Commentary & View

Migration of cortical interneurons relies on branched leading process dynamics

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Migrating cells typically reach their targets in response to a relatively wide variety of extracellular molecules. Somehow surprisingly, most cells transduce these extracellular signals into a relatively homogeneous set of cellular changes that allow them to accurately find their target position. Here we summarize the characterization of the migratory behaviour of cortical interneurons in their journey to the cerebral cortex, which seems to represent a novel type of cellular adaptation during directional guidance. Similar to other migrating cells, cortical interneurons are highly polarized cells, with a prominent leading process and a short trailing process. However, the leading process of migrating interneurons continuously branches during the migratory cycle of these cells. Leading process branches are generated in response to the extracellular environment, and seem to serve as the main mechanism that determines the migratory direction for the cell. For each migratory cycle, the branch that is best oriented towards an attractive guidance cue will become stabilized, which in turn will allow the subcellular organelles and the nucleus to progress in the right direction. This migratory process is under the strict control, among several other molecules, of members from the small Rho GTPases family proteins. Pharmacological blocking of ROCKI/II abrogates the formation of leading process branches in migrating interneurons. The resulting cells, with a single leading process, do not efficiently modify their orientation in response to extracellular guidance cues, and so they fail to complete their migration.

The processing capabilities of the central nervous system (CNS) depend on a precise cellular architecture that allows a specific matching of connexions among specific neuronal assemblies. One of the initial steps in building up these neuronal assemblies is the migration of newborn neurons from their place of origin to their final destination in the brain. In general, neurogenesis takes place in restricted areas of the CNS close to the lumen of the neuronal tube, which in most cases are at considerable distances from the territory where neurons will finally settle. Guidance mechanisms operate to ensure the proper distribution of cells and their connections in the formation of functional circuits. Growing interest in this field has increased with the discovery that alterations in neuronal migration are related to a variety of neurological pathologies. In some cases, migratory defects produce gross anatomical changes in brain structures, as in the case of lissencephaly. In other instances, even without obvious histological changes, alterations in genes related to neuronal migration have been associated with profound behavioral disturbances, as in certain forms of autism and schizophrenia.

Excitatory pyramidal cells are born in the ventricular zone of the telencephalon and reach the final destination by radial migration. The current view favours the idea that these cells use radial glia apical processes as a scaffold to reach the overlying cortical layers. In contrast, interneurons originate in the basal telencephalon, hundreds of microns away from their final destination, and so they have to undertake a long journey compared with the relatively short distances travelled by projection neurons. Interneurons migrate without strict physical guiding substrates in two well-defined steps: tangential migration, used to reach the pallium and disperse throughout the cortical surface, and radial migration, when they invade the corresponding layer of the cortex. Surprisingly, projection neurons and MGE-derived interneurons born at the same time end up populating the same layers of the cortex, suggesting that interneurons must be able to overcome a much more complex migratory pathway than their counterparts in the cortex with better efficiency. Indeed, it has been shown that interneurons migrate faster than projection neurons (40 μm/h in interneurons versus 19 μm/h in projection neurons). Thus, the cellular mechanisms used by interneurons during their migration are likely different from those used by projection neurons.

Since the initial description of the tangential migration inferred from static images, it was appreciated that migrating interneurons adopt a bipolar shape with, in most cases, a bifurcated leading process and a thin trailing process. Subsequent studies, based on dynamic experiments in embryonic cortical slices and other ex vivo preparations, have obtained a more precise picture of the migratory cycle follow by interneurons. Furthermore, it has become apparent...
that the dynamics of the branched leading process support their directional guidance during tangential migration.\textsuperscript{7,8,10} As part of the migratory cycle, the nucleus and other cell organelles advance discontinuously into the leading process reaching its bifurcation. In parallel one of the two main branches of the leading process collapse, becoming integrated in the trailing process, as the other is stabilized and bifurcates again, so the cycle is repeated. Branches are always tipped with very active growth cones, with dynamic morphological changes reminiscent of the growth cones of developing axons. However, in contrast to axon guidance, the leading process branches of migrating interneurons grow linearly without much steering, maintaining relatively constant the angle that is established between them at the time of branching. Consequently, the geometry of the leading process (i.e., the angle formed by both branches) determines the only two possible directions to be followed by the migrating neuron.\textsuperscript{8} When interneurons follow quasi-rectilinear trajectories, bifurcations form at a fairly constant angle of about 45° and typically alternate the branch that collapse or stabilize in consecutive migratory cycles. In contrast, when interneurons change direction, at least two different leading process dynamics have been observed. First, without a substantial increase in the angle between both branches, the best-aligned branch (with respect to the proper migratory pathway) can become stabilized repeatedly in several consecutive cycles, which in turn result in an effective but slow change in direction (Fig. 1A). Second, when interneurons take sharp turns (for example, during the invasion of the cortical plate away from their tangential stream in the subventricular zone), new branches will be generated at a larger angle than normal, which allow a rapid change in direction (Fig. 1B).

Migratory interneurons remain permanently polarized in at least three subcellular domains: (1) a bifurcated leading process, which constitutes its exploratory domain, (2) an elongated somatic domain behind the bifurcation, in which the translocation of the nucleus and cell organelles takes place, and (3) a trailing process, in which the collapsing branch of the leading process is periodically reabsorbed. The intracellular events that take place in each of these domains must be coordinated to couple the advancement of the cell organelles and nucleus into the stabilized branch of the leading process.

In recent years, research in several laboratories has substantially increased our knowledge on the role of the microtubule and actin cytoskeleton in the movement of these organelles within the somatic domain.\textsuperscript{7,8,10} However, the mechanisms that drive the formation and stabilization or collapse of leading process branches remain unexplored. Some of the molecules that contribute to the fine regulation of cytoskeleton dynamics are Rho effector kinases ROCKI/II. When ROCKI/II activity is pharmacologically blocked in a migrating interneuron, one of the processes retracts and the other becomes permanently stabilized. Cells with this morphology (long, unbranched leading process) do not seem to be able to change direction, failing to reach their proper destination in the cerebral cortex.\textsuperscript{8} We believe that the mechanism described here for cortical interneurons might be used by many other long-range migrating cell types for which guidance is highly dependent on chemical diffusible molecules rather than on physical guiding substrates. In this type of guidance, cellular mechanisms that allow optimization of metabolic resources, time and cell displacement are critical to reach their destination properly. In that sense, one could compare different hypothetical migrating morphologies with the one describe above. For example, cells with a branched leading process might be able to explore a wider territory in search for guidance cues than cells with a single leading process that steers for guidance information. Another advantage of cells with branched leading processes could be the ability of computing data simultaneously from the broad territory occupied by both branches (up to 50 \(\mu\)m in migrating cortical interneurons). This will be much more limited in migrating cells with a single leading process, because integration of information occurs only in the growth cone. These limitations would increase the time and distance that cells will need to find the proper way, especially when sharp changes in direction are required. An increase in the distance migrated involves a higher number of nucleokinesis, which entails an expensive energetic cost for the cells. Thus, for long migrations, neurons with a single leading process will be less efficient than those with branched leading processes. If having two branches is good, why not having more? Cells with three or more branches should be
able to recognize extracellular cues faster than cells with two leading process branches simply because their branches are oriented in more directions. However, having many branches might become inefficient if the time and resources necessary to compute very subtle differences in the extracellular territory also increases. In conclusion, more than two branches could slow down the speed of migration and increase the energetic requirements becoming an unfavourable configuration to migrate long distances.

Although this mode of migration is particularly evident in interneurons migrating from the subpallium to the cortex at all stages of their journey, we consider that it is not exclusive of these neurons. Migratory neurons from other regions of the CNS (spinal cord, thalamus and SVZa) show similar morphologies during their migratory cycle.8,9 Remarkably, even under situations of non-directed movement, migratory interneurons at the marginal zone of the cortex adopt the morphology described above,12,13 suggesting that the bifurcated leading process could be a default feature of these neurons. Interestingly, in the peripheral nervous system, a recent study suggests a similar migration mechanism in neural crest-derived enteric neurons.14 From an extended point of view, branches of migrating neurons may follow the same principles than in other organisms and cell types (e.g., Dictyostelium, neutrophils and fibroblasts), in which directed movement in shallow gradients relies on the differential stabilization of protrusions within the leading edge.15 In fact, branches are very dynamic and they behave as binary elements because a migratory cycle always finish with the collapse of one of the growth cones and its corresponding branch in benefit to the other one. This observation could be interpreted as the ability of these cells to integrate different extracellular information surrounding distant branches that will let them to follow the best oriented to the migratory pathway. Therefore, cells will probe and compute differences between at least two values within the explored field at every point in which they must decide the direction to be followed. Such a mechanism will allow accurate guidance in highly complex extracellular territories.

Despite their striking morphological differences, and therefore in the arrangement of their cytoskeletons, many eukaryotic cells move in response to chemoattractive or haptotactic gradients by choosing the best aligned to the gradient of two competing leading edge domains. It remains to be seen to what extent the similarities between the modes of movement of different cell types can be translated to common cellular and molecular mechanisms.

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