Neuronal Migration in the Developing Cerebral Cortex: Observations Based on Real-time Imaging

We have used time-lapse imaging of acute cortical slices to study the migration of neurons from their sites of origin to their positions in the developing neocortex. We found that two distinct modes of cell movement, somal translocation and glia-guided locomotion, are responsible for the radial migration of neurons generated in the cortical ventricular zone. The former is the prevalent form of radial movement of the early-born cortical neurons, while the latter is adopted by those generated later in corticogenesis. Interneurons, found to originate in the ganglionic eminence, follow tangential migratory paths to reach the developing cortex. Upon reaching the cortex, these cells seek the ventricular zone using a mode of movement that we have termed ‘ventricle-directed migration’, before they migrate to their positions in the cortical plate. In addition to these forms of movement, we report here a unique morphological and migratory behavior for a population of cortical neurons. These cells are multipolar in form, and are highly motile in the formation and retraction of their processes. Based on these morphological features, we refer to this type of cells as ‘branching cells’ and attribute the phenotype to a subset of cortical interneurons.

Introduction

According to the widely accepted model of neocortical development, first documented by the Boulder Committee (Boulder Committee, 1970), neurons of the cerebral cortex arise in the germinal ventricular zone (VZ) at the surface of the lateral ventricles. Newborn neurons migrate towards the margin of the cerebral wall to form the primordial plexiform layer or preplate (PP). This zone is then split into the superficial marginal zone (MZ) and the deeper subplate (SP) by the arrival of the cortical plate (CP) cells. These neurons accumulate in an ‘inside-out’ sequence, with newly arriving cells migrating radially past the existing neurons before stopping at the top of the CP (Berry and Rogers, 1965; Rakic, 1974). The migration of young neurons from the VZ to the CP is largely dependent on radial glia (Rakic, 1974). However, electron microscopic examination of early mouse cortex by Shoukimas and Hinds (Shoukimas and Hinds, 1978) did not reveal a consistent association between neurons and glia, even though radial glial processes were abundantly present. This prompted the speculation that at the early stages of corticogenesis, when the cerebral vesicle is relatively thin, radial glia are not required for guiding neurons. Interestingly, an earlier study using Golgi staining also suggested a mechanism for radially migrating neurons in the developing opossum cortex that is independent of glial guidance (Morest, 1970). In this mode, termed ‘perikarya translocation’, migrating neuroblasts that initially maintain processes extending to both ventricular and pial surfaces lose their ventricular attachments after terminal division and translocate their somata through pial-directed processes. However, as hypothesized by Morest (Morest, 1970), perikaryal translocation does not provide a plausible mechanism for the migration of later born cortical neurons, particularly at stages when the cortical anlage is several hundred micrometers thick. In support of Morest’s earlier work, a more recent immunohistochemical study (Brittis et al., 1995) has identified early neuronal populations in the developing rodent cortex with morphological features characteristic of cells undergoing perikaryal or somal translocation. In the light of these morphological findings, it seems possible that there may be two distinct modes of radial migration: an early, glia-independent mode, other forebrain structures such as the cerebral cortex, olfactory bulb and hippocampus? How do neurons that are generated in the subpallial region ‘know’ where to go and ‘what’ guides them towards the appropriate layers of the developing cortex? Is the information about their final destination part of their intrinsic genetic makeup or do these cells ‘acquire’ such information from guidance cues they encounter en route? While future investigations may address some of these questions, the demonstration of neurons being generated in pallial and subpallial regions has led to the study of their various modes of migration in the developing cortex. Here we review existing evidence for radial and tangential modes of migration and provide new data on a form of movement adopted by some young neurons in the developing cerebral cortex. The significance of the different modes of migration in building this complex structure is discussed.

Radial Migration: Somal Translocation and Glial-guided Locomotion

The early electron microscopical studies in fetal monkey neocortex by Rakic (Rakic, 1972) have shown that migrating neurons are closely apposed to radial glial fibers, suggesting that the glial system acts as a scaffold in their movement. Consistent with these observations, more recent immunohistochemical studies have further revealed that radial glia are present during corticogenesis and their processes span the full thickness of the cortical wall (Misson et al., 1991). However, electron microscopical examination of early mouse cortex by Shoukimas and Hinds (Shoukimas and Hinds, 1978) did not reveal a consistent association between neurons and glia, even though radial glial processes were abundantly present. This prompted the speculation that at the early stages of corticogenesis, when the cerebral vesicle is relatively thin, radial glia are not required for guiding neurons. Interestingly, an earlier study using Golgi staining also suggested a mechanism for radially migrating neurons in the developing opossum cortex that is independent of glial guidance (Morest, 1970). In this mode, termed ‘perikarya translocation’, migrating neuroblasts that initially maintain processes extending to both ventricular and pial surfaces lose their ventricular attachments after terminal division and translocate their somata through pial-directed processes. However, as hypothesized by Morest (Morest, 1970), perikaryal translocation does not provide a plausible mechanism for the migration of later born cortical neurons, particularly at stages when the cortical anlage is several hundred micrometers thick. In support of Morest’s earlier work, a more recent immunohistochemical study (Brittis et al., 1995) has identified early neuronal populations in the developing rodent cortex with morphological features characteristic of cells undergoing perikaryal or somal translocation. In the light of these morphological findings, it seems possible that there may be two distinct modes of radial migration: an early, glia-independent mode,
activated during the formation of the PP, and a later one that utilizes radial glial fibers during CP formation.

In a recent study (Nadarajah et al., 2001), we have used time-lapse imaging to demonstrate somal translocation as a distinct mode of radial movement in mouse cortical slices, and distinguished translocation from glial-guided locomotion by morphology and migratory behavior. In describing the two forms of radial movement we have shown that, during early corticogenesis, populations of cells undergo long-range somal translocation from the VZ to their positions beneath the pial surface. These cells typically showed distinct morphological features with long, radially oriented leading process terminating at the pial surface and a transient short trailing process. The migratory behavior of translocating cells is evidently distinct: firstly, as the soma advances towards the pial surface, the leading process becomes thicker and progressively shorter, while its terminal remains attached to the outer surface. Secondly, the soma of translocating cells displays continuous advancement at average speeds of 60 µm/h. Based on real-time images, it appears that somal translocation is a process of nucleokinesis in which the basal process first extends radially from the VZ to the pial surface, followed by nucleokinesis together with rapid reorganization of microtubules, resulting in shortening of the basal process. However, electron microscopical evidence would be required to demonstrate that the basal process is indeed attached to the basal lamina at the pial surface prior to translocation.

Locomoting cells, by contrast, have a free motile leading process that maintains a relatively constant length as the cell migrates forward. These cells show a characteristic saltatory pattern of migration – short bursts of forward movements interspersed with stationary phases, resulting in slower average speeds of 35 µm/h. In addition, locomoting cells appear to undergo a short-range translocation in their terminal phase of movement once their leading process reaches the MZ.

Another line of evidence that lends support for two distinct modes of radial migration comes from the analysis of mutant mice that show aberrant cortical layer formation. An anomaly consistently noted in reeler, mDab1, α3β1 integrin and VLDL mutant mice is the failure of the PP to be split by migrating CP neurons, resulting in the accumulation of CP cells beneath the PP with ill-defined layers [reviewed by Nadarajah and Parvanehs (Nadarajah and Parvanehs, 2002)]. In mice lacking Cdk5 or its activator, p35, the PP and the early CP form normally, but later-generated CP neurons collect below the SP in abnormal positions (Chae et al., 1997; Gilmore et al., 1998). The normal formation of PP in all these mutants further indicates that neurons constituting the PP may use somal translocation, a mode of migration that is independent of glial guidance and the reelin signaling pathway. Moreover, PP and early CP neurons that are generated at the onset of corticogenesis are phylogenetically older, whereas later generated cells are a more recent evolutionary addition (Marin-Padilla, 1978; Goffinet, 1983). Thus, it is conceivable that translocation is an older mode of movement in the evolution of the cerebral cortex for the transfer of PP and early CP neurons. On the other hand, glial-guided migration that is dependent on the reelin signaling pathway may have evolved to guide cells across more complex paths during late stages of cortical development, thus preserving the ‘inside-out’ pattern of corticogenesis.

**Tangential and Ventricle-directed Migration in the Developing Cortex**

Contrary to earlier studies that pointed only to radial migration as the mode of movement adopted by young cortical neurons, subsequent *in vitro* experiments, lineage analyses, and mouse chimeras have provided evidence for distinctly non-radial routes taken by cortical interneurons from their sites of origin in the GE (Walsh and Cepko, 1993; Anderson et al., 1997; Mione et al., 1997; Tan et al., 1998; Lavdas et al., 1999; Susel et al., 1999). Tracer-labeling studies have illustrated that the migration of these neurons occurs during the entire period of corticogenesis and along multiple tangential routes to their destinations in the developing cortex (Lavdas et al., 1999; Nadarajah et al., 2002). Further, while a large contingent of interneurons traverse through the intermediate zone (IZ), subsets of neurons migrate through the MZ, and at the interface of subventricular zone (SVZ) and IZ. The need for subpallial neurons to adopt multiple routes to reach the cortex or the underlying molecular mechanisms that necessitates such distinct trajectories remains unclear. Interestingly, sections from fixed embryonic brains stained for GABA or calbindin, markers of interneurons, have shown that while a large contingent of interneurons are horizontally oriented in the IZ, the number of labeled cells in the developing CP is considerably smaller (B. Nadarajah and P. Alifragis, unpublished observations). Thus, it is plausible that interneurons may need specifications that would enable them to integrate into the CP. In this regard, we have recently demonstrated that subsets of interneurons actively migrate in the direction of the cortical VZ upon reaching the dorsal telencephalon, a mode of movement that has been termed ‘ventricle-directed migration’ (Nadarajah et al., 2002). Interestingly, real-time imaging has demonstrated that neurons that migrate into the cortical VZ, after pausing for an extended period of time, resume their migration radially in the direction of the pial surface to take up their positions in the CP. The prevalence of interneurons with ventricle-directed morphological features at all stages of corticogenesis has led to the notion that these cells actively seek the cortical VZ to receive information, possibly pertaining to their layer position.

**Branching Cells in the Developing Cerebral Cortex**

Although our time-lapse studies have shown that radial and tangential pathways are the two predominant migratory routes adopted by cortical neurons (reviewed above), we have also observed a subset of cells that show distinctive morphological features and migratory behavior and have referred to them as ‘branching cells’. Using Oregon Green BAPTA-1 to label acute mouse brain slices as previously described (Nadarajah et al., 2001, 2002), we have shown that a subset of cells that appear bipolar at the onset of migration from the VZ, soon show multipolar form upon reaching the IZ (Figs 1 and 2). Examination of slices taken from mice at E13–E16 (n = 65 slices, 40 embryos, 320 moving cells) showed that, although the earliest age at which the branching cells were observed was E14, their characteristic movement was more prevalent in the later stages of corticogenesis (20% of all moving cells at E15). Branching cells are morphologically distinct from other types of migrating neurons, as they are highly motile in the formation and retraction of their processes. Figure 1 (see supplementary movie) illustrates the typical migratory behavior of one such cell that had an unbranched leading process at the onset of migration, but gave rise to branches with time as it reached the IZ. In such cases, the soma moved rapidly (1–3 µm/min) up to the branch point, paused for an extended period to retract one of the processes before resuming movement in the direction of the remaining branch which, in turn, continued to branch. The highly dynamic process of formation and retraction of branches suggests that these cells are actively exploring their environment for directional cues. Further, it appears that upon reaching the
IZ, these cells either migrate tangentially or continue their radial ascent with branched leading processes (Fig. 2).

The transient appearance of a thin trailing process (Fig. 1, \(t = 20\) min) during the movement of branching cells suggests a process of nucleokinesis that is reminiscent to somal translocation. However, the main feature of neurons that adopt somal translocation is that the leading process is anchored onto the pial surface suggesting that their direction of movement is predetermined (Fig. 3a, a'; arrows), in contrast to the exploratory behavior of branching cells. The appearance of free motile processes during migration is a feature of branching cells that is comparable to neurons undergoing glial-guided locomotion. However, a characteristic feature that distinguishes branching cells from the locomoting population, is that the latter have radially oriented leading process that remain unbranched during the course of migration while maintaining a relatively a constant length (Fig. 3c, c'; arrows). To investigate whether branching cells are present in the developing cortex, sections of fixed embryonic mouse brains (E13–E16) were stained with a panel of antibodies that label early neuronal populations. These experiments showed that although the IZ contained a subset of neurons with many branches that were positive for calbindin, the VZ was seemingly devoid of such cells (Fig. 4). It is possible that these cells, whilst being postmitotic, begin to express their

Figure 1. Time-lapse images of a branching cell labeled with Oregon Green BAPTA-1. Images were acquired every minute and each frame shows a single optical section. As the tip of the leading process reaches the IZ, it splits into two distinct branches (arrow). The soma then moves rapidly towards the branching point and pauses for an extended period of time. In the meantime, one of the two branches retracts, whereas the other splits further (arrowhead). As soon as the retraction is completed, the cell moves again rapidly towards the second branch point (arrowhead) where it pauses further. Scale bar: 50 \(\mu\)m.

Figure 2. Schematic illustration of a branching cell displaying the typical migratory behavior.

Figure 3. Time-lapse images of cells in the developing cortex illustrating the three distinct modes of migration: somal translocation (a, a'), branching cell movement (b, b'), and glia-guided locomotion (c, c'). Scale bar: 25 \(\mu\)m.
phenotype-specific markers only upon exiting the VZ. In accordance with our observations, previous time-lapse studies have illustrated that labeled cells that ascend radially from the cortical VZ display a multipolar phenotype and adopt tangential migration upon entering the IZ (O’Rourke et al., 1992).

Despite the demonstration that a subset of migrating cortical neurons are branching cells, their identity or their site of origin remains unclear. In this regard, earlier electron microscopical and real-time imaging studies have clearly demonstrated that pyramidal neurons have unbranched leading processes and migrate radially from the cortical VZ to the CP (O’Rourke et al., 1992). Based on these observations, it is unlikely that the branching cells are pyramidal neurons, although one cannot exclude the possibility of a subset of pyramidal cells that are yet to be characterized. Alternatively, it is possible that these cells are a subset of interneurons that are generated in the cortical VZ.

In support of this notion, earlier lineage studies and mouse chimeras with X-inactivated mosaics (J.G. Parnavelas, unpublished observations) (Tan et al., 1995, 1998) have demonstrated that neurons that constitute the radial clones in the developing cortex are glutamatergic, while their related siblings that show an orthogonal disposition are GABAergic interneurons. Further, the finding that in mutant mice that lack Dlx1/2 a quarter of the neurons that constitute the radial clones in the developing cerebral cortex remain (Anderson et al., 2002) suggests that a subset of interneurons are likely to be generated in the cortical VZ. Collectively, these observations together with recent experiments in ferrets (Anderson et al., 2002) indicate that while the majority of cortical interneurons are generated in the subpallium, a subset arises in the pallial proliferative zone. In this context, Rakic and colleagues (Letinic et al., 2002) have recently demonstrated that 65% of cortical GABAergic neurons in humans are generated in the proliferative zones of dorsal forebrain.

In summary, our recent studies have highlighted the various migratory modes adopted by developing cortical neurons (Fig. 5). Although our observations suggest that different modes of migration are likely to be adopted by distinct populations of cortical neurons, due to the expansion of the cerebral volume later born neurons would need to utilize more than one mode of migration to reach their destinations. Thus, it is likely that later generated cortical neurons contain the necessary molecular and cellular machinery to enable them to switch from one mode of migration to another in a temporal fashion.

Notes
We acknowledge the contributions of Drs Jan Brunstrom, Jamie Grutzendler and Alan Pearlman, and the support of the Wellcome trust.

Address correspondence to Bagirathy Nadarajah, School of Biological Sciences, University of Manchester, Manchester M13 9PT, UK. Email: bagi@man.ac.uk.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oupjournals.org

References


