Polarized Increase of Calcium and Nucleokinesis in Tangentially Migrating Neurons

Cortical interneurons originate from the ganglionic eminences and reach their final position in the cortex via tangential migratory routes. The mechanisms of this migration are poorly understood. Here we have performed confocal time-lapse analysis of cell movement in the intermediate zone of embryonic mouse cortical slices in order to directly visualize their mode of migration. Tangentially migrating neurons moved by nucleokinesis, characterized by active phases of discontinuous advances of the nucleus followed by periods of quiescence. Dissociated cells from the ganglionic eminences also showed nucleokinesis associated with an increase in intracellular calcium, [Ca$^{2+}$]. Calcium elevation was greatest in the proximal region of the leading process, a zone with a wide distribution of γ-tubulin. General increases in [Ca$^{2+}$], elicited by microperfusion with neurotransmitters did not elicit nucleokinesis. These results show that tangential migration uses nucleokinesis, a cell-intrinsic process in which calcium signalling is local, directional and highly regulated.

Keywords: calcium signalling, cytoskeleton, gabaergic neurons, neuronal migration, γ-tubulin

Introduction

Neuronal migration is a critical event in the development of the vertebrate nervous system (Hatten, 1999). In the cerebral cortex, the two basic neuronal types, pyramidal (excitatory) and non-pyramidal (inhibitory), originate from different sources and have distinct migratory routes (reviewed in Pearlman et al., 1998). Projecting pyramidal neurons originate in the ventricular zone and migrate radially to their final position in the cerebral cortex. At different stages of development, radially migrating cells move alternatively by soma translocation (Miyata et al., 2001; Nadarajah et al., 2001) or by whole cell translocation along radially oriented glial processes (reviewed in Nadarajah and Parnavelas, 2002).

Most GABA-ergic interneurons of the cortex originate in the ganglionic eminences of the basal telencephalon (De Carlos et al., 1996; Tamamaki et al., 1997; Anderson et al., 1999; Wichterle et al., 2001; Marin and Rubenstein, 2001). After leaving the proliferative zone in the MGE, they migrate following tangential pathways in different cortical compartments: the subventricular zone (SVZ), the intermediate zone (IZ) and the marginal zone (MZ). It is assumed that interneurons migrate by nucleokinesis as they traverse the intermediate zone perpendicularly to the radial glial processes, although there is no direct evidence for this. Contact with corticofugal axons at the IZ (Metin et al., 2000; Denaxa et al., 2001) hepatocyte growth factor/scatter factor (Powell et al., 2001) and TrkB signalling (Polleux et al., 2002) have been proposed to participate in controlling interneuron migration in the developing cerebral cortex. Once in the cortex, tangentially migrating neurons move in a ventricle-directed migration, with a salutary movement similar to that observed with radially migrating glia-guided neurons originated at the VZ (Nadarajah et al., 2002; Ang et al., 2003).

Changes in intracellular calcium plays a central role in numerous developmental processes, included neuronal migration (reviewed in Webb and Miller, 2003). The amplitude and frequency of calcium fluctuations are positively correlated with the rate of granule cell movement in cerebellar microexplant cultures. Interestingly, tonic elevation of [Ca$^{2+}$], results in an arrest of cell movement (Komuro and Rakic, 1996, 1998). In the case of tangentially migrating neurons in the developing cortex, there is no information about the role of calcium changes on cell migration. Tangentially migrating cells express glutamate AMPA receptors as well as GABA-A receptors (Metin et al., 2000; Poluch and Konig, 2002) whose activation produces increases in [Ca$^{2+}$] (Soria and Valdeolmillos, 2002). The activation of AMPA receptors in organotypic slice cultures lead to neurite retraction of migrating cells (Poluch et al., 2001) suggesting a role in tangential migration.

Mutations in several genes affecting nuclear migration in slime molds have suggested a role for cytoplasmic dynein, the dynactin complex and other proteins, such as NUDF and NUDC, in the process of nuclear translocation (Reinsch, 1998; Feng and Walsh, 2001; Morris, 2003). In mice and humans, mutations in the ortholog gene of NUDF (LIS1) cause lissencephaly, a brain developmental pathology affecting the process of cortical lamination (Ross and Walsh, 2001) and a cell-autonomous defect in cell migration (Hirotsume et al., 1998). In migrating cerebellar granule cells, NudC, Lis1 and cytoplasmic dynein colocalize at the microtubule organizing center (MTOC) facing the leading pole, in contrast to their widespread distribution in stationary cells, suggesting a functional interaction of these components during neuronal migration in vivo (Aumais et al., 2001).

We have studied the movement of tangentially migrating cells through the IZ in cortical slice cultures from embryonic (E13–14) mouse and in dissociated cell cultures from the MGE. In both experimental conditions, migrating neurons display a characteristic somal translocation or nucleokinetic movement. Nuclear displacement occurs simultaneously or immediately following a local [Ca$^{2+}$], increase in the leading process near the nucleus, suggesting that a localized calcium signal is necessary to elicit nucleokinesis.

Materials and Methods

Animals

C57 strain mice were mated overnight and vaginal smears examined the next morning. The day of sperm positivity was taken as embryonic.
Pregnant mice were deeply anaesthetized with choral hydrate (i.p.) and the fetuses (E13.5–14.5) extracted by caesarean. Experimental procedures involving live animals were carried out in accordance with the guidelines set by the European Community and were approved by the Animal Care Committee of the authors’ institution.

**Slice Culture**

Slices were prepared and cultured as previously described (Soria and Valdeolmillos, 2002). Whole brains were dissected out and embedded in warm (41°C) 4% low-melting point agarose (Sigma, St Louis, MO) and rapidly cooled. Coronal cortical slices 500 µm thick were cut with a Vibratome (VT1000S; Leica, Germany). Caudal sections were used in which both the medial ganglionic eminence (MGE) and lateral ganglionic eminence (LGE) were present. The slices were cultured on top of Millicell membranes (Millipore, Bedford, MA) of 0.4 µm pore diameter in Neurobasal medium (Life Technologies Gaithersburg, MD) supplemented with B27 (1:50; Life Technologies), 6.5 mg/ml glucose, 0.1 mM glutamine and 50 mg/ml penicillin/streptomycin for periods ranging from 24 to 48 h. We used confocal Oregon-green BAPTA microfluorescence for the analysis of nuclear movements and [Ca^{2+}] measurements. Experiments were carried out on slices at 23–26°C continuously superfused (1 ml/min) in ACSF medium. For the experiments described in this study, 75 slices from 45 animals were processed.

**Time-lapse Experiments**

Cortical slices were removed from the culture medium and incubated for 1 h in Neurobasal medium containing 10 µM Oregon-green BAPTA AM (Molecular Probes, Eugene, OR), dissolved in 0.09% dimethyl sulfoxide and 0.006% pluronic acid. In some experiments we used Fluo-3 as a calcium indicator with essentially the same results. Slices were transferred to the stage of an upright Leica DMLFA microscope coupled to a confocal spectral scanning head (Leica TCS SL) and viewed through 10–60x water immersion objectives. Oregon-green BAPTA labeling was excited with the 488 nm line of an argon laser and the fluorescence emitted between 500 and 540 nm measured via a photomultiplier tube. We recorded XYZ stacks of images at a frequency of one stack every 1–3 min for periods of up to 60 min. To analyze cell movement, z projection images of every stack were projected in a single image, that we call XYZ projection. This image represents a two-dimensional history of a given cell within a region (see for example the XYZ image in Fig. 2). With this procedure it is immediately obvious when a cell moves with respect to another. To avoid photodamage of the slice and photo bleaching of the indicator, laser excitation was kept to the minimum compatible with a good signal to noise ratio. Changes in Oregon-green fluorescence are expressed as the ratio between fluorescence at the beginning of the experiment and at a given time-point. Drugs were micro-perfused from a patch pipette positioned above the cell of interest by pressure injection of 10–60 s and the fluorescence ratio (F350/F380) calculated using the U7568 software from AQUA-COSMOS package.

**Dissociated MGE Cells**

Fetuses (E13.5) were decapitated and heads immediately placed in chilled L15 medium. Brains were isolated and the MGE dissected in L15 medium and transferred to DMEM supplemented with N_{2} in which cells were dissociated by five gentle passages through a pipette tip. Cells dissociated from one brain were resuspended in 1 ml of medium and plated on glass coverslips treated with polylysine (mol. wt 70–150 000 at 0.01%) and laminin (50 µg/ml). Each coverslip received 0.1 ml of the cell suspension and 4 h later 0.9 ml of medium was added to each culture. Cultures were maintained at 37°C in 5% CO_{2} atmosphere.

Time-lapse analysis of dissociated cells (24–48 h after plating) was performed in an inverted microscope (DMIRB; Leica) while maintaining the culture at 34–35°C with continuous superfusion (1 ml/min) in ACSF medium as described above. Cells were incubated 60 min in medium containing Fura-2 AM (5 µM; Molecular Probes, Eugene, OR), dissolved in 0.09% dimethyl sulfoxide and 0.006% pluronic acid. The fluorophore was excited at 350 and 380 nm and the emission at 510 nm recorded with an ORCA C4742 camera (Hamamatsu Photonics). Images were acquired every 15–60 s and the fluorescence ratio (F350/F380) calculated using the U7568 software from AQUA-COSMOS package.

**Immunocytochemistry**

Dissociated cells were fixed in a 4% paraformaldehyde solution containing 4% sucrose for 30 min at 37°C. The following antibodies were used in this study: monoclonal anti-gamma-tubulin (GTU88 from SIGMA) at 1:500 dilution revealed with a secondary antibody antimouse IgG labelled with Cy2 from Jackson and anti-glutamic acid decarboxylase 65–67 (Sigma) at 1:1000 dilution revealed with an antirabbit IgG conjugated to Cy3 (Sigma).

**Results**

To determine the tangential cells mode of migration in slice cultures from embryonic (E13–14) mice, we performed confocal time-lapse observations at the IZ zone in cortical areas close to the ganglionic eminences (Fig. 1d). Cells were loaded with Oregon-green BAPTA-AM, a fluorescent calcium indicator. Some cells showed the bipolar morphology typical of tangentially migrating cells, with thick leading processes directed dorsomedially and thin trailing processes. During prolonged observations these cells could be seen to alternate between periods of rest and of nucleokinesis (Fig. 1b). Plotting nuclear displacement of the neuron shown in time-lapse series demonstrates the existence of phases of no movement (initial 20 min) and of motility (minutes 22–38; Fig. 1c). The difference between fluorescence levels in the nuclear region prior to nuclear translocation (Fig. 1b, minute 22) and during its onset (minute 26) suggest a transitory increase in [Ca^{2+}], associated with nuclear displacement (see below). For a time-lapse view of nucleokinesis, see Movie 1 of the Supplementary Information. Detailed analysis of the active phase of movement in several cells revealed a variable pattern of nuclear translocation (Fig. 1d). In some cells the speed of nuclear translocation was constant, while in others, the nucleus moved in a more irregular fashion, with periods of displacement at different speeds interspersed by periods of no movement (Table 1). During the active phase of movement, the nuclear translocation phase in cells moving in the slices lasted for 41.3 ±11.9 min, with a mean speed of 0.55 ±0.38 µm/min. At the magnification used in our experiments, typically 30–40 somata were visible and only one or two of those cells were seen to move in periods of up to 60 min, indicating that the number of cells displaying nucleokinesis at a given time is low. This suggests that under our experimental conditions the period between active nucleokinetic phases is relatively long. Similar results were obtained when cells were labeled with calcine (not shown) instead of the calcium indicator suggesting there are not calcium buffering effects responsible for the relative low number of cells showing nucleokinesis at a given moment. It has been shown that in co-culture experiments migrating cells spend ~50–70 min in a quiescent state (Polleux et al., 2002). We have not further analyzed the relative durations of the active and quiescent phases of migration.

In our experiments we define nucleokinesis as the movement of the cell nucleus within an otherwise stationary cell without retraction or elongation of the leading and trailing
processes. However, in slices bulk loaded with fluorescent indicators, it is difficult to follow the fine details of the leading and trailing processes due to the high levels of fluorescence from the surrounding tissue. To overcome this problem we have taken advantage of the fact that the leading process of many tangentially migrating cells is often branched in a bifurcation that can be used as a reference mark for the nuclear displacement. As shown in Figure 2, cellular movement is characterized by the sliding of the nucleus inside the leading process. At time 0 the nucleus (white arrow, Fig. 2) enters the leading process. Active nuclear movement continues up to minute 34, at which time the nucleus reaches the bifurcation (arrowhead, Fig. 2), where it remains for the rest of the time under study. Whole cell locomotion would involve a simultaneous displacement of nucleus and branching point of the leading process, without a shortening of the distance between them.

Calcium signalling plays a central role in different kinds of cell movement, including neuronal migration (Komuro and Rakic, 1996, 1998), but its role in the tangential migration of interneurons has not been previously explored. We investigated the presence of [Ca\(^{2+}\)] changes during nuclear translocation. The changes in fluorescence intensity shown in Figure 1 already suggest a role for calcium signalling in this process. However, an unequivocal localization of the fluorescence intensity changes with calcium indicators excited at a single wavelength is hindered by the changes in cell morphology during the active phase of movement.

To obtain a better spatial resolution of the [Ca\(^{2+}\)] changes associated with nucleokinesis, we performed additional experiments in dissociated culture of cells from the MGE. Twenty-

**Table 1**
Comparison of nucleokinetick velocities in cells from slice cultures (recorded at 26°C) and dissociated cells (recorded at 34°C)

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<th>Velocity (µm/min)</th>
<th>Mean ± SD</th>
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<tr>
<td>Slices (n = 11)</td>
<td>0.29–1.72</td>
<td>0.55 ± 0.38</td>
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<tr>
<td>Dissociated (n = 10)</td>
<td>0.24–3.79</td>
<td>1.43 ± 1.11</td>
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Figure 1. Nucleokinesis in a neuron migrating tangentially. (a) The photomicrograph shows the region, indicated by the frame, were measurements were made. MZ, marginal zone; VZ, ventricular zone; IZ, intermediate zone; GE, ganglionic eminence. Scale bar = 200 µm. (b) Sequence of frames showing confocal projection images (stack of eight planes acquired at 1.5 µm z-intervals) taken at the time (min) indicated in each frame. The arrow in each frame shows the position of the nucleus at the beginning of the sequence. At time 0, the cell has a prominent soma and bipolar morphology. The cell remains in a resting phase during the first 20 min of the sequence and then the nucleus begins to enter the leading process, acquiring a fusiform morphology (frame 22). The nucleus continues advancing inside the leading process up to minute 38, at which time it stops. In the last frame, the arrow shows that the position initially occupied by the nucleus is now the thin and long trailing process. Scale bar = 10 µm. (c) Plot of nuclear displacement measured during the 40 minute experiment shown in a. (d) Plot of nucleokinesis in representative cells. Only the active phase of movement is represented. The pattern of movement ranges from continuous (i.e. #1) to variable speed (#2, #3).
four hours after plating dissociated neurons show a variety of morphologies including some with bipolar shape (Fig. 3a). Time-lapse sequences of a bipolar cell revealed nucleokinetic movements similar to those described in the slice cultures and characterized by the sliding of the nucleus inside the thicker process. The average speed of nuclear displacement in dissociated cells (Table 1) is about three times faster than that observed in the slices (see Table 11). This difference could be explained as a result of differences in the temperatures (34°C for dissociated cells versus 25°C for the slice cultures) at which time-lapse analyses were performed. Other factors, such as the laminin substratum in which dissociated cells are plated, could also account for these differences.

Measurements with the ratiometric calcium indicator Fura-2 revealed an elevation of \([\text{Ca}^{2+}]_i\) during nucleokinesis (Fig. 3). Remarkably, the maximal \([\text{Ca}^{2+}]_i\) rise was consistently located in the area just ahead of the nucleus (Fig. 3a; images at 13 and 20 min), pointing the direction of the nuclear displacement. Figure 3c shows the time-course of the fluorescence ratio in the two regions marked by the blue and red dots while the soma is translocating towards the right. During the first half of the sequence (10–20 min) the blue and red lines show ratio intensities at the soma and leading process, respectively: \([\text{Ca}^{2+}]_i\) is 25% higher at the leading process. During the second part of the sequence (minutes 25–35) the \([\text{Ca}^{2+}]_i\) values on the red circle, now at the soma, decrease while those at the blue area, located outside the cell, are at background levels. This asymmetric distribution of \([\text{Ca}^{2+}]_i\) is maintained as the nucleus advances (see Movie 2 of the Supplementary Information). We have analysed active phases of nucleokinesis in 26 cells (individual recording time range, 15–100 min; total recording, 393 min) In 20 cells (313 min of recording), the nucleus moved significant distances in the direction of the polarized \([\text{Ca}^{2+}]_i\) (as shown in Fig. 3). Four other cells moved with short (less than

Figure 2. Nucleokinesis proceeds inside the leading process and stops at the branching point. Confocal projection images taken at the time (min) indicated in each frame. The arrowhead shows that the position of the branch remained constant during the experiment. The white arrow mark the position of the nucleus at each time. The last panel shows the projection of all the images (see Materials and Methods) during the 50 min experiment. Only the nucleus has shown movement, the branch and neighbouring cells have remained stationary. The white arrows show the position of the nucleus at times 0 and 50 min. Scale bar = 10 μm.

Figure 3. Nucleokinesis and calcium changes in dissociated cells from the MGE. (a) Phase contrast and pseudo-colour images of Fura-2 fluorescence ratios (F350/F380) from the same cell, taken at the times indicated (minutes). During nucleokinesis, the maximal \([\text{Ca}^{2+}]_i\), is always located just ahead of the nucleus (images at 13 and 20 min). (b) Plot of nuclei displacement from experiment shown in a. (c) Time course of the mean F350/F380 ratio measured in two fixed regions of the recording field marked by the blue and red areas respectively. From minutes 10 to 20 the blue and red lines show ratio intensities at the soma and leading process respectively: \([\text{Ca}^{2+}]_i\) is 25% higher at the leading process. From minutes 25 to 35 the blue circle is located outside the cell giving background values of ratio intensities while the values on the red circle, now showing \([\text{Ca}^{2+}]_i\), at the soma, decreases. Recordings were performed at 34°C, 24 h after plating. Scale bar = 10 μm.

Figure 4. Gamma-tubulin is polarized towards the leading process in interneurons. (a) Nomarski interference images showing nucleokinesis, taken at the times indicated (upper panel). At 15 min the cultures were fixed and processed for GAD 65–67 (left lower panel) and γ-tubulin (right lower panel) in the experimental bath. We invariably found that γ-tubulin is polarized in the leading process (four out of four experiments). γ-Tubulin shows a wider distribution in bipolar moving neurons than in cells with a multipolar or fibroblast-like morphology. (b) γ-Tubulin is concentrated in a single spot, probably corresponding to the centrosome, in a multipolar stationary neuron.
Figure 3

Figure 4
the soma length) back and forward displacements and \([\text{Ca}^{2+}]_i\) changes were perinuclear. In the two remaining cells, the direction of movement was opposite to the maximum \([\text{Ca}^{2+}]_i\). In summary, in 20 out of 22 cases in which we observed a clear nucleokinetetic movement we observed simultaneously a polarization of \([\text{Ca}^{2+}]_i\) in the direction of movement. Polarization of \([\text{Ca}^{2+}]_i\) was also observed in some cells in which there were not soma displacement during the recording time. We conclude that, under the experimental conditions of our study, polarization of \([\text{Ca}^{2+}]_i\) is a necessary although not sufficient event for nucleokinesis.

This polarized calcium signal suggests the participation of precise mechanisms and structures able to maintain \([\text{Ca}^{2+}]_i\) asymmetries for prolonged periods of time in highly dynamic cells (Berridge, 1998; Delmas and Brown, 2002).

Nuclear displacement occurred in isolated cells as well as in cells making contacts with other cells or axonal projections. This observation supports the view that nuclear translocation is an intrinsic property of these cells which can be manifested independently of the guidance contacts used to direct migration within the developing cortex in vivo. Interestingly, cerebellar granule cells follow an intrinsic migratory program, reflected by changes in their migratory behaviors when contact with other cells is absent (Yacubova and Komuro, 2002a).

The microtubular cytoskeleton participate in nucleokinesis (Rivas and Hatten, 1995; Rakic et al., 1996; Reinsch, 1998) Dissociated interneurons, visualized as GD65–67 immunopositive cells, showed γ-tubulin clearly polarized at one pole of the nucleus. To test whether the polarized distribution of γ-tubulin correlated with the leading process, cells showing soma translocation after time lapse experiments were fixed and immunolabelled with anti-GD65–67 and anti-γ-tubulin (Fig. 4a). We found that the direction of nuclear movement (toward the right in Fig. 4a) coincided consistently with the diffuse γ-tubulin staining pattern, directly demonstrating that microtubules in the leading process are preferentially oriented with their minus ends toward the nucleus. Interestingly, γ-tubulin shows a wider distribution in migratory neurons than that observed in multipolar stationary neurons (Fig. 4b) and fibroblasts. A distribution of γ-tubulin similar to the one reported here, and colocalizing with LIS1 and Nudel proteins has been described in early differentiating cortical neurons (Feng et al., 2000).

The experimental data in dissociated cells confirm the results obtained in cortical slices and suggest that a localized calcium signal is necessary to elicit nucleokinesis. To test this hypothesis more directly, the effects of general increases in calcium on nucleokinesis were analysed. Our previous work (Soria and Valdeolmillos, 2002) has shown that activation of NMDA, AMPA/kainate and GABAa receptors (Metin et al., 2000; Poluch and Konig, 2002) leads to \([\text{Ca}^{2+}]_i\) increase in tangentially migrating cells. Furthermore, the activation of AMPA receptors in organotypic slice cultures leads to neurite retraction of migrating cells (Poluch et al., 2001) which suggests a role in tangential migration.

A cell with migratory morphology and a branched leading process in the slice (Fig. 5a) was challenged with a brief puff of kainate delivered by a pressure pulse (100 ms duration) through a patch pipette. Fluorescence before, during and after kainate application was measured. Kainate elicited an immediate and transitory \([\text{Ca}^{2+}]_i\) increase in the soma, the initial part of the leading process and the branches distal to its bifurcation (Fig. 5b) that was not followed by nucleokinesis. The same results were obtained with NMDA and the GABAa agonist muscimol. We also searched for neurotransmitter responses in cells that had displayed nucleokinesis previously (Fig. 5c,d). Kainate also induced a transitory increase in \([\text{Ca}^{2+}]_i\) in these cells (graph insert), but it was not followed by an immediate movement of the soma. Thirty minutes after kainate stimulation, a slight backward movement (4 μm, see frame at minute 60) was followed by a small forward movement. Other cells in the slice which also showed an increase in \([\text{Ca}^{2+}]_i\), did not move in response to kainate. Neurotransmitters play an important role in neuronal migration (Behar et al., 1999, 2001; Simonian and Herbison, 2001; Ishiuchi et al., 2002). The lack of a neurotransmitter effect in our observations would suggest that their role in neuronal migration is not directly linked to the nucleokinetetic process.

**Discussion**

The results presented in this study show that tangentially migrating neurons in the IZ use nucleokinesis as a mode of migration. The spatial and temporal distribution of \([\text{Ca}^{2+}]_i\), observed in migrating cells, suggest that calcium signalling is a local, directional and highly regulated process during nucleokinesis.

Two basic modes of movement, whole cell locomotion and soma translocation, have been described in migrating neurons. Nuclear translocation was first inferred from morphological studies of developing brain (Boo and Morest, 1990) and visualized in cerebellar slices (Hager et al., 1995) and developing cortex (O’Rourke et al., 1992). Glia-mediated migration of cerebellar neurons involve saltatory phases along the glial processes by translocation of the soma (Gasser and Hatten, 1990). In cortical slices, movement by whole cell locomotion and by somal translocation, have been described for the radial migration of neurons (Nadarajah et al., 2001). So both modes of movement presumably represent different phases of cell advancement rather than reflecting cell-type specific or region-specific properties.

The term ‘nucleokinesis’ was applied first to the nuclear movement observed in bipolar human lung adenocarcinoma cells (Klominek et al., 1991). The nuclei of these cells are transported to the opposite end of the cell, while gross cell shape and position remain unchanged. In slice cultures loaded with the nucleic acid stain SYTO 83 in addition to Oregon-green we found that the cell soma is almost entirely occupied by the nucleus indicating that the observed soma translations correspond to nuclear displacement (not shown). The sliding of the nucleus changes its shape, from round to fusiform, as it enters the initial part of the leading process. Once the soma translocation is initiated, the movement is steady although the rate of movement is irregular, alternating slow and rapid phases. This migratory dynamic is similar to the one found in the final stages of radially migrating cells as their leading process reaches the marginal zone (Nadarajah et al., 2001). The main difference would be that radially migrating cells retract their leading process once they reach the marginal zone, while tangentially migrating neurons ought to repeat the same process several times along their migratory route, resulting in a ‘saltatory-like’ mode of migration.

Likewise, it has been recently shown that GABAAergic interneurons migrating tangentially within the marginal zone...
extend a leading process into the cortical plate where the cell translocates its cell body towards the end of its leading process (Ang et al., 2003). Therefore nucleokinesis seems to be a mechanism widely used by different types of neurons at different developmental stages.

In our recordings we have seen group of cells migrating tangentially arranged in parallel and in some occasions in close contact. However, we have never observed coordinated movements of cells, suggesting that nucleokinesis relays on a mechanism independent for every cell. In fact, nuclear translocation of MGE migrating cells seems to be a cell intrinsic process since it can be observed also in isolated cells in the dissociated cultures.

The effect of neurotransmitters in neuronal migration has been analyzed in different migratory subsets of neurons. In general, it has been found that the effects of the neurotrans-

Figure 5. Kainate perfusion increases [Ca^{2+}], but does not promote nucleokinesis. (a) Confocal projection images taken at the times indicated (min). The frame marked ‘Kainate’ was sampled 10 s after a 100 ms pulse of kainate (nominal concentration in the pipette was 50 µM). The last frame was acquired 30 min after kainate challenge. (b) Changes in fluorescence measured at the circular regions marked in the second frame. (c) Confocal projection images taken at the time indicated (min). The position of the cell nucleus displaying nucleokinesis is marked by the arrow. (d) Plot of nucleokinesis and fluorescence changes in the cell identified in c. During the first 27 min of recording the nucleus moves 19 µm. When kainate is microperfused at 30 min, there is an increase in calcium measured as an increase in fluorescence (F/Fo graph insert). In the following 20 min the nucleus does not move and at 30 min post-challenge a backward movement is initiated (4 µm, see frame at minute 60) followed by a small forward movement, as shown in frame 100 min.
mitters are very variable, depending on the species and the preparation studied (Komuro and Rakic, 1996, 1998; Fueshko et al., 1998; Behar et al., 1999, 2001; Simonian and Herbon, 2001; Owens and Kriegstein, 2002; Yacubova and Komuro, 2002b). In tangentially migrating cells, the activation of glutamate and GABA receptors (Metin et al., 2000; Poluch and Konig, 2002) increases calcium (Soria and Valdeolmillos, 2002). The activation of AMPA receptors in organotypic slice cultures lead to neurite retraction of migrating cells (Poluch et al., 2001) suggesting a role in tangential migration. However, the [Ca^{2+}]i changes elicited by the neurotransmitters were not able to induce nucleokinesis in previously resting cells nor in cells which have shown a previous soma translocation. The lack of a direct effect of neurotransmitters observed here may be reconciled with the above mentioned observations if the role of neurotransmitters in migration is not directly linked to the nucleokinetic process. We have found that during the active phase of nucleokinesis there is a good correlation between the direction of movement and [Ca^{2+}]i polarization (Fig. 3). The fact that a generalized [Ca^{2+}]i increase in cerebellar granule cells does not result in an increase, but rather in a significant decrease in the rate of cell movement (Komuro and Rakic, 1996) and the results presented here, suggest that the calcium changes associated with the translocation of the soma are spatially and temporally regulated so that nucleokinesis is activated at the precise place and time.

Migrating granule cells of the cerebellum showed a cage-like distribution of microtubules encircling the nucleus (Rivas and Hatten, 1995) and there are other evidences showing the involvement of microtubules in the process of soma translocation. We hypothesize that the local calcium elevation in the proximal part of the leading process is related to the dynamic modification of cytoskeletal components taking place at this pole of the nucleus. Changes that are reflected also in the wider distribution of γ tubulin observed in migrating neurons as compared to stationary multipolar neurons (Fig. 4b).

In differentiated neurons the cytoskeleton is polarized with axonal microtubules oriented with their plus ends towards the periphery while dendritic microtubules are randomly oriented. Such a polarization is related to the differential distribution of microtubule associated proteins tau and MAP2 predominating in axons and dendrites respectively. However, in migrating cerebellar granule neurons the orientation of Mts does not follow the above mentioned distribution. Thus, the leading processes of migrating granule cells in situ, which will be transformed in dendrites, showed uniform microtubule orientation with their plus ends towards the direction of migration, while microtubules in the trailing process, which will become the axon, are randomly oriented (Rakic et al., 1996). GABA positive cells with bipolar morphology in the developing cortex show a high expression of MAP2 in their leading processes (Tamamaki et al., 1997; Poluch and Konig, 2002). Gamma tubulin, a marker of nucleating minus ends of Mts, concentrates in the pole of the nucleus facing the leading process, suggesting that plus ends of microtubules are oriented in the direction of migration in the leading process. Therefore, in contrast with the typical distribution of Mts in differentiated neurons, the orientation of microtubules in migrating cells might reflect the direction of migration rather than their final transformation into axons or dendrites.

The distribution of other proteins implicated in nuclear translocation like LIS1, NUDC and NUDEL, in dissociated cells at early times after plating (Feng et al., 2000) and cerebellar migrating neurons (Aumais et al., 2001) is similar to the distribution of gamma-tubulin. LIS1-NudE interactions may be crucial for maintaining the dynamic stability of microtubules in migrating neurons and their presence could contribute to the microtubule shortening and translocation of the nucleus in the proximal part of the leading process. The wider distribution of gamma-tubulin we have observed at the leading pole of the nucleus in migrating neurons, suggest that the minus ends of microtubules in the leading process do not nucleate at a single point until LIS1 and NUDEL are sorted to the axon (Sasaki et al., 2000).

The effect of calcium could be mediated by DCAMKL1, a protein kinase with homology to doublecortin (DCX). DCAMKL1 stimulates polymerization of purified tubulin and contains a domain encoding for a putative Ca^{2+}/calmodulin dependent protein kinase (Lin et al., 2000). CAM kinases are activated downstream of [Ca^{2+}], transients and phosphorylation of cytoskeletal components such as DCX by DCAMKL1 or other kinases could represent a rapid mechanism for linking calcium signalling to microtubule reorganization.

In conclusion our results directly show that tangentially migrating neurons in the IZ use soma translocation as a mode of migration. Nuclear displacement is associated to a local and sustained [Ca^{2+}]i increase in the leading process near the nucleus, providing a link between internal or external migratory signals and cytoskeletal reorganization.

Supplementary Information

**Movie 1. Nucleokinesis of a Tangentially Migrating Neuron**
Confocal projection images of a neuron migrating through the IZ. The arrow in the first frame points to the nucleus at the beginning of the sequence. The cell remains in a resting phase during the first half of the sequence, then the nucleus moves, advancing inside the leading process and stops at the end of the sequence. Total recording time was 40 min.

**Movie 2. Nucleokinesis and Calcium Changes in Dissociated Cells**
Sequential images of Fura-2 fluorescence ratio (pseudo-color coded) superimposed on 380 fluorescence images. During nucleokinesis the maximal [Ca^{2+}]i is located ahead of the nucleus at the beginning of the sequence. The cell remains in a resting phase during the first half of the sequence, then the nucleus moves, advancing inside the leading process and stops at the end of the sequence. Total recording time was 18 min.

**Notes**
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