

Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

The Wanderlust of Newborn Neocortical Interneurons

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Review of Tanaka et al. (<http://www.jneurosci.org/cgi/content/full/29/5/1300>)

For proper formation of the cerebral cortex, immature neurons must travel from their birthplace within the walls of the lateral ventricle to their final destinations throughout the brain. This process requires active migration over long distances and failure of neurons to properly migrate carries serious consequences for the organism. In humans, disruption of the genes that direct neuronal migration can cause severe mental retardation or even death (Gleeson and Walsh, 2000). Understanding the mechanisms for neuronal circuit formation will lead to a better understanding of basic design principles for brain architecture and the development of potential therapies for the treatment of brain disorders caused by aberrant migration.

During development, newborn neurons use distinct forms of migration depending on their origin. In the mammalian neocortex, projection neurons are born in the ventricular zone that lines the basal surface of the cortex. These neurons migrate in a straight line from the ventricular zone along radial glia fibers. Fate-mapping experiments have revealed that, as a consequence of this form of migration, projection neurons do not distribute widely throughout the cortex. Instead, neurons derived from the same radial glia come to reside nearby within the cortex,

forming a column of related neurons around one radial fiber (Rakic, 2007).

In contrast to projection neurons, interneurons distribute throughout the cortex via both radial and tangential migratory paths, but how these cells disperse so broadly remains a mystery. A recent study by Tanaka et al. (2009) published in *The Journal of Neuroscience* has revealed a novel migratory behavior of interneurons that may facilitate the dispersion of these cells throughout the cortex. The authors used time-lapse imaging of explants from the embryonic brain to follow the migratory paths of fluorescently labeled newborn interneurons.

Migrating interneurons in the marginal zone were previously discovered by this group to move in multiple directions (Tanaka et al., 2006). However, in the GAD67-GFP transgenic mice used, GFP was expressed in all migrating interneurons and, due to the high density of cells labeled, the behavior of individual cells could not be determined. In the current paper, Tanaka et al. (2009) used site-directed electroporation in GAD67-GFP mice to specifically and sparsely label interneurons, making it possible to examine the morphology and migratory trajectories of individual cells. The authors electroporated a plasmid containing the gene for a red fluorescent protein into the medial ganglionic eminence of GAD67-GFP mice. The medial ganglionic eminence is part of the ventral ventricular zone from which many types of cortical interneurons are generated. By varying the electroporation parameters, the authors were able to titrate the number of red fluorescent cells

and achieved sparse labeling. Thus, for most of their experiments, cells double labeled with GFP and a red fluorescent protein were chosen for imaging. Once interneurons reached the marginal zone, explants were removed from the brain and placed under a confocal microscope for long-term imaging.

With this technique, the authors produced time-lapse movies that reveal an unexpected migratory behavior of interneurons in the marginal zone. To quantify this behavior, the authors used a metric they term deflection angle. First, they determined the preferred direction of the cell, which is the direction between the initial and final positions of the soma. To calculate the deflection angle, they then took a weighted average of the angle between the neuron's current heading and the preferred direction over all time points. Cells traveling with a deflection angle of $>30^\circ$ were classified as "wandering." Instead of migrating in straight lines, these cells changed directions frequently and moved in an undirected manner. Depending on the age of the animal, 35–68% of interneurons in the marginal zone met the criteria for wandering behavior. Also, unlike neurons that migrate along glial fibers (Hatten, 1990), many of the cells in these movies were morphologically complex.

Is the wandering behavior of interneurons a random walk, similar to the diffusion of gas particles? The cellular movements from the time-lapse movies can provide an answer here. For purely deterministic motion, the distance a particle travels is proportional to time elapsed.

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Conversely, in a random walk, net displacement is proportional to the square root of time elapsed. Surprisingly, the movements of labeled cells in the marginal zone fit a random walk model, a behavior that has not previously been described for other types of migrating neurons. This observation leads to a number of additional questions. Is this unique form of migration essential for cortical formation? If so, what is its cellular basis?

Random walk behavior could emerge from several distinct mechanisms. Migrating interneurons might reach their targets by trial and error in a complex environment of external cues, as has been hypothesized for synapse formation (Rankin and Cook, 1986). This model is consistent with the morphology of the migrating neurons in the supplemental movie, since tips of the neuronal processes display swellings that resemble axonal growth cones, suggesting that these neurons could be sensing cues in their environment. However, the authors favor the hypothesis that interneuron migration in the marginal zone is a cell-autonomous process that is independent of external stimuli, such as guidance cues, and resembles diffusion. A consequence of this provocative idea is that the ultimate placement of each interneuron is not predetermined. Further experiments are required to support this claim. For example, fate-mapping experiments could determine the extent to which the final location of specific interneurons is predetermined.

The authors point out that the wandering behavior may result from the loss of guidance cues when the marginal zone is removed from the embryonic brain and placed *in vitro*. However, they find that labeled cells found in the marginal zone of

histological sections taken from the intact brain share the same complex multipolar morphology as the labeled migratory cells *in vitro*. In contrast, neurons that move in a straight line have a simple, bipolar morphology reflecting a commitment to their route (Hatten, 1990). Further experiments are necessary to both validate the *in vitro* observation and understand the mechanism of the random-walk behavior.

After documenting the migratory behavior of cells, the authors attempt to identify factors that anchor interneurons in the marginal zone. One candidate is the receptor CXCR4, expressed in migrating interneurons, and its ligand CXCL12, produced in the meninges above the marginal zone. Knock-out mice that lack either gene exhibit improper interneuron migration. To test whether CXCR4/CXCL12 signaling plays a role in wandering migration in the marginal zone, the authors electroporated plasmids expressing CRE recombinase into the ventricular zone of CXCR4^{loxP} conditional knock-out mice. Loss of CXCR4 disrupted the laminar patterning of interneurons, decreasing the relative number of interneurons in the marginal zone while increasing the number in the cortical plate. While providing a link between interneuron accumulation in the marginal zone and genes that affect migration, this experiment does not address the main point of this paper, namely that wandering migration in the marginal zone is a mechanism for interneuron dispersion. In knock-out mice for either CXCR4 or CXCL12, the laminar position of interneurons is disrupted, but there is no evidence of any defect in horizontal dispersion (Stumm et al., 2003).

The paper clearly demonstrates a novel form of migration in newborn interneu-

rons in the marginal zone of the developing cortex. The supplemental movie beautifully documents this distinct and remarkable form of migration. Further study of the molecular mechanisms of this mode of migration will be necessary to establish whether it plays an important role in the intact organism. However, the paper provides strong support for the idea that directly observing cellular behavior can provide both insights into and constraints on potential developmental mechanisms.

References

- Gleeson JG, Walsh CA (2000) Neuronal migration disorders: from genetic diseases to developmental mechanisms. *Trends Neurosci* 23:352–359.
- Hatten ME (1990) Riding the glial monorail: a common mechanism for glial-guided neuronal migration in different regions of the developing mammalian brain. *Trends Neurosci* 13:179–184.
- Rakic P (2007) The radial edifice of cortical architecture: from neuronal silhouettes to genetic engineering. *Brain Res Rev* 55:204–219.
- Rankin EC, Cook JE (1986) Topographic refinement of the regenerating retinotectal projection of the goldfish in standard laboratory conditions: a quantitative WGA-HRP study. *Exp Brain Res* 63:409–420.
- Stumm RK, Zhou C, Ara T, Lazarini F, Dubois-Dalcq M, Nagasawa T, Höltt V, Schulz S (2003) CXCR4 regulates interneuron migration in the developing neocortex. *J Neurosci* 23:5123–5130.
- Tanaka DH, Maekawa K, Yanagawa Y, Obata K, Murakami F (2006) Multidirectional and multizonal tangential migration of GABAergic interneurons in the developing cerebral cortex. *Development* 133:2167–2176.
- Tanaka DH, Yanagida M, Zhu Y, Mikami S, Nagasawa T, Miyazaki J, Yanagawa Y, Obata K, Murakami F (2009) Random walk behavior of migrating cortical interneurons in the marginal zone: time-lapse analysis in flat-mount cortex. *J Neurosci* 29:1300–1311.