α3β1 integrin modulates neuronal migration and placement during early stages of cerebral cortical development

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Summary

We show that α3 integrin mutation disrupts distinct aspects of neuronal migration and placement in the cerebral cortex. The preplate develops normally in α3 integrin mutant mice. However, time lapse imaging of migrating neurons in embryonic cortical slices indicates retarded radial and tangential migration of neurons, but not ventricular zone-directed migration. Examination of the actin cytoskeleton of α3 integrin mutant cortical cells reveals aberrant actin cytoskeletal dynamics at the leading edges. Deficits are also evident in the ability of developing neurons to probe their cellular environment with filopodial and lamellipodial activity. Calbindin or calretinin positive upper layer neurons as well as the deep layer neurons of α3 integrin mutant mice expressing EGFP were misplaced. These results suggest that α3β1 integrin deficiency impairs distinct patterns of neuronal migration and placement through dysregulated actin dynamics and defective ability to search and respond to migration modulating cues in the developing cortex.

Key words: Cerebral cortex, Migration, Adhesion

Introduction

Normal development of the mammalian cerebral cortex requires the coordinated migration of postmitotic neurons from the ventricular zone to the outermost layer of the developing cortical plate. Migrating neurons travel through varying intercellular environments in distinct orientations, past previously generated neuronal cohorts of the cortical plate, in order to form layers with distinct patterns of synaptic connectivity (D'Arcangelo et al., 1995; Hatten, 2002; Marin et al., 2003; Nadarajah et al., 2001; Nadarajah and Parnavelas, 2002; O'Rourke et al., 1992; Rakic, 1972; Yakubova and Komuro, 2002). Abnormalities in neuronal migration and layer formation lead to abnormal placement and connectivity of cortical neurons, an underlying cause of many congenital brain disorders in humans (Hong et al., 2000; Ross and Walsh, 2001). The specific cell-cell adhesion-related mechanisms that determine how neurons migrate and coalesce into distinct layers in the developing cerebral cortex remains to be fully characterized. Integrins, heterodimeric cell-surface receptors that serve as links between the extracellular matrix (ECM) and the internal cytoskeleton, modulate a the adhesive behavior of a cell in response to multiple environmental cues (Clark and Brugge, 1995; Condic and Letourneau, 1997; Feng and Walsh, 2001; Galileo et al., 1992; Grabham and Goldberg, 1997; Hynes, 2002; Juliano, 2002; Lawson and Maxfield, 1995; Pinkstaff et al., 1999; Schmid et al., 2003; Sheppard, 2000). α3 integrin (Itga3 – Mouse Genome Informatics) is a major integrin subunit expressed by neurons in the developing cortex, and mice homozygous for a targeted mutation in the α3 integrin gene die during the perinatal period with severe defects in the development of the kidneys, lungs, skin and brain (Anton et al., 1999; De Arcangelis et al., 1999; DeFreitas et al., 1995; Hodivala-Dilke et al., 1998; Kreidberg et al., 1996). Disrupted neuronal laminar organization in the α3 integrin mutant cerebral cortex suggests an impairment of proper neuronal migration and placement in the absence of α3 integrin signaling.

We have tested this hypothesis using real-time analysis of neuronal migration in wild type and α3β1 integrin-deficient embryonic cerebral cortex. BrdU birthdating indicates that the preplate splits normally in the α3 integrin mutant cortex. However, radial and tangentially directed neuronal migration that follows, proceeds at a significantly slower rate in the absence of α3β1 integrin. By contrast, ventricular zone directed migration is not affected in mutant cortices. Deficits in neuronal migration are accompanied by the inability of α3β1 integrin-deficient migrating neurons to display the characteristic probing extensions and retractions at their leading and trailing edges. Real-time imaging of actin dynamics in the leading edges of wild-type and α3 integrin$^{-/-}$ cortical cells indicates a significant deficit in the dynamic activity of actin filaments that underlie filopodial and lamellipodial activity in α3 integrin$^{-/-}$ cells. This deficit is rescued by ectopic expression of α3 integrin in α3 mutant cells.

Furthermore, intercross between α3 integrin mutant mice and a Thy1-GFP transgenic mouse line expressing GFP specifically in layer 6 (Feng et al., 2000) indicates misplacement of neurons normally destined for layer 6 in α3 integrin mutant cortices. Similar displacement is also evident in calbindin or calretinin positive upper layer neurons. The inability to migrate normally and deficits in the ability to engage cues such as fibronectin or reelin, which are present along the migratory route, may underlie the misplacement of neurons in the cerebral cortex of α3 integrin mutant mice.

Materials and methods

Mutant mouse strains

Generation and characterization of targeted mutation in mouse α3 integrins was described by Kreidberg et al. (Kreidberg et al., 1996). Genotypes of the embryos used were determined by PCR as described earlier (DiPersio et al., 1997). Thy1-GFP transgenic lines expressing GFP in layer 6 (line I; a gift from Drs G. Feng and J. R. Sanes) were phenotyped as described by Feng et al. (Feng et al., 2000).

BrdU birthdating studies

Pregnant mice were injected intraperitoneally with BrdU (7.5 mg/kg body weight, dissolved in saline; Boehringer-Mannheim) on embryonic days 10.5 or 11.5. At E16.5, brains were removed, fixed in 70% ethanol, embedded in paraffin wax, cut into cut into 10 μm thick coronal sections, and processed for BrdU labeling as described earlier (Anton et al., 1996). Comparison between sections from different embryos were obtained from identical cortical regions corresponding approximately to posterior frontal, parietal and anterior occipital areas.

Preparation of embryonic cortical slices for time-lapse imaging

Coronal slices (150 μm) of embryonic day 15 wild-type and littermate α3 integrin mutant cortices were incubated with 12.5 μm Oregon Green 488 BAPTA-1 AM (Molecular Probes #O-6807) diluted in neurobasal medium at 37°C for 1 hour. Slices were then washed three times in DMEM/10% FBS medium and cultured on gel matrix (1 mg/ml)-coated glass bottom microwell petri dishes (MatTek) overnight. Labeled neurons in the intermediate zone of medial cerebral wall from regions approximately corresponding to parietal, occipital cortical areas were then imaged repeatedly every 10-25 minutes for 2-3 hours using a Zeiss Pascal inverted laser-scanning microscope equipped with live tissue incubation chamber (see Fig. S2A in the supplementary material). The rate of migration of the monitored cells was measured using LSM5 Pascal program (Zeiss). To label tangentially migrating neurons from the ganglionic eminence, 0.5 μl of Oregon Green 488 BAPTA-1 AM (250 μM) was applied to ganglionic eminence of cortical slices with a pulled glass micropipette (see Fig. S2B in the supplementary material). Slices were then processed and imaged as described earlier. In some experiments, slices were infected with adenoviral vectors expressing GFP (3.125×10^8 vector genomes/ml media; gift from Dr K. Fisher, Tulane University) for 1 day before imaging of labeled neurons. The number of all protrusions and retractions in the leading and trailing edges of GFP labeled cells were counted. Activity index indicates number of extensions or retractions/hour.

Electroporation of cortical cells

E14 cortices from wild-type and α3 null embryos were briefly dissociated into small aggregates in ice-cold DMEM+10% FBS, electroporated with 3 μg of pEGFP-actin (BD Biosciences), α3 integrin (gift of Dr Kreidberg, Harvard Medical School), PH domain from Akt-EGFP or Rac-EGFP plasmid DNA (gift of Dr Snider, UNC) using the Mouse Neuron Nucleofector kit (Amaxa, Cologne, Germany) as per the manufacturer's instructions. The electroporated tissue aggregates were then dissociated and plated in DMEM+10% FBS on glass bottom microwell dishes coated with poly-D-lysine (0.5 mg/ml) and ECM gel matrix (2 mg/ml; Sigma-Aldrich). After 24-48 hours in vitro, time lapse images of transfected cells were recorded for 15 minutes at 30 second intervals using a Zeiss Pascal inverted laser-scanning microscope equipped with a live tissue incubation chamber. α3 integrin expression in the rescue experiments was confirmed by immunolabeling of rescued cells (GFP positive) with anti-α3 integrin antibodies (Becton-Dickinson).

Analysis of actin microspikes and PH-Akt EGFP labeled leading edge protrusions

The leading edges of EGFP-actin expressing cells were overlaid with a 1000 μm^2 box. Individual microspikes inside this area were identified and the changes in their net length were measured. The percentage actin microspikes that underwent dynamic changes were also measured. In PH-Akt EGFP transfected cells, leading edges were overlaid with a 1000 μm^2 box and the number of active protrusions in this area was counted.

Antibodies

Cortical interneurons were immunostained with polyclonal anti-Calretinin (Chemicon Ab5054) or Calbindin (Chemicon Ab1778) antibodies. α3 integrin antibodies were obtained from BD Transduction Labs (#611045), Chemicon (AB1920), or generously provided by Dr DiPersio, Albany Medical College (Ab #8-4).

Embryonic cortical neuron adhesion assay

To measure changes in the response of wild type and α3 integrin mutant cortical neurons to different, biologically relevant ECM substrates in vitro, we modified an assay described by Hordivala-Dilke et al. (Hordivala-Dilke et al., 1998). Briefly, 24-well plates were coated first with poly-lysine overnight, followed by fibronectin or laminin (10 μg/ml) for 1 hour. Plates were then blocked with bovine serum albumin (10 mg/ml) for 1 hour. E16 cortical cells were suspended in serum-free DMEM and plated out at 50,000 cells per well. After incubation for 1 hour at 37°C, non-adherent cells were washed off with HBBS and adherent cells fixed with 4% paraformaldehyde. Adhered neurons were visualized with Tuj1 antibodies. Number of neurons in 10 sample, 0.2 mm^2 fields were counted in each well. Data shown were based on four independent experiments.

Results

The preplate splits normally in α3 integrin mutant cortex

To investigate the pattern of earliest neuronal migration that results in the invasion of the preplate and its splitting into marginal zone and subplate, we labeled newly generated neurons with BrdU at E10.5 and 11.5, and analyzed the extent of their migration in the cerebral cortex at E16.5. In wild-type mice, E10.5 labeled BrdU cells were found primarily in the marginal zone and some in the subplate, whereas E11.5 labeled BrdU cells were found mainly in the subplate and some in the marginal zone (Fig. 1A,C). Similar patterns of distribution were also seen in littermate mutant cortices (Fig. 1B,D). Together, these results suggest that in α3 integrin mutant mice, the preplate splits normally into the marginal zone and subplate.

Neuronal misplacement in α3 integrin mutant cortex

BrdU birthdating studies at later embryonic stages (E14 or E16) indicate significant deficits in the final placement of neurons in distinct layers in α3 integrin mutant cortex (Anton

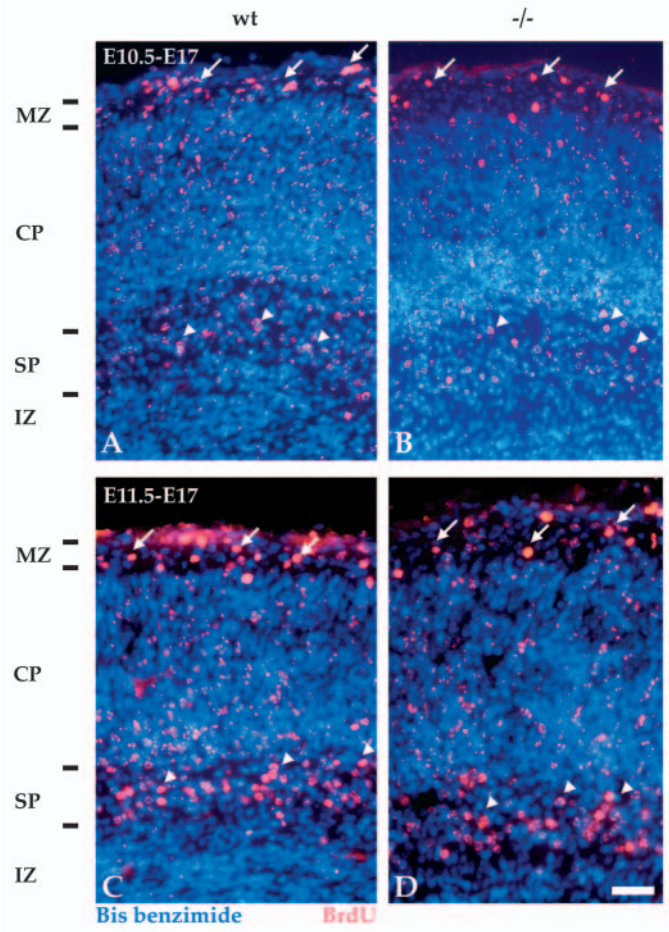

Fig. 1. Development of the preplate in α3 integrin-deficient mice. Neurons that are destined to the preplate of cerebral cortex were labeled at birth (E10.5 or E11.5) with BrdU, and their location was analyzed at E17, following the splitting of the preplate into marginal zone and subplate by invading migratory neurons. BrdU immunoreactivity was visualized with Cy3 and nuclei were counterstained with bis benzimide. When neurons were birthdated at E10.5, in wild-type mice, BrdU-labeled neurons were found in the marginal zone (arrows) and subplate layers (arrowheads) of cerebral cortex, as expected (A). Similar distribution was also seen in α3 integrin-deficient cortex (B). When neurons were birthdated at E11.5, most of the labeled neurons in wild-type cortex were found in subplate and few in the marginal zone (C). Identical pattern of labeling is also evident in α3 integrin-deficient cortex (D). Together, these results indicate that the preplate develops and splits normally in the absence of α_3 integrin. MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone. Scale bar: 60 μm.

et al., 1999). To further explore this deficit, we crossed α3 integrin$^{+/-}$ mice with the Thy1-GFP transgenic lines expressing GFP in layer 6 (line I; Fig. 2) (Feng et al., 2000). At postnatal day 0 (P0), GFP expression is limited to deep layer neurons in distinct medial and lateral domains of the occipital region of the cortex (Fig. 2B). Analysis of GFP-positive neuronal distribution in Thy1-GFP-positive, α3 integrin$^{-/-}$ mice indicated that layer 6 neurons are malpositioned, for the most part below their target destination, at postnatal day 0 (Fig. 2C,D, see Fig. S1 in the supplementary material). The apical dendrites of these neurons also appear to be misoriented when compared with those of the wild-type neurons (Fig. 2C′,D′). Radial orientation of α3 integrin mutant apical dendrites towards pial surface deviated by an average of 32±6.6°. By contrast, mean deviation of wild-type dendrites is 7±3.1° [significant at $P<0.05$ when compared with mutants (Student's t-test); $n=75$ for wild type and for mutant]. To assess possible malpositioning of upper layer interneurons, we immunolabeled wild-type and α3 integrin deficient cortices with calretinin and calbindin antibodies. Anti-calretinin and calbindin antibodies primarily label distinct groups of non-pyramidal, interneuronal cell populations in layers I-III and layers III/IV, respectively (Hof et al., 1999). Calbindin is also diffusely expressed in some neurons in the deeper layers. In wild-type sections, as expected, anti- calretinin and calbindin antibodies labeled distinct groups of neurons in layers I-III and layers III/IV, respectively (Fig. 2E,G). This distinct, well-organized laminar distribution of calbindin- and calretinin-positive neurons is not clearly evident in α3 integrin mutant cortex (Fig. 2F,H; see Fig. S1 in the supplementary material for quantification of neuronal distribution of calbindin- and calretinin-positive neurons), although many calbindin or calretinin positive neurons make it to the cortical plate in α3 integrin mutants. Analysis beyond P0 is not possible in these mice because of the perinatal lethality of the α3 integrin mutation. Nevertheless, the neuronal positional defect of upper and deeper layer neurons in α3 integrin mutants suggests deficits in normal neuronal migration. We therefore investigated the patterns of migration in the developing cerebral wall of α3 integrin deficient mice in real time.

In vivo assay for neuronal migration in α3 integrin mutant cortex

Migrating neurons in the intermediate zone of wild-type and α3 integrin$^{-/-}$ embryonic cerebral wall were labeled with Oregon Green BAPTA-1 AM. Tangentially migrating interneurons from the ganglionic eminence were also labeled at their origin. Radial, tangential, and ventricular zone directed neuronal migration in these embryonic cortical slice preparations were repeatedly monitored for 2-3 hours (Fig. 3; see Figs S2, S3 in the supplementary material). In wild-type slices, radial and tangential neuronal movement occurred at an average rate of 27±3.2 μm/hour and 43±5.4 μm/hour, respectively (Fig. 3A,E,G). By contrast, the rates of radial and tangential neuronal migration were reduced by 40% and 33%, respectively, in α3 integrin mutants (Fig. 3B,F,G). Migration of neurons towards the direction of ventricular zone however, appears not to be affected in α3 integrin$^{-/-}$ cortex (Fig. 3C,D,G). Analysis of isolated neurons at higher magnification indicates that leading and trailing processes develops normally in α3 integrin mutant neurons. Extension and retraction of these processes are essential components of neuronal movement in cerebral cortex. However, compared with wild-type neurons, leading and trailing processes of α3 integrin mutant neurons display reduced (–28%) protrusive and retractive activity (Fig. 4).

Altered actin dynamics in α3 integrin mutant cortical cells

The slower rate of migration and the reduction in extension and retraction of processes in α3 mutant cortical cells suggested possible deficits in integrin-linked dynamic regulation of actin microfilaments at the growth edges. We investigated this by

transfecting E14 wild-type and α3 integrin mutant embryonic cortical cells with pEGPF-actin and evaluating actin dynamics at the growth edges of the transfected cells. The fluorophore-labeled actin is readily incorporated into the actin cytoskeleton, thus allowing in vivo time lapse recordings. Images were collected every 30 seconds for 10 minutes from the leading edges of transfected cells. The actin dynamics was significantly impaired in α3 integrin null cells (Fig. 5). The rate of polymerization or depolymerization of actin microspikes within a 1000 μm^2 area at the leading edge was measured over time. In wild-type cells, actin microspike elongation or retraction occurred at a rate of 1.27±0.06 μm/minute (n=51), whereas in α3 integrin null cells it occurred at a rate of 0.28±0.02 μm/minute (n=53, significant at P<0.01, Student's t-test). The percentage of actin microspikes that showed any changes in length also decreased significantly in α3 integrin null cells (wild type, 73±3%; mutant 30±2%). When α3 integrin was re-expressed in mutant cells, the rate of actin microspike elongation or retraction was restored to 1.06±0.06 μm/minute (n=50). Furthermore, the percentage of microspikes that underwent changes in length was also increased to levels comparable with wild-type values, following re-expression of α3 integrin (wild type, 73±3%; mutant + α3 integrin, 65±4%; difference between wild type and mutant + α3 integrin is not significant at P<0.01, Student's t-test). The altered actin cytoskeleton dynamics at the leading edges of α3 integrin mutant cells may underlie the decreased cell motility and contribute to the impaired activity at the leading edges, normally needed for proper migration (Edmondson and Hatten, 1987; Rivas and Hatten, 1995). To further investigate the dynamics of leading edges, we electroporated embryonic cortical cells with EGFP fused to the PH domain of Akt (PH-Akt-EGFP). This fusion protein is directed to the growth cones (Bondeva et al., 2002; Markus et al., 2002), which are analogous to the leading processes of the migrating neurons (Song and Poo, 2001). Visualization of activity at the growth tips with this probe indicates that wild-type neuronal cells showed rapid movement of growth cones and filopodial protrusions (Fig. 6A). The mean number of protrusions in a 1000 μm^2 area of the leading edge during 10 minute interval is 20.2±0.95 (n=50). The extension and retraction rate of these protrusions is 3.2±0.11 μm/min (n=50).

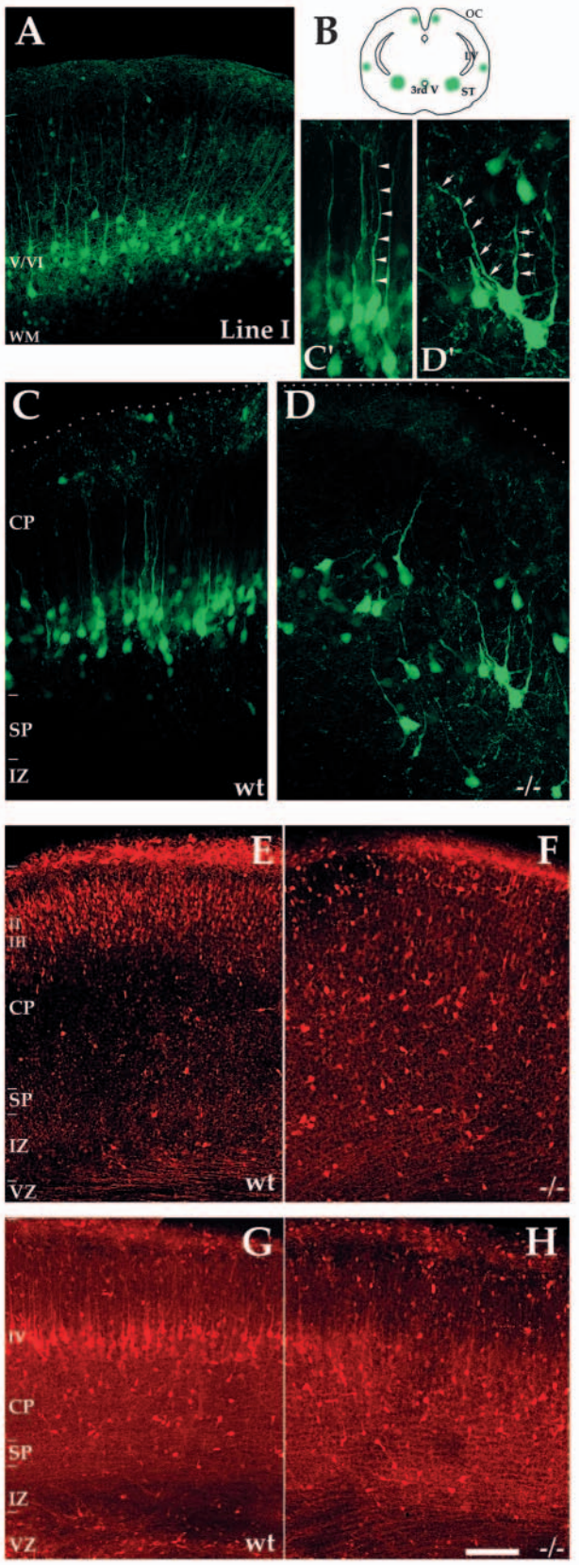

Fig. 2. Disrupted cortical neuronal placement in α_3 integrin-deficient mice. α3 integrin-deficient mice were crossed with Thy1-GFP line-I (Feng et al., 2000), known to express GFP in layer VI neurons (A). At P0 (B), GFP expression is limited to distinct medial and lateral domains of the occipital region of the cortex. Expression of GFP was also noticed in the striatal area and in an area surrounding third ventricle (B). In wild-type lateral occipital cortex at P0 (C), layer VI neurons express GFP, whereas in α3 integrin-deficient cortex (D), GFP-positive neurons were ectopically placed, away from their destination in layer VI. Higher magnification images of GFP-labeled neurons in wild-type (C′, arrowheads) and α3 integrin mutant (D′, arrows) cortex show disrupted apical dendrite orientation in α3 integrin neurons. Immunolabeling with anti- calretinin or calbindin antibodies indicates malpositioning of cortical interneurons in α3 integrin mutants. In P0 wild-type cortex, anti-calretinin (E) or calbindin (G) antibodies primarily label bands of neurons in newly formed layers I-III and III/IV, respectively. By contrast, calretinin- (F) or calbindin (H)-expressing neurons are diffusely distributed in α3 integrin mutant cortex. OC, occipital cortex; ST, striatum; 3rd V, third ventricle; LV, lateral ventricle; CP, cortical plate; SP, subplate; IZ, intermediate zone; VZ, ventricular zone; WM, white matter. Scale bar: 100 μm in A; 75 μm in C,D; 60 μm in C′,D′; 180 μm in E-H.

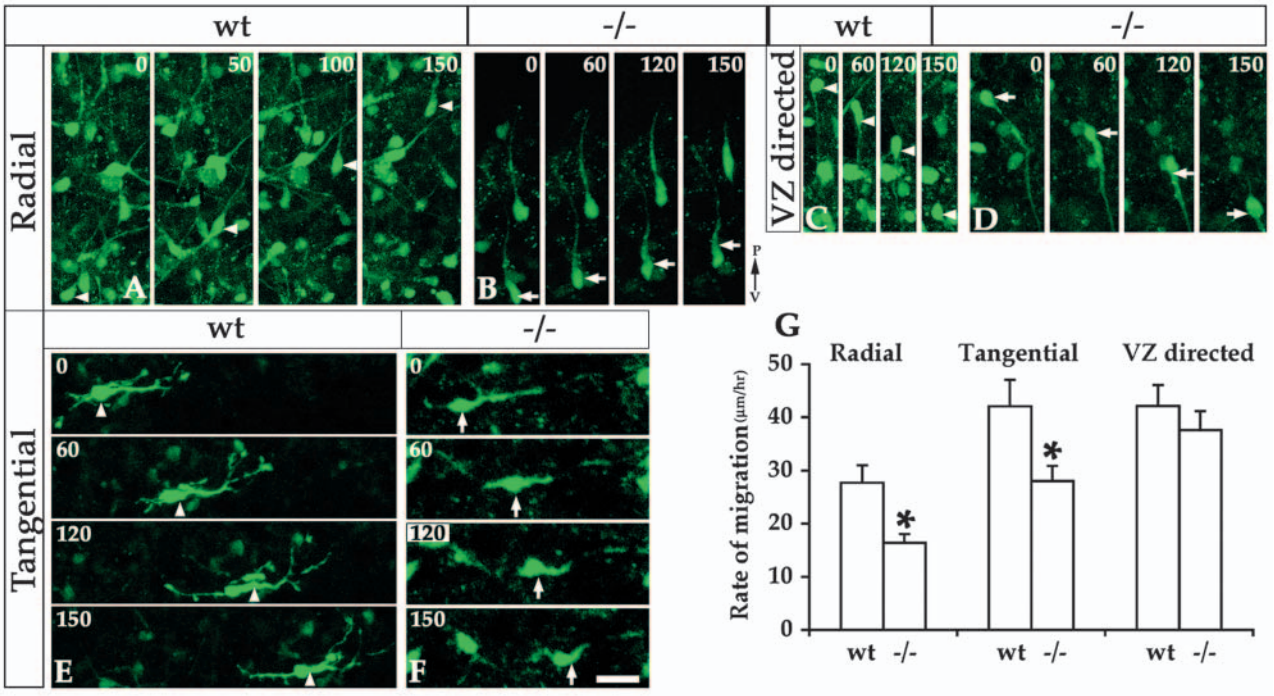

Fig. 3. Disrupted neuronal migration in α3 integrin deficient cortex. Neurons in wild-type and mutant E15-E16 cortices were labeled with BAPTA green. Labeled cells in the intermediate zone of the slices, migrating in radial direction (A,B), towards the ventricular zone (C,D) or in tangential orientation (E,F) were repeatedly monitored. (A,C,E,G) In wild-type cortex, neurons migrated radially, tangentially and towards the ventricular zone at an average rate of 27±3.2 μm/hour, 43.5±5.4 μm/hour and 43±3.9 μm/hour, respectively. Arrowheads in A,C,E indicate sample migrating wild-type cells. (B,F,G) In α3β1 integrin-deficient cortex, the rates of radial and tangential migration of neurons were significantly reduced to 16±1.6 μm/hour, 29±3.1 μm/hour, respectively. By contrast, no significant differences were noticed in the rate of ventricular zone directed neuronal migration (C,D; wild type, 43±3.9 μm/hour; mutant, 37±4.1 μm/hour). Arrows in B,D,F indicate sample migrating α3 integrin mutant cells. n=80 for radial wild type and mutant; n=80 for tangential wild type, n=75 for mutant; n=50 for wild type, ventricular zone directed, n=20 for mutant. Data shown are mean±s.e.m.; asterisk (G), significant when compared with controls at P<0.01 (Student's t-test). Time elapsed since the beginning of observations are indicated in minutes. P and V, direction of the pial and ventricular surfaces, respectively. Scale bar: 50 μm in A-D; 40 μm in E,F. (Also see the Figs S2, S3 and Movies 1, 2 in the supplementary material.)

By contrast, mutant growth cones displayed significantly less activity at the leading edge (mean number of protrusions is 10.2±0.68, mean extension and retraction rate is 1.3±0.05 μm/minute, n=50; Fig. 6B). Re-expression of α3 integrin in mutant cells restored growth cone activity (mean number of protrusions 17.2±1.02, mean extension and retraction rate is 2.9±0.19 μm/minute, n=50; Fig. 6C; not significant at P<0.01 when compared with wild type, Student's t-test). Similar differences in leading edge activity were also noticed when wild-type and mutant cortical cells were electroporated with another growth cone-directed fusion protein, the small GTPase Rac1 (Rac1-EGFP; data not shown).

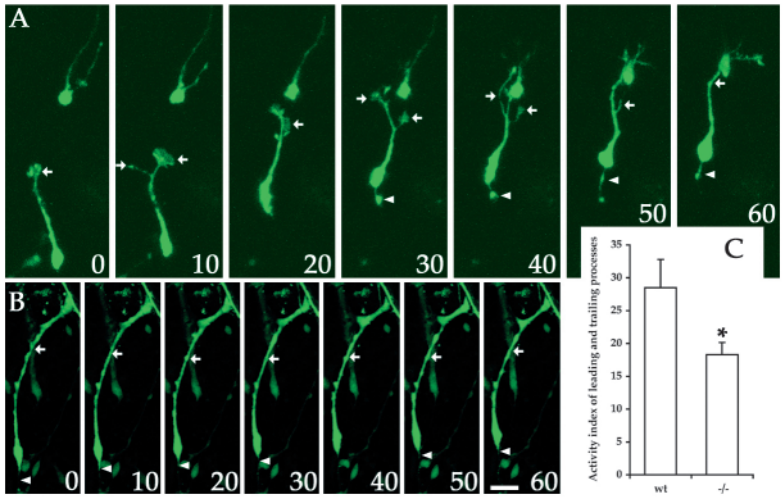

Fig. 4. Altered dynamics of leading and trailing edges of migrating neurons in α3 integrin-deficient cortex. Time-lapse imaging of GFP-labeled neurons in the wild-type cortex indicates active extensions and retractions of the leading (arrow) and trailing processes (arrowhead) of migrating neurons (A). By contrast, α3 integrin mutant neurons displayed reduced protrusive activity in their leading and trailing processes (B). Quantification of extensions and retractions (activity index) indicates a 28% reduction in α3 integrin mutant cells. Cells shown are from the intermediate zone of E16 cortex. Time elapsed since the beginning of observations are indicated in minutes. Number of cells analyzed: n=24, wild type; n=28, mutant. Data shown are mean±s.e.m.; asterisk (C), significant when compared with controls at P<0.01 (Student's t-test). Scale bar: 25 μm. (Also see Movies 3, 4 in the supplementary material.)

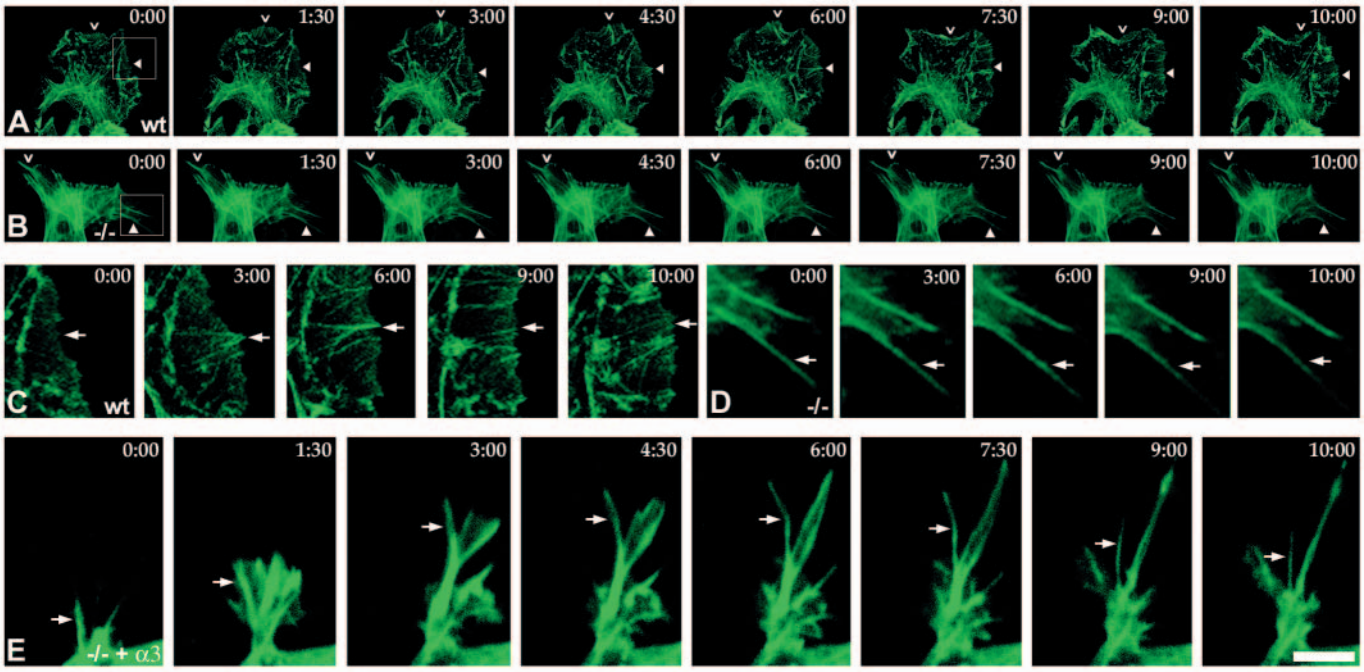

Fig. 5. Deficient actin dynamics in α3 integrin mutant cortical cells. E14 cortical cells were electroporated with EGFP-actin and time-lapse images of the actin cytoskeleton at the leading edges was recorded after 48 hours. The actin cytoskeleton of the tips of the wild-type cells shows very active remodeling (A; see actin microspikes in regions marked with arrowheads, ˅ indicates dynamic changes in their assembly and disassembly). (C) Higher magnification view of the outlined area in A. Arrows indicate actin microspikes undergoing remodeling. By contrast, actin cytoskeleton of leading edges of the α3-deficient cells display significantly reduced dynamic activity (B; compare actin microspikes in regions marked with arrowheads, compare ˅ with similarly marked actin microspikes in A). (D) Higher magnification view of the outlined area in B. Arrows indicate actin microspikes that are considerably less dynamic than those from wild-type cells. (E) Re-expression of α3 integrin rescued actin dynamics deficits in α3 integrin mutant cells. Arrow indicates an actin microspike undergoing dynamic remodeling at the leading edge of an α3 mutant cell transfected with α3 integrin DNA. Time elapsed since the beginning of observations is indicated in minutes. Scale bar: 10 μm in A,B; 3 μm in C-E. (Also see Movies 5-7 in the supplementary material.)

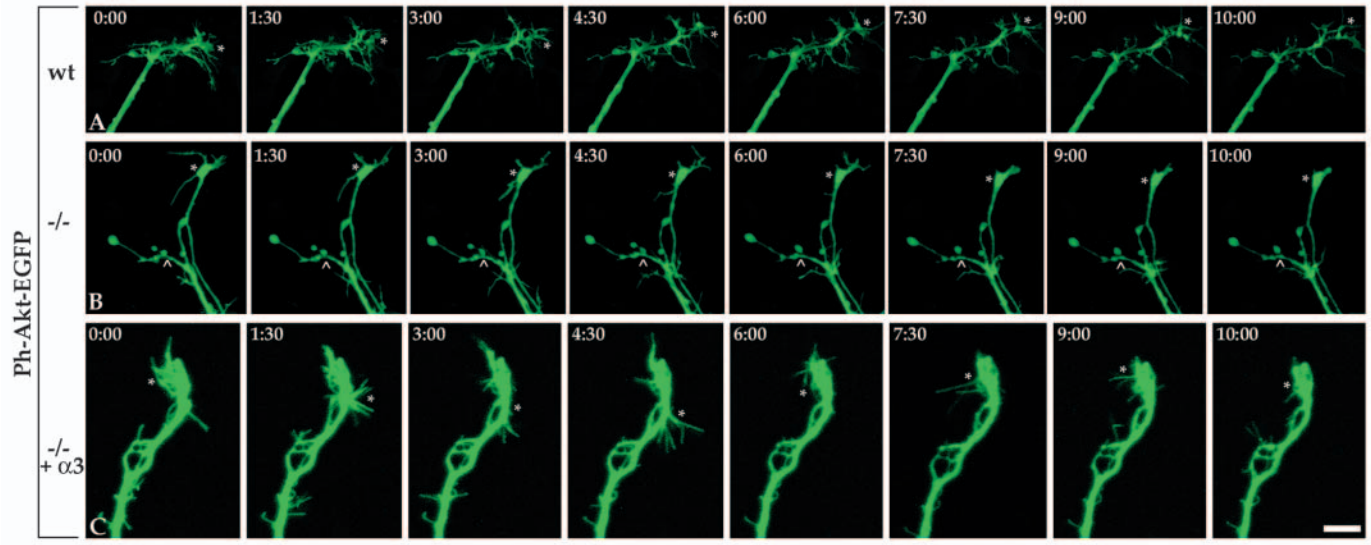

Fig. 6. Altered filopodial activity in the leading edges of α3 mutant cortical cells. E14 neuronal cells from wild-type or mutant cortices were electroporated with PH-Akt-EGFP (A,B) and time-lapse images of the growth cone ends of neuronal processes were recorded after 48 hours. PH-Akt-EGFP is targeted to active growth cones, thus enabling the evaluation of leading edge activity. In PH-Akt-EGFP transfected wild-type cells (A), filopodia emerge in large numbers from many spots along the leading edge (A; see activity in regions marked with asterisks) and appear to intensely sample the environment of the cell. By contrast, fewer filopodia emerge from the two adjacent leading edges (^, *) of PH-Akt-EGFP transfected α3 integrin mutant cortical cells (B), and their ability to probe the cellular environment appear to have been retarded (compare activity in regions marked with asterisks in A and B). Re-expression of α3 integrin in α3 integrin-deficient cells rescued the deficits in filopodial activity (C; see active region marked with an asterisk). *n*=67 (wild type), *n*=70 (mutant and mutant + α3 integrin). Time elapsed since the beginning of observations is indicated in minutes. Scale bar: 20 μm. (Also see Movies 8-10 in the supplementary material.)

Discussion

Targeted mutation of the α3 integrin gene results in defective cortical laminar organization. The preplate develops normally in α3 integrin mutants, but subsequent migration to the cortical plate is disrupted. α3β1-deficient neurons display a reduced rate of migration, altered actin dynamics and a general deficit in their ability to probe their cellular milieu with filopodial and lamellipodial activity. The inability of α3 integrin mutant neurons to engage in cell-cell recognition and adhesion interactions needed for normal migration and the lack of ability to respond appropriately to crucial positional cues such as fibronectin or reelin may contribute to the defective neuronal placement in the α3 integrin-deficient cortex.

α3β1 integrin function during neuronal motility

Earlier studies indicated that α3β1 integrins could modulate neuron-glial recognition cues during neuronal migration and placement in cortex (Anton et al., 1999; Dulabon et al., 2000; Sanada et al., 2004). Real-time monitoring of migrating neurons in the embryonic cerebral cortex provides, at a mechanistic level, information about how signals that are transduced by α3β1 integrin affect neuronal migration in the developing cerebral cortex. Radially and tangentially directed neuronal migration is affected, with accompanying deficits in actin dynamics and protrusive edge activity of motile neurons. α3 integrin can influence oriented neuronal movement in multiple ways. For example, α3 integrins can influence neuronal response to cues such as fibronectin (Hodivala-Dilke et al., 1998), which is present along the radial glial migratory routes in cerebral cortex (Pearlman and Sheppard, 1996; Sheppard et al., 1991; Sheppard et al., 1995; Stettler and Galileo, 2004). Consistent with this possibility is the observation that α3 integrin mutant embryonic cortical neurons display enhanced (+62±0.06%) adhesion to fibronectin substrate in vitro (see Fig. S4 in the supplementary material). Increased adhesion to ECM cues present along the radial glial migratory guides may thus play a role in reducing the rate of migration of α3 integrin mutant neurons. Whether deficits in radial neuronal migration can indirectly affect the tangential migration of neurons in α3 integrin mutant cortex remains unclear. However, recent studies indicate that netrin 1, a diffusible guidance protein, can bind to α3β1 integrin and regulate hepatocyte growth factor (HGF) induced haptotaxis of epithelial cells on netrin 1 (Yebra et al., 2003). HGF is a motogen for tangentially migrating neurons (Powell et al., 2001) and thus it is conceivable that netrin-α3β1 interactions may similarly regulate HGF activity during tangential neuronal migration in embryonic cerebral cortex.

In keratinocytes, α3β1 integrin has been shown to promote cell spreading on laminin 5 and actin fiber assembly and organization (DeMali et al., 2003; Hodivala-Dilke, 1998). Integrin α3β1-deficient keratinocytes also fail to polarize and engage in oriented migration (Choma et al., 2004). Furthermore, cell polarity during oriented cell migration involves the accumulation of PIP3 in the leading edges of the cells (Funamato et al., 2002; Wang et al., 2002; Weiner et al., 2002). Lack of PIP3 dynamics at the leading edge as indicated by the PH domain of Akt-GFP fusion probe in α3 integrin mutant cells also suggests a role for α3β1 integrin in maintaining polarity during oriented neuronal migration.

Disruption in pial or vascular ECM assembly (Blackshear et al., 1997; Graus-Porta et al., 2001; McCarty et al., 2002; Moore et al., 2002) or deficits in ECM components perlecan (Costell et al., 1999), laminin α_5 chain (Miner et al., 1998) or laminin γ_1 nidogen binding site (Halfter et al., 2002) has been shown to disrupt corticogenesis. α3β1 is required for MMP9-mediated ECM remodeling and assembly during keratinocyte cell motility. α3β1 integrin may similarly influence ECM remodeling in the developing cerebral cortex. It is thus possible that in α3β1 integrin-deficient cortex, deficits in the ability to bind and respond to ligands in the environment, to remodel ECM or maintain cell polarity may contribute to retarded neuronal migration.

Intriguingly, migration of neuroblasts towards the ventricular zone occurs at a normal rate in α3 integrin mutants. These neurons are thought to originate in the ganglionic eminence and migrate towards ventricular zone to obtain positional information, prior to radial migration towards the cortical plate (Nadarajah et al., 2002). Lack of significant α3β1 integrin effect in this mode of migration is indicative of the specific role integrin mediated cell-cell adhesion can play in regulating distinct patterns of migration in the developing nervous system. The behavioral response of a cell to the ECM is an integrated response determined by the specific components of the ECM present, and the subset of integrins expressed by that cell. Thus it will be instructive to determine the repertoire of integrins expressed in neurons migrating in distinctly different orientations during the development of cortex.

α3 integrin-actin interactions

An important outcome of integrin- ECM engagement induced cytoplasmic signaling is the promotion of actin assembly (DeMali and Burridge, 2003; DeMali et al., 2003). In regions of cells where integrins first engage their ligands, such as the leading edges, a high degree of integrin dependent actin polymerization activity is evident. Here, integrins are linked to actin filaments by actin-binding proteins, such as talin, filamin and α-actinin. Members of the Mena/VASP family are also important regulators of actin filament assembly at the leading edges, where they are thought to indirectly interact with integrins to target actin polymerization to new integrin-ECM contact sites (Calderwood et al., 2000). Thus, deficiency of α3 integrin at the leading edge of migrating neurons may drastically affect the ability of the cell to polymerize actin and, consequently, impair local reorganization of the actin network needed for dynamic protrusions at the leading edges. Recent studies also suggest that changes in the activation of integrin-actin linking protein, such as talin or actin dynamics itself, could influence integrin function in an inside-out manner (Bennet et al., 1999; Calderwood et al., 2000; Cram and Schwarzbauer, 2004; Hynes, 2002; Kim et al., 2003). How such mechanisms are affected in the α3 integrin-deficient cortical neurons remains to be elucidated.

α3β1 integrin function during neuronal placement and cortical layer formation

β1 integrin in the cerebral cortex can dimerize with at least 10 different α subunits, including α3. To date, no other β integrin subunit has been shown to associate with α3 integrin subunits. α3β1 integrin also can associate with itself and with members of the tetraspanin family of transmembrane proteins, and may transdominantly inhibit other integrins (Sriramarao et al., 1993;

Symington et al., 1993; Hynes, 2002; Hodivala-Dilke et al., 1998). Cortical layer formation is disrupted following cre-lox-mediated inactivation of β1 integrins in cortical neurons and glia from around embryonic day 10.5 (Graus-Porta et al., 2001; Forster et al., 2002). Defective meningeal basement membrane assembly, marginal-zone formation and glial end feet anchoring at the top of the cortex are thought to lead to this phenotype. Whether the lack of pial anchoring of radial glial cells in β1-deficient cortex affect their ability to function as neuronal stem cells or as neuronal migratory guides, and thus contribute to the defective placement of neurons in the cortex is unclear. The varied, non-overlapping cortical phenotypes of β1, α1, α3, α6 and αv-null mice may reflect the transdominant, transnegative or compensatory influences distinct integrin receptor dimers may exert over each other and the ECM ligands in the developing cerebral cortex (Bader et al., 1998; Fassler and Meyer, 1995; Gardner et al., 1999; Georges-Labouesse et al., 1998). For example, in vitro binding of a ligand to a signal transducing integrin or inactivation of specific integrin subunits can initiate a unidirectional signaling cascade affecting the function of the target integrin in the same cell (Blystone et al., 1999; Hodivala-Dilke et al., 1998; Simon et al., 1997). Elucidating how such integrin crosstalk regulates patterns of neuronal migration in the developing cortex will be crucial to fully understand the specific role of distinct integrins in corticogenesis.

Pathways of migration and cell-cell interactions during migration crucially influence layer formation and phenotypic specification of different classes of cerebral cortical neurons (Anderson et al., 1997; Parnavelas, 2000; Sanada et al., 2004). The changing patterns of adhesive interactions mediated by integrins during neuronal translocation across the cerebral wall may not only control the trajectory of neurons, but may also trigger the developmental programs needed for progressive acquisition of distinct cortical neuronal phenotypes. Evaluation of whether the neurons that have undergone altered patterns of migration and placement in the absence of α3 integrin subunit develop the full complement of layer-specific characteristics of distinct cortical neurons awaits the generation of cell-type specific or inducible α3 integrin mutant mouse models. However, the results shown here demonstrate the significance of α3 integrin-mediated signaling in distinct patterns of neuronal migration and the eventual positioning of neurons in specific layers of the developing cortex.

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Supplementary material

Supplementary material for this article is available at
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