signaling is not involved in controlling the occurrence of granule cell turning.

cAMP Signaling Differentially Controls Turning Frequency and Migration Speed. Analysis of the response to alterations of cAMP signaling indicates that the relationships between the turning frequency and the migration speed can be categorized into three groups (Kumada et al., 2009). The first group has the migration speed and turning frequency change in the same direction. For example, inhibiting PKA with PKI results in increases in both turning frequency and migration speed of granule cells. The second group has alterations in turning frequency, but not in migration speed, as seen in the case of inhibiting AC with 9CP-Ade, or inhibiting cAMP signaling with Rp-cAMPS. The third group has alterations in migration speed, but not in turning frequency, as seen in the case of stimulating AC with forskolin, or stimulating cAMP signaling with Sp-cAMPS. These results indicate that the cellular mechanisms underlying the cAMP-dependent alterations of the turning frequency and the migration speed of granule cells partially overlap with each other.

Control of Turning Mode by cAMP and cGMP Signaling. Recent *in vitro* and *in vivo* studies show that cAMP and cGMP signaling control the mode of granule cell turning (Kumada et al., 2009). First, *in vitro* studies reveal that in microexplant cultures of the early postnatal cerebellum, inhibiting cAMP signaling with Rp-cAMPS or inhibiting PKA with PKI increases the occurrence of the first mode of granule cell turning, but decreases the occurrence of the second mode (Fig. 5). In contrast, stimulating AC with forskolin decreases the occurrence of the first mode of turning, but increases the occurrence of the second mode. Stimulating cGMP signaling with Br-cGMP increases the occurrence of the first mode of turning, but decreases the occurrence of the second mode. Inhibiting cGMP signaling with Rp-8-pCPT-cGMPS or ODQ does not alter the modes of turning. Second, *in vivo* studies demonstrate that the injection of Rp-cAMPS into the subarachnoid space between the skull and the surface of the cerebellum of P10 mice increases the occurrence of L-shape turning of granule cells at the EGL-Ml border, but decreases the occurrence of T- and Y-shape turning. The injection of Br-cGMP increases the occurrence of L-shape turning of granule cells at the EGL-Ml border, but decreases the occurrence of Y-shape turning.

Inhibiting cAMP signaling or stimulating cGMP signaling increases the occurrence of the first mode of turning *in vitro* and the L-shape turning *in vivo* (Kumada et al., 2009). Because both the first mode of turning and the L-shape turning are characterized by the turning of the tip of the leading process to a new

direction, inhibiting cAMP signaling or stimulating cGMP signaling enhances the turning of the tip of the leading process, but inhibits the bifurcation of the tip of the leading process, which is required for the second mode of turning and the Y-shape turning.

Possible Mechanisms Underlying the Regulation of Granule Cell Turning by cAMP and cGMP Signaling. Cellular mechanisms underlying cyclic nucleotide-controlled granule cell turning remain to be examined, but there are some hints. It has been shown that changes in the ratio of cAMP/cGMP or the cytoplasmic cAMP gradients affect neuronal growth cone turning (Song et al., 1998; Nishiyama et al., 2003; Munck et al., 2004), suggesting that the alterations of cAMP and cGMP signaling may induce the turning of the leading process of migrating granule cells. Furthermore, it has been reported that cAMP and cGMP signaling affect the distribution of F-actin in the somata of migrating neurons (Haase and Bicker, 2003), suggesting that alterations of cAMP and cGMP signaling are prerequisite for the reorientation of the soma to a new direction of migration. Moreover, phosphorylation by PKA can switch off the activity of oncoprotein 18, a regulator of microtubule dynamics (Gradin et al., 1998). Thus, the changes in PKA activity may play a role in changing the direction of cell movement by controlling the behavior of the leading process by altering the microtubule dynamics.

ROLE OF CALCIUM AND CYCLIC NUCLEOTIDE SIGNALING IN GRANULE CELL MIGRATION DEFICITS IN FETAL ALCOHOL SPECTRUM DISORDERS AND FETAL MINAMATA DISEASE

Using mouse models of fetal alcohol spectrum disorders (FASD) and fetal Minamata disease (FMD), recent studies reveal how exposure to alcohol and methylmercury (MeHg) adversely affects granule cell migration. In this section, we will discuss the role of Ca^{2+} and cyclic nucleotide signaling in the deficits of granule cell migration observed in FASD and FMD.

Role of Ca^{2+} and Cyclic Nucleotide Signaling in Granule Cell Migration Deficits in a Mouse Model of FASD

Fetal Alcohol Spectrum Disorders (FASD). Prolonged exposure to alcohol during gestation and lactation is correlated with a pattern of abnormal development in newborns known as FASD (Riley et al., 2011; Paintner et al., 2012; Coriale et al., 2013). The most serious feature of FASD is disturbance of the central nervous system (Marcus, 1987; Riley and McGee, 2005; Welch-Carre, 2005). Multiple aspects of the developmental program are involved in the alcohol-induced malformation of the brain. Among them, the most striking abnormalities appear to involve the impairment of neuronal and glial migration (Miller, 1986, 1993). In the brains of FASD patients, 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 (Clarren et al., 1978; Riley and McGee, 2005; Welch-Carre, 2005). Aberrations of brain stem and cerebellar development, in large part related to faulty cell migration, are also especially frequent, along with the migrational disturbances of schizencephaly and polymicrogyria (Clarren et al., 1978; Peiffer et al., 1979).

Animal Models for Studying FASD. Pre- and/or neonatal exposure to alcohol induces long-term neuromorphologic, neurochemical and behavioral changes in human infants, leading to the diagnosis of FASD (Marcus, 1987; Riley and McGee, 2005; Welch-Carre, 2005). Some of these changes are also observed in experimental animals (Cudd, 2005). In mice and rats, the early postnatal period is equivalent to fetal development in humans (Kornguth et al., 1979), and alcohol exposure causes a reduction of their brain weight, especially in the cerebellum (Kornguth et al., 1979; Sakata-Haga et al., 2001; Dikranian et al., 2005). Due to the obvious limitations of human studies, animal models of FAS have been used to further document the phenomenon of alcohol-induced defects in brain development and to study underlying mechanisms (Cudd, 2005).

Exposure to Alcohol Inhibits the Translocation of Granule Cells in the Developing Cerebellum. Even single exposures to ethanol can cause deficits in granule cell translocation in the P10 mouse cerebellum (Kumada et al., 2010). An intraperitoneal (i.p.) injection of 1, 3, or 5 g/kg b.w. of ethanol into P10 mice results in elevations of blood ethanol levels to 15.2 m*M* (1 g/kg b.w.), 45.7 m*M* (3 g/kg b.w.), and 81.7 m*M* (5 g/kg b.w.) one hour after injection. Furthermore, a single i.p. injection of 1, 3, or 5 g/kg b.w. of ethanol results in the reduction of the number of migrating granule cells in the ML, PCL, and IGL of the cerebellum 1 day after injection in a dose-dependent manner. These results suggest that even a single exposure to

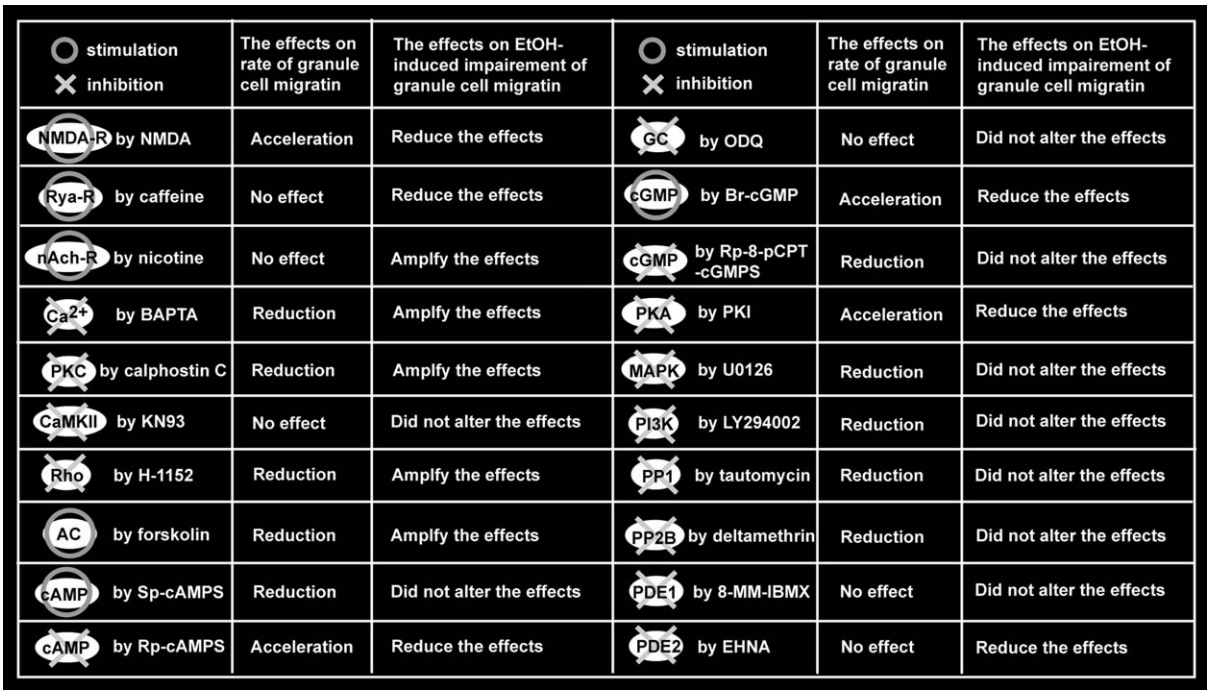
ethanol can prevent granule cells from entering the ML.

Alcohol Directly Inhibits Granule Cell Migration. Time-lapse recordings of cell movement in the cerebellar slices of P10 mice reveal that the administration of ethanol immediately slows the migration of granule cells along the migratory pathway (Kumada et al., 2006, 2007). Although 2.5 m*M* ethanol fails to alter the speed of granule cell migration, ethanol at concentrations ranging from 10 m*M* to 100 m*M* significantly inhibits the migration of granule cells in a dose-dependent manner. Furthermore, in microexplant cultures of P0-P3 mouse cerebella, ethanol at concentrations ranging from 25 m*M* to 200 m*M* significantly inhibits the migration of isolated granule cells, which migrate in the absence of cell-cell contact, suggesting that ethanol directly inhibits the migration of granule cells (Kumada et al., 2006).

Role of Ca^{2+} Signaling in Alcohol-Induced Deficits of Granule Cell Migration. Even low levels of ethanol are known to modulate the functions of voltage-gated and ligand-gated Ca^{2+} channels by binding to a hydrophobic pocket on the proteins (Walter and Messing, 1999), suggesting the involvement of Ca^{2+} signaling in ethanol-induced deficits in granule cell migration. Indeed, time-lapse recordings of intracellular Ca^{2+} levels reveal that ethanol exposure decreases the Ca^{2+} spike frequency in the granule cell somata in a dose-dependent manner (Kumada et al., 2006, 2011). These results led to the experiments testing whether enhancing Ca^{2+} signaling ameliorates the effects of ethanol on granule cell migration. Importantly, increasing internal Ca^{2+} release with caffeine or Ca^{2+} influxes with NMDA significantly reduces the effects of 25 to 100 m*M* ethanol on granule cell migration (Kumada et al., 2006). These results indicate that ethanol affects granule cell migration, at least in part, by inhibiting Ca^{2+} signaling.

Role of cAMP and cGMP Signaling in Alcohol-Induced Deficits in Granule Cell Migration. Ethanol exposure markedly increases cAMP levels but decreases cGMP levels in the P10 mouse cerebellum (Kumada et al., 2006), suggesting that cAMP and cGMP signaling play a role in the ethanol-induced deficits in granule cell migration. As expected, time-lapse recordings of cell movement show that inhibiting cAMP signaling with Rp-cAMPS completely reverses the effects of 25–100 mM ethanol on the speed of granule cell migration (Kumada et al., 2006). Likewise, inhibiting PKA activity with PKI reduces the effects of 25 to 50 m*M* ethanol on migration. In contrast, stimulating cAMP signaling with forskolin enhances the effects of 25 m*M* ethanol on migration. Furthermore, stimulating cGMP signaling with Br-cGMP markedly reduces the effects of 25 to 100 m*M* ethanol on granule cell migration, whereas inhibiting cGMP signaling with Rp-8-pCPT-cGMPS does not change the effects of ethanol on migration. Moreover, inhibiting the activity of cyclic nucleotide phosphodiesterases 2 (PDE2) with EHNA, which blocks the cGMP-dependent cleavage of cAMP and cGMP, reduces the effects of ethanol on granule cell migration. These results indicate that the effects of ethanol on granule cell migration are highly sensitive to changes in the activity of the cAMP and cGMP signaling pathways: stimulating cAMP signaling amplifies the effects of ethanol on granule cell migration, whereas inhibiting cAMP signaling or stimulating cGMP signaling reduces the effects of ethanol on the migration (Kumada et al., 2006). Because exposure to ethanol increases cAMP levels and decreases cGMP levels, ethanol may cause the slowdown of granule cell migration by stimulating cAMP signaling and inhibiting cGMP signaling.

Ca^{2+} and Cyclic Nucleotide Signaling Require the Activation of Multiple Downstream Targets for Reversing the Effects of Alcohol on Granule Cell Migration. To ameliorate the effects of ethanol on granule cell migration, Ca^{2+} and cyclic nucleotide signaling require the activation of multiple but distinct downstream effectors (Kumada et al., 2006). For example, stimulating internal Ca^{2+} release with caffeine requires the activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), calcineurin, protein phosphatase 1 (PP1), Rho GTPase, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI_3K). Stimulating Ca^{2+} influxes with NMDA requires the activation of protein kinase C (PKC), CaMKII, calcineurin, PP1, MAPK and PI_3K. Inhibiting cAMP signaling with Rp-cAMP requires the activation of PKC, CaMKII, calcineurin, PP1, Rho GTPase and PI_3K. Stimulating cGMP signaling with Br-cGMP requires the activation of PP1, Rho GTPase, MAPK and PI_3K. The roles of signaling molecules on granule cell migration and the effects of these molecules on alcohol-induced deficient in granule cell migration are summarized in Fig. 6. Possible cellular mechanisms underlying the effects of ethanol on the cAMP-PKA, Ca^{2+}-PKC, and cGMP-PKG signaling pathway in migrating granule cells are schematically presented in Fig. 7.



stimulation / inhibition	The effects on rate of granule cell migratin	The effects on EtOH-induced impairement of granule cell migratin	stimulation / inhibition	The effects on rate of granule cell migratin	The effects on EtOH-induced impairement 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	Amplfy the effects	cGMP by Rp-8-pCPT -cGMPS	Reduction	Did not alter the effects
Ca^{2+} by BAPTA	Reduction	Amplfy the effects	PKA by PKI	Acceleration	Reduce the effects
PKC by calphostin C	Reduction	Amplfy 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	Amplfy the effects	PP1 by tautomycin	Reduction	Did not alter the effects
AC by forskolin	Reduction	Amplfy 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

Figure 6 The roles of signaling molecules on granule cell migration and the effects of these molecules on alcohol-induced impairment of granule cell migration.

The Inhibitory Effect of Alcohol on Granule Cell Turning. Ethanol exposure also affects the frequency and mode of granule cell turning *in vivo* and *in vitro* (Kumada et al., 2010). *In vivo* studies demonstrate that a single i.p. injection of ethanol (1, 3, or 5 g/kg b.w.) into P10 mice significantly decreases the number of turning granule cells at the EGL-ML border in a dose-dependent manner. Ethanol exposure also alters the ratio of T-, Y-, and L-shape granule cell turning at the EGL-ML border. Furthermore, *in vitro* studies reveal that in microexplant cultures of the early postnatal cerebellum, ethanol reduces the frequency of autonomous turning of isolated granule cells in a dose-dependent manner.

Reduction of the Effects of Alcohol on the Granule Cell Turning by Controlling Ca^{2+} and Cyclic Nucleotide Signaling. Experimental manipulations of Ca^{2+} and cyclic nucleotide signaling ameliorate the effects of ethanol on granule cell turning (Kumada et al., 2010). For example, stimulating Ca^{2+} influx with NMDA and internal Ca^{2+} release with caffeine, or inhibiting cAMP signaling with Rp-cAMPS and PKA with PKI increases the frequency of granule cell turning in the presence of ethanol. Furthermore, stimulating cGMP signaling with Br-cGMP, or inhibiting the activity of PDE2 with EHNA increases the frequency of granule cell turning in the presence of ethanol. These results indicate that stimulating Ca^{2+} and cGMP signaling, or inhibiting cAMP signaling demonstrably reduces the effects of ethanol on granule cell turning.

Manipulating Ca^{2+} and Cyclic Nucleotide Signaling Mitigates the Deficits in Granule Cell Migration in a Mouse Model of FASD. *In vitro* studies demonstrate that manipulations of Ca^{2+} and cyclic nucleotide signaling reduce the effects of ethanol on granule cell migration, suggesting that experimental alterations of this signaling might also reduce the effects of ethanol on granule cell migration *in vivo*. As expected, the injection of caffeine, Rp-cAMPS, or Br-cGMP into the subarachnoid space between the skull and the surface of the P10 mouse cerebellum completely reverses the effects of ethanol on the number of migrating granule cells in the ML, PCL, and IGL and the speed of migration (Kumada et al., 2006). These results suggest that controlling Ca^{2+} and cyclic nucleotide signaling provides potential therapeutic targets for ameliorating the deficits in neuronal cell migration in the brains of patients with FASD (Kumada et al., 2007, 2010, 2011; Jiang et al., 2008).

Role of Ca^{2+} and Cyclic Nucleotide Signaling in Granule Cell Migration Deficits in a Mouse Model of FMD

Abnormal Brain Development with Fetal Minamata Disease (FMD). Many infants of mothers from

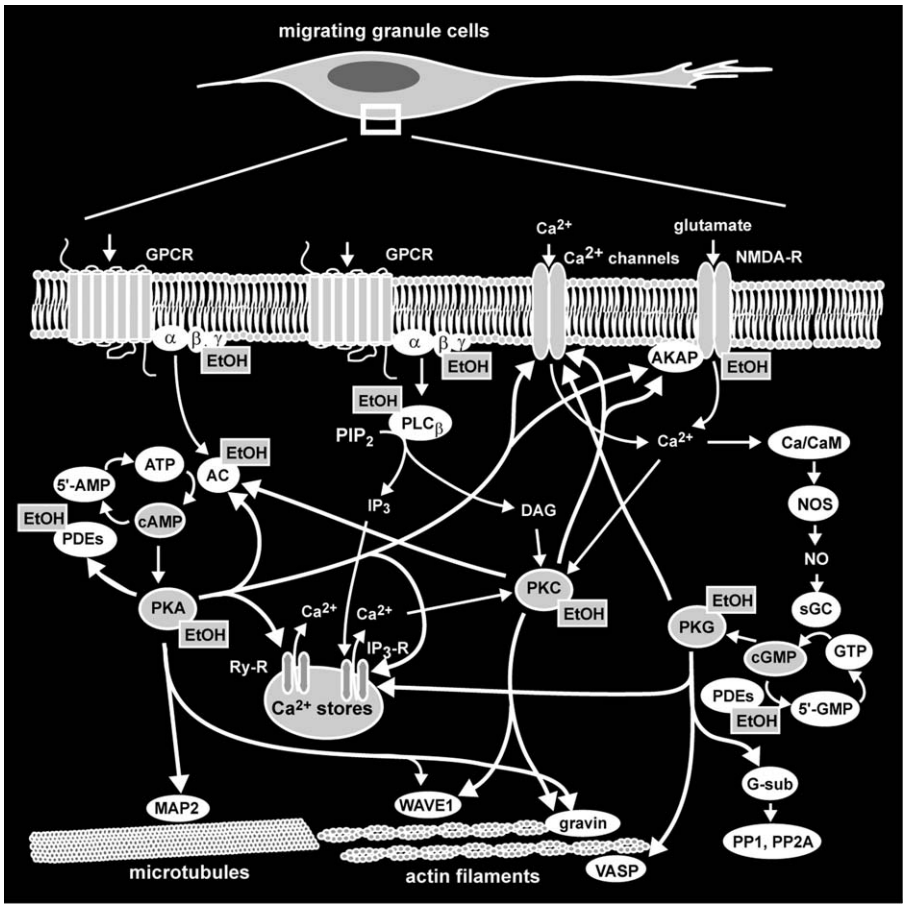


Figure 7 Schematic drawing showing how ethanol affects the cAMP-PKA, Ca^{2+}-PKC, and cGMP-PKG signaling pathway in the migrating granule cells. GPCR, G-protein coupled receptors; NOS, nitric oxide synthase; NO, nitric oxide; sGC, soluble guanylyl cyclase.

Minamata Bay born after 1955 developed characteristic neurological symptoms, including mental retardation and cerebral palsy (Harada, 1964; Synder, 1971; Eto, 1997). Later it was discovered that methylmercury (MeHg) induces congenital intoxication via the transplacental transfer of mercury to the fetus (Harada, 1978). This medical phenomenon is known as fetal Minamata disease (FMD). The expression of clinical symptoms in FMD varies widely, ranging from mildly affected individuals to severe mental retardation and complete physical incapacitation (Harada, 1964, 1978; Synder, 1971; Eto, 1997). Characteristic neuropathological changes are summarized as follows: (1) bilateral cerebral atrophy and hypoplasia with a decrease in number of cortical neurons, (2) many cortical neurons appeared to be malformed, reduced in size, or had shrunken neuritic processes, (3) cerebellar atrophy and hypoplasia with a reduction in the cerebellar granule cell layer, (4) hypoplasia of the corpus callosum, (5) dysmyelination of white matter, and (6) hydrocephalus (Matsumoto and Takeuchi, 1965; Amin-Zaki et al., 1974; Eto et al., 1992; Ekino et al., 2007). The most susceptible brain regions to MeHg-mediated injury are the cerebellum and the cerebrum (Takeuchi et al., 1962; Chang et al., 1980; Eto, 1997). There are large numbers of ectopic granule cells in the cerebellum of FMD patients (Choi et al., 1978; Choi, 1986), suggesting that granule cells are very vulnerable to MeHg exposure.

MeHg Exposure Remains a Major Public Health Issue. MeHg exposure remains a major public health concern because of the natural and anthropogenic release of inorganic mercury into the aquatic environment, where it is biotransformed by algae and bacteria into MeHg (Crinnion, 2000; Pirrone, 2001). MeHg can pass along the food chain, leading to humans through consumption of MeHg-contaminated fish (such as tuna). Once inside the body of pregnant women, MeHg readily crosses the placental and blood-brain barriers with a direct toxic effect on the fetus

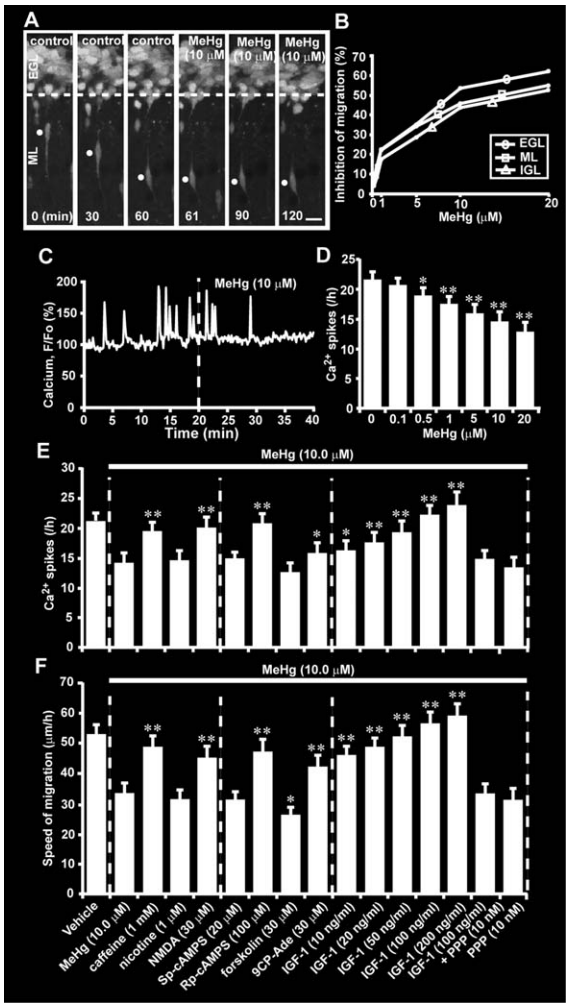


Figure 8 MeHg inhibits granule cell migration by reducing Ca^{2+} spike frequency. A. Time-lapse images showing that the application of 10 μ*M* MeHg inhibits the migration of a granule cell in the ML of a cerebellar slice obtained from a P10 mouse. White circles mark the granule cell soma. Elapsed time is indicated on the bottom of each photograph. Bar: 12 μm. B. Inhibition of the speed of granule cell migration in the EGL, ML, and IGL by MeHg. C. Reduction of the recurrence of spontaneous Ca^{2+} spikes in granule cell soma by 10 μ*M* MeHg. D. Histograms showing a dose-dependent reduction in the Ca^{2+} spike frequency of granule cells by MeHg. Bars: S.D. E & F. Changes in the effects of MeHg (10 μ*M*) on (E) Ca^{2+} spike frequency of granule cells and (F) the speed of migration by caffeine, nicotine, NMDA, Sp-cAMPS, Rp-cAMPS, forskolin, 9CP-Ade, IGF-1, IGF-1 + PPP, or PPP. Bars: S.D. In D, E, and F, single ($p < 0.05$) and double ($p < 0.01$) asterisks indicate statistical significance.

(Lapham et al., 1995; Clarkson, 1997). The fetus serves as a "mercury trap" in pregnant mothers, resulting in higher tissue concentrations of mercury than in the maternal tissues (Amin-Zaki et al., 1974). Aside from placental transfer, the developing infant can also be exposed to mercury via the mother's milk.

MeHg Inhibits Granule Cell Translocation in the Developing Cerebellum. Recent studies demonstrate that upon i.p. injection of MeHg for four consecutive days (P6–P9), the number of migrating granule cells in the ML, PCL and IGL of P10 mouse cerebellum is reduced in a dose-dependent manner (Fahrion et al., 2012). The use of a TUNEL assay indicates that low to moderate levels of MeHg do not alter the number of $TUNEL^{+}$ granule cells in the EGL, while higher levels of MeHg increase the number. Therefore, the reduction of the number of migrating granule cells in the ML, PCL and IGL by moderate levels of MeHg is mainly due to the inhibition of granule cell translocation, while the reduction of the number by high levels of MeHg is due to the inhibition of translocation as well as the increase in cell death.

MeHg Directly Inhibits Granule Cell Migration. Real-time recordings of cell movement in cerebellar slices of P10 mice reveal that the application of MeHg immediately decelerates granule cell migration along their migratory pathway (The EGL, ML, IGL) (Fig. 8A, B) (Fahrion et al., 2012). Furthermore, time-lapse recordings of cell movement in microexplant cultures of P0-P3 mouse cerebella show that MeHg slows the migration of isolated granule cells in a dose-dependent manner (Fahrion et al., 2012). These results indicate that exposure to MeHg directly inhibits the migration of granule cells.

MeHg Reduces Ca^{2+} Spike Frequency of Migrating Granule Cells. MeHg is known to disrupt intracellular Ca^{2+} homeostasis and reduces N-type Ca^{2+} channel currents in granule cells (Marty and Atchison, 1997; Sirois and Atchison, 2000), suggesting that MeHg inhibits granule cell migration by altering Ca^{2+} signaling. As expected, time-lapse recordings of intracellular Ca^{2+} levels show that the application of MeHg results in the reduction of the Ca^{2+} spike frequency of granule cells in a dose-dependent manner (Fig. 8C, D) (Fahrion et al., 2012). These results suggest that MeHg decelerates granule cell migration by inhibiting Ca^{2+} signaling, specifically reducing the Ca^{2+} spike frequency.

Increasing Ca^{2+} Spike Frequency Reduces the Effects of MeHg on Granule Cell Migration. If MeHg slows down granule cell migration via reducing Ca^{2+} spike frequency, increasing Ca^{2+} spike frequency may reduce the effect of MeHg on migration. Indeed, *in vitro* studies demonstrate that in microexplant cultures of the early postnatal mouse cerebellum, stimulating Ca^{2+} signaling with caffeine or NMDA

markedly reduces the effect of MeHg on Ca^{2+} spike frequency and the speed of granule cell migration (Fig. 8E, F) (Fahrion et al., 2012). Similarly, inhibiting cAMP signaling with Rp-cAMPS or 9CP-Ade reduces the effects of MeHg on Ca^{2+} spike frequency and migration speed (Fig. 8E, F). In addition, the application of insulin-like growth factor 1 (IGF-1) at concentrations between 10 and 200 ng/mL eliminates the effect of MeHg on the Ca^{2+} spike frequency in a dose-dependent manner, and completely reverses the effect of MeHg on the migration speed (Fig. 8E, F). These results indicate that increasing the Ca^{2+} spike frequency ameliorates the effect of MeHg on the speed of granule cell migration *in vitro*.

Reduction of the Effects of MeHg on Granule Cell Translocation in the Developing Cerebellum by Manipulating Ca^{2+}, cAMP, and IGF-1 Signaling. *In vitro* studies suggest that controlling Ca^{2+}, cAMP, and IGF-1signaling mitigates the effects of MeHg on granule cell translocation in the developing cerebellum. Importantly, *in vivo* studies demonstrate that the injection of caffeine, Rp-cAMPS, or IGF-1 into the subarachnoid space between the skull and the surface of the cerebellum for two consecutive days (P8-P9) significantly reduces the effect of MeHg on the number of migrating granule cells in the ML, PCL and IGL of the P10 mouse cerebellum (Fahrion et al., 2012). These results indicate that the deficits in MeHg-induced granule cell translocation in the developing cerebellum can be rescued by stimulating Ca^{2+} and IGF-1signaling or inhibiting cAMP signaling.

MeHg-Induced Deceleration of Granule Cell Migration in the Developing Cerebellum is Mitigated by Controlling Ca^{2+}, cAMP, and IGF-1 Signaling. Using *in vivo* live-recordings of cell movement, recent studies show that the deceleration of granule cell migration in the developing cerebellum by MeHg exposure is effectively counteracted by stimulating Ca^{2+} and IGF-1 signaling, or inhibiting cAMP signaling (Fahrion et al., 2012). For example, the injection of caffeine, Rp-cAMPS, 9CP-Ade, or IGF-1 into the dorsal surface of the P10 mouse cerebellum significantly reduces the effect of MeHg on the speed of granule cell migration in the EGL. These results provide new clues for searching potential therapeutic treatments for babies with MeHg intoxication.

CONCLUSIONS

In this review, we described the recent studies examining the role of Ca^{2+} and cyclic nucleotide signaling in granule cell migration. We also indicated the role of Ca^{2+} and cyclic nucleotide signaling as potential therapeutic targets for the deficits in granule cell migration in patients with FASD and FMD. Ca^{2+} and cyclic nucleotide signaling have a wide variety of downstream targets, which are directly and indirectly involved in the regulation of granule cell migration through the control of the assembly and disassembly of cytoskeletal elements. To date, the molecular mechanisms of how Ca^{2+} and cyclic nucleotide signaling regulate neuronal cell migration by altering the activity of their diverse down-stream targets remain to be determined. Furthermore, little is known about the role of second messenger signaling other than Ca^{2+} and cyclic nucleotide signaling in controlling granule cell migration. Moreover, the question of whether controlling Ca^{2+} and cyclic nucleotide signaling also reduces the effects of alcohol and methyl mercury on the migration of immature neurons in the developing cerebrum should be examined.

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