

presence of a process that has a cumulative effect rather than a random effect throughout the recording period. This could be related to fatigue or the trauma inflicted by the electrode on local synaptic circuitry, or other factors. This cumulative process may have also contributed to the directional tuning changes across time.

Furthermore, some of the examples of time-dependent response changes in M1 are from neurons with very low peak discharge rates (Rokni et al., 2007). This suggests that these neurons were on the margins of the task-related population and made only a minor contribution to task performance. While the activity changes in

those neurons might be statistically significant, it is not clear to what degree they are functionally significant or provide strong evidence for an unstable motor representation.

Despite these reservations, the study by Rokni et al. (2007) is important. It draws attention to the possibility that the movement representation in the motor cortex is not as stable as is generally assumed. It presents some interesting speculations about the implications of an unstable redundant motor representation on motor cortex function. It suggests that sensory signals from the periphery are used not only for feedback correction for errors and to guide motor learning,

but also to maintain the motor representation within a range of equivalent functional states against debilitating drift caused by stochastic noise in adaptive components of the system. Finally, it makes strong predictions that should be readily testable by experiments.

#### REFERENCES

- Li, C.S.R., Padoa-Schioppa, C., and Bizzi, E. (2001). *Neuron* 30, 593–607.
- Padoa-Schioppa, C., Li, C.S.R., and Bizzi, E. (2004). *J. Neurophysiol.* 91, 449–473.
- Rokni, U., Richardson, A.G., Bizzi, E., and Seung, H.S. (2007). *Neuron* 54, this issue, 653–666.

## Surfing on Calcium Waves

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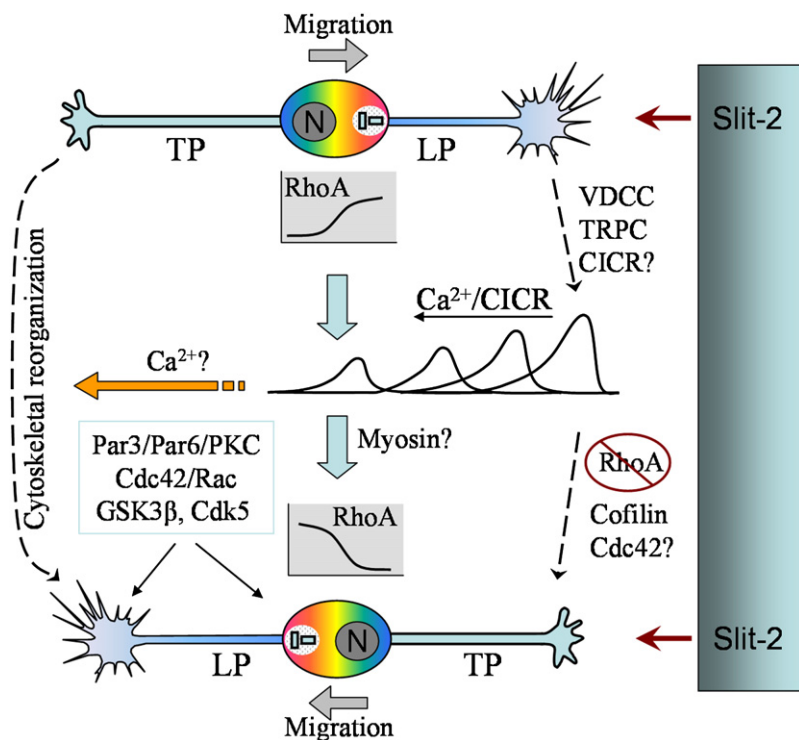
**A key question in brain development is how migration of neuronal precursors is guided to establish the ordered laminar layers. In the April 20, 2007 issue of *Cell*, Guan et al. show that the leading process of migrating cerebellar granule neurons senses repulsive Slit molecules by generating a Ca<sup>2+</sup> wave that propagates to the soma to cause reversal of cell polarity and migration.**

Guided migration of immature neurons from their birthplace to the final destination constitutes the basis for highly ordered cytoarchitecture, specific synaptic connectivity, and complex function of the nervous system (Bronner-Fraser, 1994; Hatten, 1999; Kriegstein and Noctor, 2004; Rakic, 1995). Recent evidence indicates that several axon guidance molecules are also involved in directing neuronal migration, suggesting that the same set of membrane receptors and cytoplasmic transduction mechanisms may be used for these two forms of neuronal motility (Ayala et al., 2007). Most of the migrating neurons exhibit a highly polarized morphology with a leading

and a trailing neurite process. Directed movement of the neuron typically requires three distinct steps: extension of the leading process, translocation of the soma and nucleus, and retraction of the trailing process. The leading process has a growth-cone-like motile tip similar to the axonal growth cone and is responsible for the process' extension. Successful migration of the cell also requires the translocation of the soma, which involves the detachment of the somal adhesion to the substrate and movement of the nucleus ("nucleokinesis") and other cytoplasmic organelles, and perhaps uses mechanisms distinct from those operating at the growth cone. In vitro obser-

variations of cultured cerebellar granule cells indicate that both the leading growth cone and the soma exhibit saltatory, but coordinated, advancement (Hatten, 1999). However, two different modes of neuronal migration have been observed in vivo, one relying primarily on somal translocation, with its long leading process remaining attached to the pial surface, and the other involving coordinated movement of the short leading process and soma (Ayala et al., 2007). Therefore, the extension of the leading process and the somal translocation may be differentially coupled in different modes of migration.

How does a migrating neuron detect extracellular cues? If only the leading



**Figure 1. Reversal of Neuronal Polarity and Migration by Slit-2-Induced Ca<sup>2+</sup> Waves**

The diagram depicts the main findings from the study by Guan et al. and some open questions. The cerebellar granule neuron exhibits a bipolar morphology with a leading process (LP) containing a prominent growth cone and a trailing process (TP). The RhoA is asymmetrically active in the soma with the higher activity at the front of the soma (shown as a pseudocolor gradient). The frontal Slit-2 application causes a local elevation of [Ca<sup>2+</sup>]<sub>i</sub>, potentially through voltage-dependent Ca<sup>2+</sup> channels (VDCC) and transient receptor potential (TRPC) channels. The signals downstream of local Ca<sup>2+</sup> elevation that induce growth cone collapse remain unclear, although Guan et al. showed that RhoA is not involved, leaving other candidates (e.g., cofilin and other GTPases) open. Slit-2 also initiates a Ca<sup>2+</sup> wave through CICR that propagates to the soma, which likely results in an asymmetric elevation of [Ca<sup>2+</sup>]<sub>i</sub> to reverse the asymmetry of RhoA, leading to the reversal of the somal translocation and the cell polarity. Based on the previous findings, the centrosome may also relocate to the opposite side during the reversal of migration. However, what signal or signals cause the trailing process to transform into a leading process with high motility remain poorly understood (orange arrow with the question mark). Conceivably, the transformation requires changes in many cytoskeletal structures and signaling components. A number of signaling components, including Par molecules, Rho GTPases, and GSK3β and Cdk5, are likely involved in both the somal translocation and the conversion of the trailing process to a leading process. Many other potential players (Ayala et al., 2007) are excluded for simplicity.

growth cone is responsible for the signal detection, a long-range signaling mechanism between the growth cone and the soma is required for proper coordination of the motility of these two distant parts of the neuron. Furthermore, coordinated retraction of the trailing process, as the cell advances, must involve actin-based contractile machinery and degradation of the adhesion complex, as suggested by studies of nonneuronal cell migration (Horwitz and Parsons, 1999; Sheetz et al., 1998). In a study in the April 20, 2007 issue of *Cell*, Poo and his collab-

orators have tackled these questions using an elegant culture system in which potential guidance molecules can be delivered to different parts of the migrating cerebellar granule cell in the dish (Guan et al., 2007). They present compelling imaging data demonstrating that the growth cone of the leading process senses Slit gradients by generating a Ca<sup>2+</sup> wave that propagates to the soma, triggering the repolarization of the granule cell morphology and the reversal of migration direction.

Cerebellar granule neurons in culture display a bipolar morphology with

spontaneous movement following a leading process. To test which part or parts of the neuron sense extracellular cues, the authors employed local application of Slit-2, a repulsive molecule for axons and migrating neurons, to the tip of the leading edge (frontal), the soma, or the trailing process. The authors found that only frontal Slit-2 application caused the leading edge to collapse, which was followed by reversal of the cell polarity and migration. Specifically, the direction of somal translocation was reversed and, interestingly, the original trailing process assumed a growth cone morphology and exhibited active motility. Using high-resolution ratiometric Ca<sup>2+</sup> imaging, the authors determined that frontal Slit application elicited a local elevation of intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) in the growth cone that gradually propagated as a wave to the soma (Figure 1). Using bath application of high concentrations of ryanodine to block Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR), the authors demonstrated that Slit-2-induced Ca<sup>2+</sup> elevation at the growth cone was slightly affected, but at the soma it was completely abolished (together with the reversal of somal translocation). Therefore, CICR mediates the propagation of a Ca<sup>2+</sup> wave from the growth cone to the soma to induce the reversal of translocation. The requirement and sufficiency of the Ca<sup>2+</sup> wave at the soma for reversal of migration was elegantly tested by either local blockade of CICR with high ryanodine or local activation of CICR by frontal application of a low concentration of ryanodine. These data thus provide convincing evidence that Ca<sup>2+</sup> elevation at the soma from a Ca<sup>2+</sup> wave emanating at the growth cone is the cause of the reversal of migration.

Previous studies have established a positive correlation between the rate of cell movement and the amplitude and frequency of [Ca<sup>2+</sup>]<sub>i</sub> fluctuations in the cell body through NMDA receptors and voltage-dependent Ca<sup>2+</sup> channels, but no reversal of migration was observed (Komuro and Kumada, 2005). Therefore, the reversal of somal translocation by frontal application of low concentrations of ryanodine

indicates that either CICR functions distinctly from  $\text{Ca}^{2+}$  influx through membrane depolarization or that the  $\text{Ca}^{2+}$  wave carries the directional instructions in a spatiotemporal pattern. The lack of high-resolution imaging applied to investigate the spatiotemporal pattern of  $[\text{Ca}^{2+}]_i$  in the soma during reversal leaves this question open. Another important conclusion from the study by Guan et al. (2007) is that growth cone behavior and somal translocation are independent, and in this regard, it is important to show that the growth cone collapse is not linked to the reversal of somal translocation. Otherwise, one might suspect that the reversal of migration may simply result from growth cone collapse of the leading process, which would force the neuron to reverse the direction. The separation between growth cone and somal responses is supported by the finding that local blockade of CICR in the soma (by high concentrations of ryanodine) blocked the reversal of somal translocation without affecting the growth cone collapse induced by frontal Slit-2 application. The most convincing piece of evidence comes from the frontal application of low concentrations of ryanodine, after which both the growth cone and soma exhibited a similar elevation of  $\text{Ca}^{2+}$  through CICR and the soma reversed its direction of translocation, but no apparent collapse of the leading process was observed. Therefore, Slit-2 molecules appear to trigger two distinct effects: the large elevation of  $[\text{Ca}^{2+}]_i$  that is responsible for collapse and the initiation of a  $\text{Ca}^{2+}$  wave through CICR that propagates to the soma and causes the reversal of translocation. While the mechanism underlying the  $\text{Ca}^{2+}$  elevation at the growth cone by Slit-2 remains unclear, these data demonstrate the amazing ability of  $\text{Ca}^{2+}$  to produce short-range and long-range effects on different parts of a neuron, which could be a key for coordinating the various cellular activities in a highly polarized neuron.

What is the downstream effector or effectors of  $\text{Ca}^{2+}$  in the reversal of migration direction? An even more intriguing question is, "How is the trailing process transformed into a leading

process during the reversal of migration?" This is particularly puzzling since the  $\text{Ca}^{2+}$  signals appear to only reach the soma, rather than reach all the way to the end of the trailing process (Figure 1). The authors indicate that the reversal of cell polarity and migration is mediated by the small GTPase RhoA, which is preferentially localized in the soma with accumulation toward the leading front, but then redistributed when the neuron is induced to reverse its orientation by frontal application of Slit-2 molecules. Experiments using high and low concentrations of ryanodine were performed to demonstrate that  $[\text{Ca}^{2+}]_i$  elevation at the growth cone is both necessary and sufficient to induce redistribution of RhoA expression. Finally, using fluorescence resonance energy transfer (FRET) to observe active RhoA, the authors claim that the reversal of migration may also be due to the inhibition of RhoA at the frontal end and the redistribution of active RhoA. Since RhoA inhibition has been shown to actually promote nerve growth, it is unlikely that inhibition of RhoA activity halts the migration of the neuron. Rather, the redistribution of RhoA is probably involved in changing the polarity of the migrating neuron. Thus, the mechanism by which the neuron stops migrating in the forward direction and begins migrating in the reverse direction is not yet known. It also remains to be seen how the trailing process acquires the motility of a leading process during the reversal of migration.

The study has clearly illustrated several intriguing and exciting findings on the role of long-range  $\text{Ca}^{2+}$  signaling and RhoA in repolarizing and reversing migrating neurons. However, several outstanding issues remain to be addressed, particularly those regarding the role of the cytoskeleton. For example, the mechanism by which  $\text{Ca}^{2+}$  waves induce the redistribution of RhoA has not been addressed by this study, although the authors propose a cortical myosin-dependent mechanism in which a front-to-rear  $\text{Ca}^{2+}$  gradient may cause a myosin-dependent rearward flow of cortical F-actin, thus pulling RhoA to the rear. Furthermore,

the effects of the  $\text{Ca}^{2+}$  wave on the cytoskeletal structures in the soma were not clear. Migrating neurons have a specialized motility apparatus consisting of a perinuclear tubulin cage that holds the nucleus in the rear of the cell, an actin network around the soma, and microtubules that project to the leading process extending in the direction of migration. Recent studies also indicate that the movement of the centrosome into the leading process precedes nucleokinesis and that the centrosome coordinates cytoskeletal dynamics in response to mPar6 $\alpha$ -PKC $\zeta$  and GSK3 $\beta$ -mediated signaling (Higginbotham and Gleeson, 2007; Solecki et al., 2006). It would be of great interest to see if the  $\text{Ca}^{2+}$  wave elicited by Slit-2 causes the relocation of the centrosome to the new leading process following reversal of migration (Figure 1). Clearly, further elucidation of the molecular link between the  $\text{Ca}^{2+}$  signals and these known cellular components involved in migration would significantly advance our knowledge of directed migration during brain development.

One question that begs for answers is the *in vivo* relevance of the findings described by Guan et al. (2007). The observed migration of cerebellar granule neurons in culture more closely resembles cortical rather than cerebellar migration *in vivo*. Granule cells of the cerebellum first migrate tangentially in the external granule layer (EGL), then extend a T-shaped bipolar process which become the parallel fiber axons, followed by radial migration of the cell body into the internal granule layer (IGL) where the dendrites form (Komuro and Yacubova, 2003). Radial migration of granule neurons along glial fibers involves the extension of a prominent leading process with a growth cone-like motile tip. However, the trailing processes do not retract as the cell body moves toward the IGL. The reversal of migration observed in culture almost never happens in cerebellum *in vivo* (Solecki et al., 2004). Furthermore, the identification of the function of Slit-2 repulsion during development remains to be revealed. As Slit-2 is expressed by

Purkinje cells and its receptor Robo2 by granule cells (Marillat et al., 2002), Guan et al. suggest that the repulsive action of Slit-2 prevents the premature migration of EGL cells to the IGL. Therefore, Slit-2 may be potentially involved in controlling the transition of granule cell migration from tangential migration to radial directions. In this case, Slit-2 expression in the Purkinje cells or Robo2 expression on the granule cells would need to be downregulated at the time that the granule cells begin migrating radially. Alternatively, Slit-Robo signaling could be silenced by crosstalk with other guidance signaling pathways (Grunwald and Klein, 2002). Regardless of these outstanding questions, the exciting findings on long-range  $Ca^{2+}$  signaling reversing neuronal polarity and migration have opened up a door for more studies

that will provide a better understanding of the developmental events that pack billions of neurons into highly organized structures for complex functions and how their failure contributes to human migration disorders.

**REFERENCES**

Ayala, R., Shu, T., and Tsai, L.H. (2007). *Cell* 128, 29–43.

Bronner-Fraser, M. (1994). *FASEB J.* 8, 699–706.

Grunwald, I.C., and Klein, R. (2002). *Curr. Opin. Neurobiol.* 12, 250–259.

Guan, C.B., Xu, H.T., Jin, M., Yuan, X.B., and Poo, M.M. (2007). *Cell* 129, 385–395.

Hatten, M.E. (1999). *Annu. Rev. Neurosci.* 22, 511–539.

Higginbotham, H.R., and Gleeson, J.G. (2007). *Trends Neurosci.*, in press. Published online April 7, 2007.

Horwitz, A.R., and Parsons, J.T. (1999). *Science* 286, 1102–1103.

Komuro, H., and Kumada, T. (2005). *Cell Calcium* 37, 387–393.

Komuro, H., and Yacubova, E. (2003). *Cell. Mol. Life Sci.* 60, 1084–1098.

Kriegstein, A.R., and Noctor, S.C. (2004). *Trends Neurosci.* 27, 392–399.

Marillat, V., Cases, O., Nguyen-Ba-Charvet, K.T., Tessier-Lavigne, M., Sotelo, C., and Chedotal, A. (2002). *J. Comp. Neurol.* 442, 130–155.

Rakic, P. (1995). *Proc. Natl. Acad. Sci. USA* 92, 11323–11327.

Sheetz, M.P., Felsenfeld, D.P., and Galbraith, C.G. (1998). *Trends Cell Biol.* 8, 51–54.

Solecki, D.J., Govek, E.E., and Hatten, M.E. (2006). *J. Neurosci.* 26, 10624–10625.

Solecki, D.J., Model, L., Gaetz, J., Kapoor, T.M., and Hatten, M.E. (2004). *Nat. Neurosci.* 7, 1195–1203.

## The Sodium “Leak” Has Finally Been Plugged

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Most electrophysiologists generally do not speak highly of leak currents. In reality, these conductances represent a crucial functional mechanism by which neurons control resting membrane potentials. A new study in *Cell* by Lu et al. has surprisingly confirmed the identity of the long-sought voltage-insensitive sodium leak conductance to be encoded by the third branch of the voltage-gated sodium and calcium channel family.

Nerve cell resting membrane potentials are set by a complex interplay of ionic pumps, transporters, and channels, most of which have been well characterized at the molecular level. Missing from this molecular description has been the identification of the voltage-insensitive background sodium “leak” conductance that helps to maintain resting potentials depolarized to the  $\sim$ –90 mV potassium equilibrium potential. While the background sodium conductance has

variously been speculated to result from voltage-gated sodium channels, “leaky” potassium channels, transporters, and TRP channels, until a recent report in *Cell* (Lu et al., 2007) there has been no definitive evidence one way or the other as to the molecular nature of this conductance so crucial to setting membrane resting potential.

To set the story up, between the late 1980s and into the 1990s, molecular cloning and exogenous expression studies revealed that a family of 20

four-domain type  $\alpha$  subunits encode the known voltage-gated sodium ( $Na_v1.1$ – $1.9$  and  $Na_x$ ) and calcium ( $Ca_v1.1$ – $1.4$ ,  $Ca_v2.1$ – $2.3$ ,  $Ca_v3.1$ – $3.3$ ) channels (Figure 1). Much subsequent work went into defining the structure-function relationships concerning sodium and calcium channel permeation, gating, modulation, and trafficking. However, a 1999 report of the existence of a third branch on the four-domain voltage-gated ion channel genetic tree left the somewhat