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A novel mode of tangential migration of cortical projection neurons

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Abstract

Projection neurons of the developing cerebral cortex are generated in the cerebral ventricular zone and subsequently move to the developing cortical plate via radial migration. Conversely, most inhibitory interneurons originate in the ganglionic eminences and enter the developing cortical plate by tangential migration. Using immunohistochemical analysis together with tracer labeling experiments in organotypic brain slices, we show that a portion of cortical projection neurons migrates tangentially over long distances. Lineage analysis revealed that these neurons are derived from *Emx1+* cortical progenitors and express the transcription factor Satb2 but do not express GABA or Olig1. In vitro and in vivo analysis of *reeler* mutant brains demonstrated that although *reeler* mutation does not influence tangential migration of interneurons, it affects the tangential migration of cortical projection neurons.
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Keywords: Cerebral cortex; Reelin; Transcription factor; Migration; Cre; Transgenic; Cell fate; Subiculum

Introduction

The cerebral cortex is the part of the forebrain responsible for cognitive functions. Early in development, the forming forebrain is subdivided into two separate domains: the ventral telencephalon, with two distinct proliferating cell masses (MGE and LGE), and the dorsal telencephalon from which the cerebral cortex will develop. A unique feature of brain development is that newborn neurons migrate from their site of birth in the proliferative zones to their final position. In recent years, there has been a great interest in mechanisms that control neuronal migration. Defects in the migration of cortical neurons are associated with several neurological disorders in humans (Clark, 2004). Different neuronal populations use different modes of migration (Marin and Rubenstein, 2003; Nadarajah et al., 2003). For example, the excitatory cortical neurons travel from the cortical proliferative zone (called the Ventricular Zone VZ) to their final destination in the cortical plate (CP) primarily by radial migration, whereas the vast majority of inhibitory interneurons are born in the ganglionic eminences and invade the cortex by tangential migration.

In the mature neocortex, neurons are arranged in six layers, numbered I (most superficial) to VI. Neurons within each layer share common properties including time of birth, morphology, connectivity and physiology (McConnell, 1995). The first postmitotic cortical neurons form a transient structure called the preplate (PP). Subsequent generations of neurons split the preplate into two zones, the superficial marginal zone (MZ) and the subplate (SP), and accumulate between these layers to form the cortical plate (CP). With the exception of layer I (MZ), all layers are formed with an inside-out pattern, in which the deep layers are laid down first, followed by the addition of the more superficial layers (Berry and Rogers, 1965). A key molecule in

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this process is Reelin, an extracellular matrix protein secreted by Cajal–Retzius cells in the MZ that when mutated, causes a disruption in neuronal migration (D'Arcangelo, 2001; D'Arcangelo et al., 1995; Drakew et al., 1998; Ogawa et al., 1995). The brains of *reeler* mice have multiple neuroanatomical defects, including an inversion of the cortical layers (Caviness, 1982). Additionally, although Reelin does not affect tangential migration of interneurons, it affects their radial migration at postnatal ages (Hevner et al., 2004).

As cortical development proceeds, an additional proliferative zone appears on top of the VZ which is called the subventricular zone (SVZ). This proliferative zone will initially give rise to neurons that will predominately occupy the upper cortical layers and subsequently to glia (Goldman, 1995; Tarabykin et al., 2001). Several genes are known to be expressed in the SVZ. One of them, *Satb2*, encodes for a protein that belongs to a family of transcription factors that appear to regulate tissue-specific organization of chromatin, by binding to core unwinding elements within the Matrix Attachment DNA Regions (Britanova et al., 2005). Using Satb2 as a marker of cortical SVZ neurons, we present here evidence of two findings in neuronal migration: that a subpopulation of projection neurons born in the cortical VZ migrates tangentially over long distances and also that this process is disrupted in *reeler* mutants. Our data support an update to the current model on the modes of migration of cortical neurons.

Results

The expression of Satb2, but not that of Nurr1 and Sip1, is shifted dorsally in the reeler mutant subiculum

To acquire a better understanding of the role of Reelin in the migration of SVZ neurons, we used Satb2 as a marker to examine the migration of SVZ neurons towards their final position.

The expression of Satb2 is confined to the cerebral cortex with strict lateral and medial boundaries (Britanova et al., 2005). Satb2 is initially detected at E13.5 in a subset of neurons in the neocortical SVZ. Later in development, it is expressed in the upper layers of the CP, as well as in the IZ. By E18.5, the majority of Satb2 expressing cells predominately occupy the upper part of the cortical plate (our unpublished data).

We compared by in situ hybridization the expression pattern of Satb2 in WT and *reeler*−/− brains at P2 to investigate how the radial migration of SVZ neurons expressing Satb2 (Satb2+) is affected in *reeler* mutant brains. Fig. 1c demonstrates that in WT P2, brains, *satb2* was expressed in the cortical plate, with higher expression levels in the upper layers. In addition, *satb2* was also expressed in the superficial part of the subiculum, but not in the CA1 region of the hippocampus marking the boundary between the subiculum and the hippocampus. In the *reeler*−/− neocortex, the typical inversion in the expression pattern was clearly visible (Fig. 1d). Unexpectedly, however, in the *reeler*−/− mutant brains, we noticed that the expression domain of *satb2* was shifted dorsally (Fig. 1d). We next investigated whether the dorsal shift in the boundary of *satb2* expression was due to a misspecification of the subiculum in the *reeler*−/− brains. We analyzed the expression patterns of two other transcription factors, *Sip1* (Smad Interacting Protein) and *Nurr1*, in WT and *reeler*−/− brains.

Sip1 was originally identified in a yeast two-hybrid screen through its binding to R-Smad (Verschueren et al., 1999). In situ hybridization analysis in WT brains showed that *Sip1* is expressed in the neocortex and in the CA1–CA3 fields of the hippocampus but not in the subiculum. Therefore, it can be used to mark the boundary between the neocortex and the subiculum as well as the boundary between the subiculum and the hippocampus (Fig. 1e). In situ hybridization analysis of *Sip1* in *reeler*−/− brains revealed that both the boundaries between the subiculum and the neocortex dorsally and the subiculum and the hippocampus ventrally, are maintained. Our results show that the dorsal shift in the expression pattern of *satb2* in the *reeler*−/− mutant brains is not due to an expansion of the hippocampus. Furthermore, the gap in the expression pattern of *Sip1* that marks the subiculum is maintained in the *reeler*−/− animals suggesting that the subiculum is properly specified (Fig. 1f).

Nurr1 is specifically expressed in the subiculum in WT P2 brains (Fig. 1g; Gray et al., 2004). *In situ* hybridization analysis in *reeler*−/− brains showed that Nurr1 is still expressed suggesting that Reelin does not have an instructive role in the subiculum (Fig. 1h). However, despite the fact that the subiculum is properly specified in *reeler*−/− brains, it is reduced in size suggesting that the reduction in the expression of *satb2* we observed is due to a reduction in the number of neurons in the subiculum.

Birthdating of satb2 cells in subiculum

To investigate the origin of *satb2* neurons in the subiculum, we performed birth dating analysis for these neurons by injecting BrdU in pregnant animals at E13.5, E14.5, E15.5 and E16.5 followed by double immunohistochemistry for BrdU and Satb2 in P2 brains (Fig. 2a). Cell counts of Satb2+ neurons, double labeled with BrdU showed that the majority of Satb2+ neurons in the subiculum were born at E14.5 (45%) and only a few neurons were generated at E13.5 (26%) or E15.5 (29%) (Fig. 2c, red line). In the lateral cortex, however, Satb2+ neurons were generated gradually after E13.5 (23%) peaking at E15.5 (35%) (Fig. 2c, green line). Our birth dating analysis suggests that in the subiculum Satb2+ neurons are generated in a narrow window of time.

Taking into account the neurogenic gradient of cortical development (lateral regions are developmentally more advanced than more medial ones) as well as the fact that at E14.5 *satb2* is not highly expressed in the developing subiculum, we expected to see a shift in the peak of BrdU+/Satb2+ neurons in the subiculum to later ages. This discrepancy in our birthdating analysis prompted us to investigate whether the Satb2+ neurons in the subiculum were born locally, or if they were born at a different site and migrated tangentially to the subiculum.

Migration analysis of Satb2+ neurons in WT brain slices

To show if Satb2+ neurons were migrating tangentially to the subiculum, we performed migration assays in brain slices

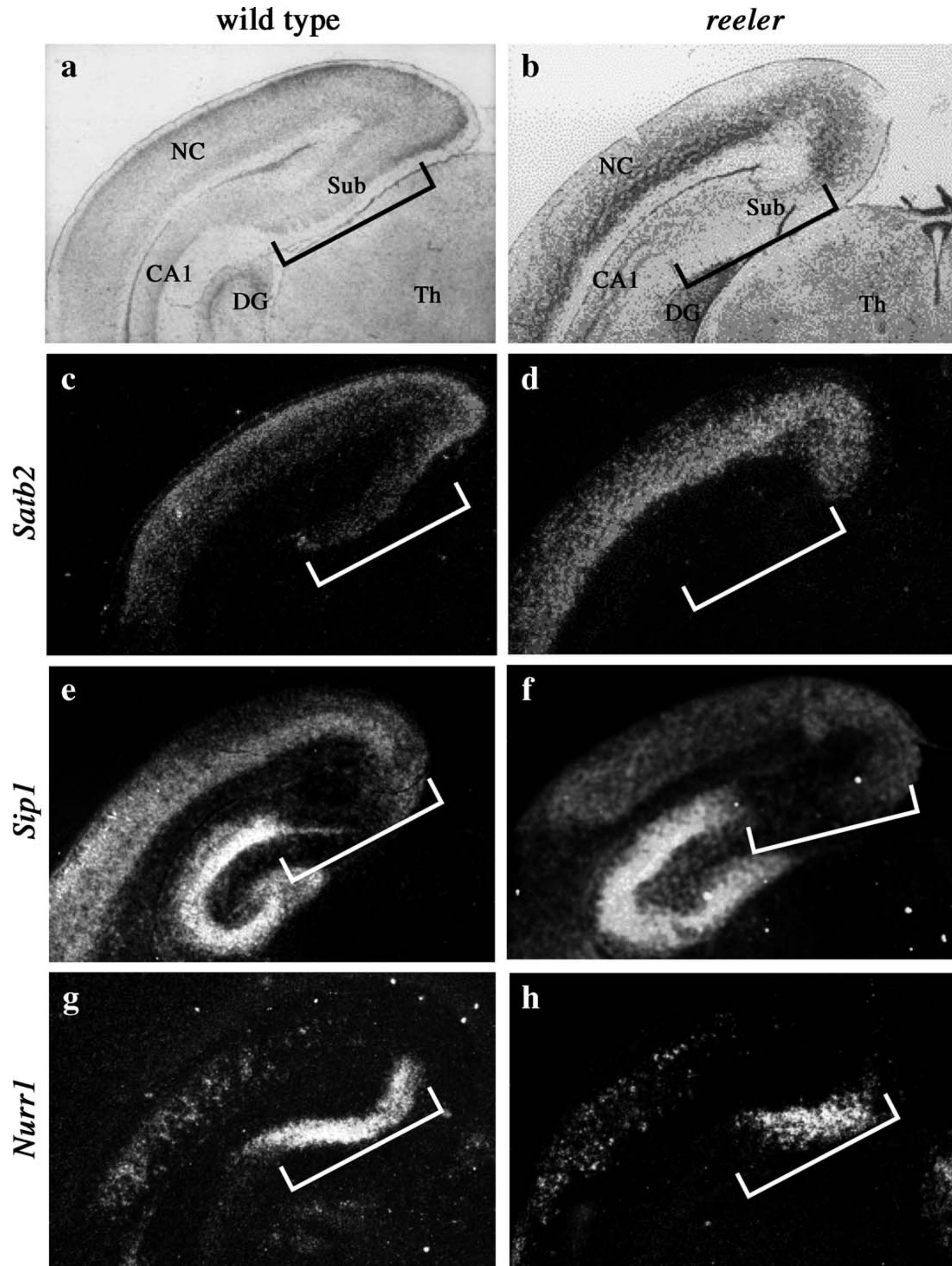

Fig. 1. Marker analysis in P2 WT and *reeler*−/− brains. Marker analysis by in situ hybridization in P2 WT (a, c, e) and "reeler" (b, d, f) brain sections. (a, b) Bright-field views. (c) In WT brains *Satb2* is predominately expressed in upper layer neurons, as well as in the superficial part of the subiculum. (d) In the "reeler" brains, the expression pattern of *Satb2* is inverted in the neocortex and severely reduced in the subiculum (bar). (e) In WT brains, *Sip1* marks the boundaries between the subiculum with the cortex and with the hippocampus. (f) The boundaries are maintained in the "reeler" brains. (g) In WT brains, *Nurr1* is specifically expressed in the subiculum. (h) In the *reeler* brains, *Nurr1* is still expressed marking the subiculum which is reduced in size. CA1: CA1 region of hippocampus, DG: dentate gyrus, NC: neocortex, Sub: subiculum, Th: thalamus.

prepared from E14.5 WT brains (the time in which the majority of the subicular Satb2+ neurons are born). CMFDA (Alifragis et al., 2002) was focally injected into the SVZ of the lateral cortex. The slices were then incubated at 37° to allow cells that picked up the dye to migrate away from the injection site. Forty-eight hours later, the slices were fixed, resectioned and immunohistochemistry was performed to see whether any of the labeled migrating neurons were positive for Satb2 (a schematic representation can be seen in Figs. 3a and 4a). As shown in Fig. 3a, confocal images were acquired adjacent to the injection site as an internal control to our experiment (box 1 in Fig. 3a). Furthermore, confocal images were also acquired from the

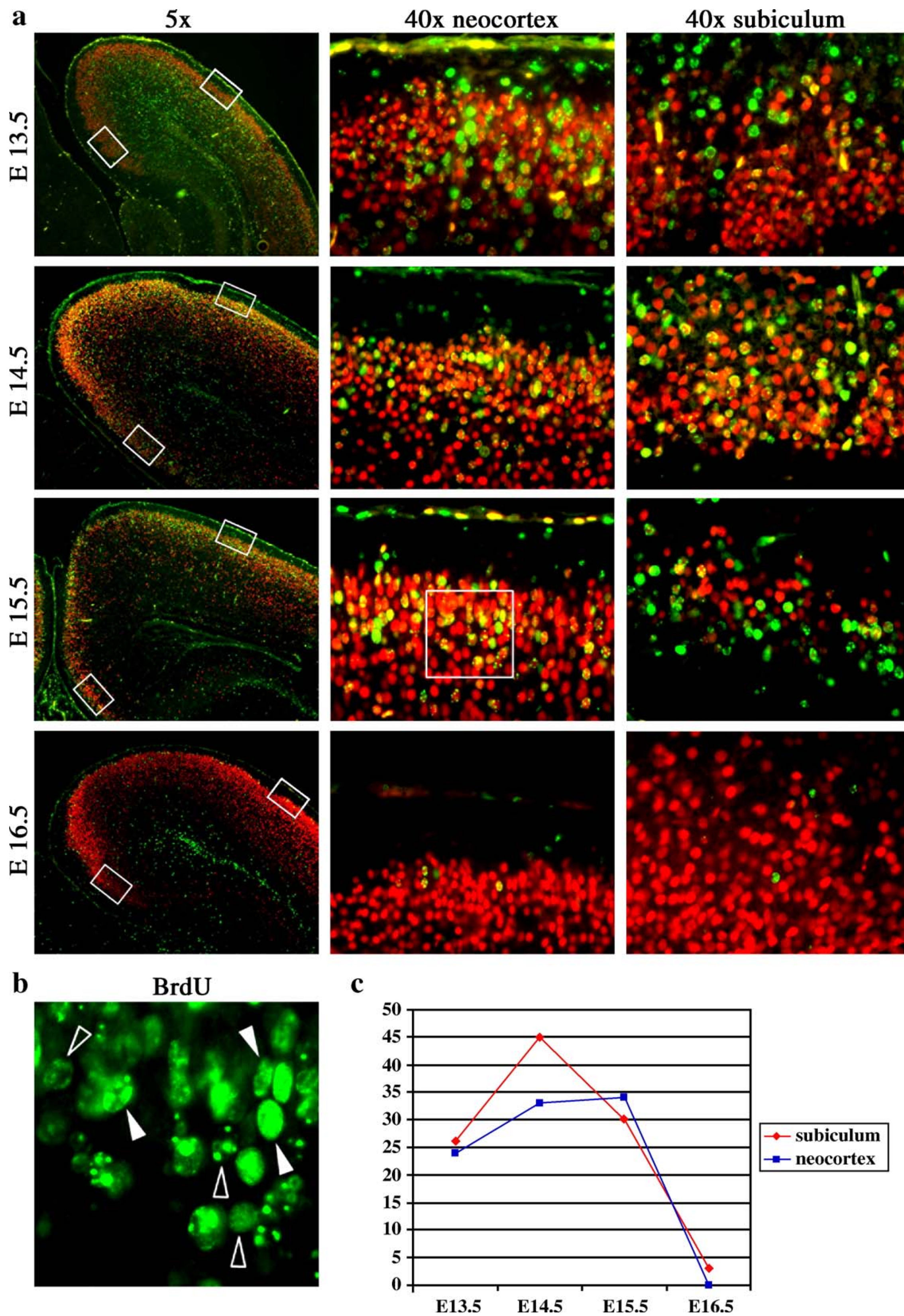

Fig. 2. Birth dating analysis of Satb2+ cells in the subiculum and the neocortex. (a) Double labeling for BrdU (green) and Satb2 (red) in brain sections of P2 pubs. Pregnant dams were given a single pulse of BrdU at E13.5, E14.5, E15.5 or E16.5. (b) Examples of cells that were counted as BrdU positive are depicted by arrowheads. (c) Graphic representation of the birth dating analysis in the neocortex and the subiculum showed that the majority of Satb2+ cells in the subiculum were born at E14.5.

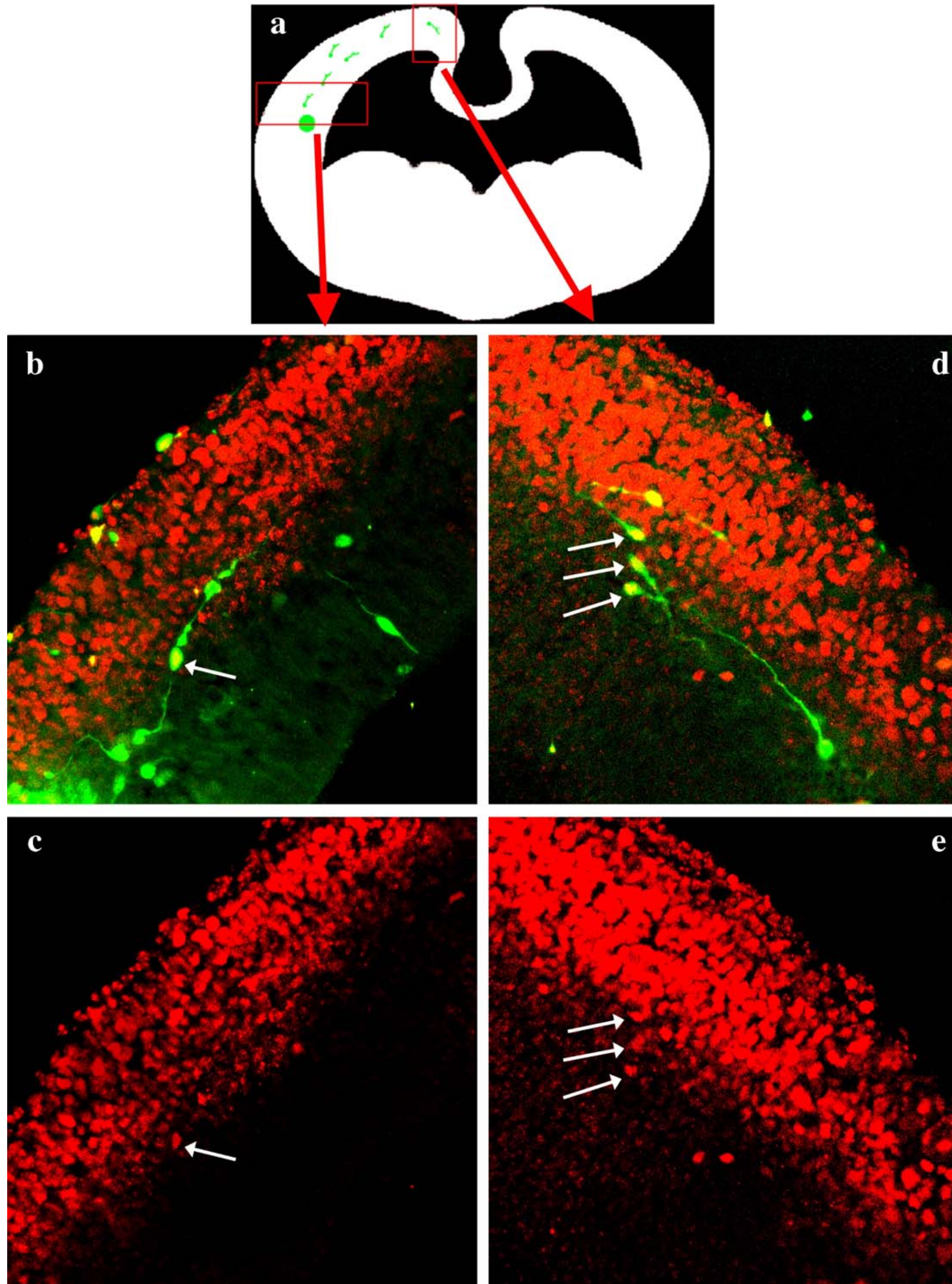

Fig. 3. Characterization of tangentially migrating cells in the cortex of WT animals. Placement of CMFDA-coated particles in the cortical SVZ of WT brain slices obtained from embryonic mouse brains at E14.5 results in migrating dye-labeled cells (green) in the dorsal cortex after 2 DIV. (a) Schematic representation of brain slices showing the site of the injection of the dye. The boxes represent the areas from which confocal images were taken. (b and d) Examination of sections stained for Satb2 showed that next to the injection site, several dye labeled neurons (green) were positive for Satb2 (red). (c and e) Examination of sections stained for Satb2 showed that in the dorsal cortex as well several dye labeled neurons (green) were positive for Satb2 (red) suggesting that these neurons migrate tangentially. Arrows indicate examples of double labeled cells.

dorsal cortex (box 2 in Fig. 3a) to determine whether any of the tangentially migrating neurons were positive for Satb2. To unambiguously show colocalization of CMFDA labeling with antibody staining, images were collected sequentially between frames in the z-plane using Argon and Krypton lasers.

As shown in Figs. 3b and d (arrowhead), several neurons near the site of the injection were identified positive for Satb2. Supporting our hypothesis that a subset of Satb2+ neurons migrate tangentially, images taken at the level of the dorsal cortex (Figs. 3c, e arrowheads) showed a number of tangentially

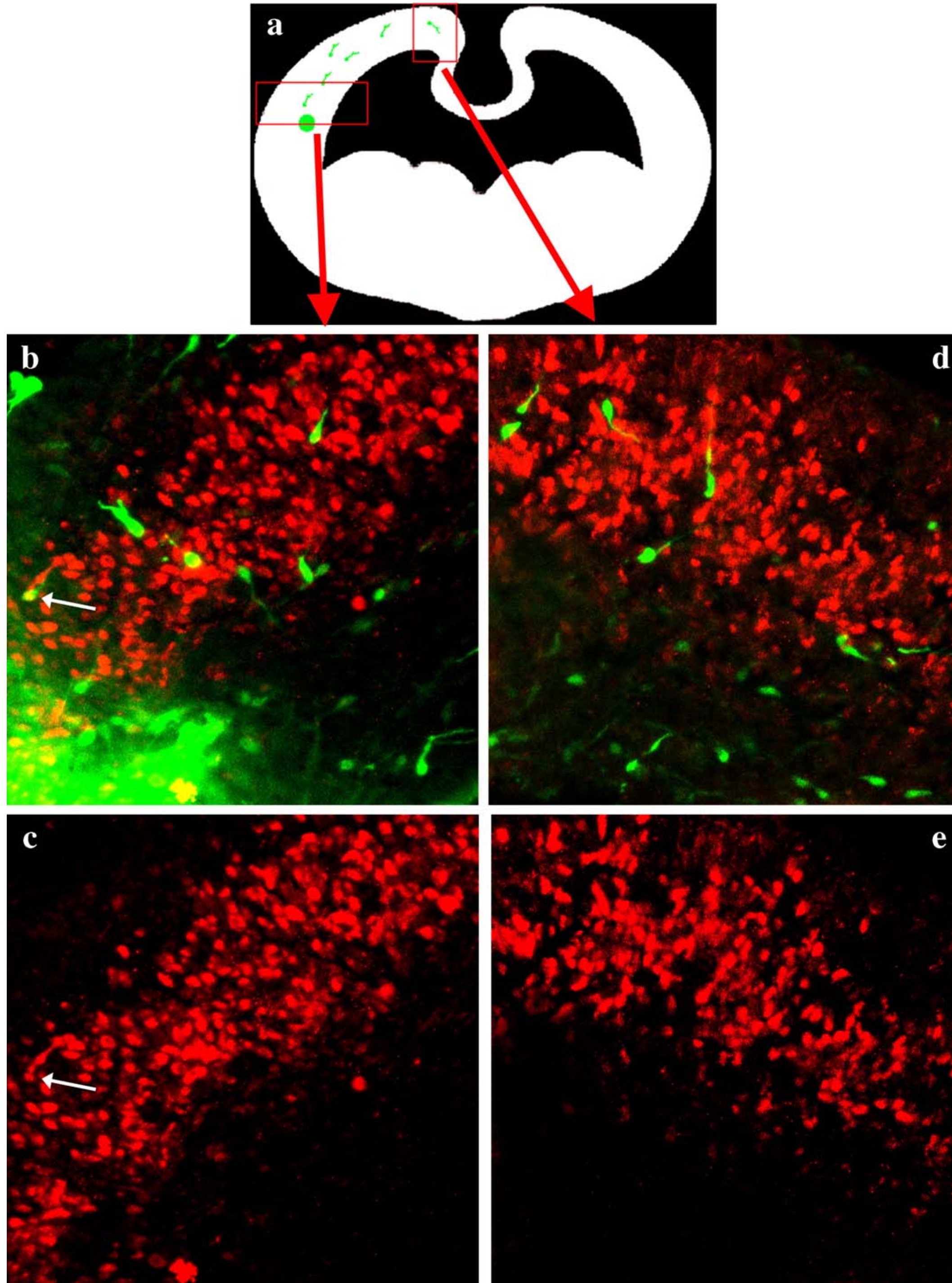

Fig. 4. Characterization of tangentially migrating cells in the cortex of *reeler*−/− animals. Placement of CMFDA-coated particles in the cortical SVZ of brain slices obtained from *reeler*−/− embryonic mouse brains at E14.5 results in migrating dye-labeled cells (green) in the dorsal cortex after 2 DIV. (a) Schematic representation of brain slices showing the site of the injection of the dye. The boxes represent the areas from which confocal images were taken. (b and d) Examination of sections stained for Satb2 showed that next to the injection site several dye labeled neurons (green) were positive for Satb2 (red). (c and e) Examination of sections stained for Satb2 showed that in the dorsal cortex tangential migration of Satb2+ neurons (red) was severely reduced. Arrows indicate examples of double labeled cells.

migrating neurons expressing Satb2. Cell counts showed that 26% of the labeled neurons in the dorsal cortex ($n=230$ migrating cells counted from WT brain slices taken from two separate litters) were also positive for Satb2. Interestingly, all tangentially migrating Satb2+ neurons were located at the upper part of the IZ. None of the Satb2+ migrating neurons were detected at the lower IZ, which at this age seems to be the preferred avenue of cortical interneurons that migrate from the ganglionic eminences in the cortex. The migration assays in WT brain slices supported our hypothesis that a subset of subicular Satb2+ neurons are not born in the subicular VZ, but arrive at the subiculum by tangential migration.

Migration analysis of Satb2+ neurons in reeler−/− brain slices

Our results so far indicated that the reduction in the expression of *satb2* in the subiculum of *reeler−/−* brains could be a result of a migrational defect of tangentially migrating Satb2+ neurons. We reasoned thus that this phenotype could be due to one of three possibilities: either the absence of Reelin produced a general defect in the tangential migration of both interneurons and projection neurons in the cortex, or that the absence of Reelin affected only the tangential migration of Satb2+ neurons, or alternatively, Satb2+ neurons were still able to migrate tangentially towards the dorsal cortex but were not allowed, in the absence of Reelin, to enter the subiculum from the neocortex.

To distinguish between these possibilities, we performed migration assays in brain slices prepared from E14.5 *reeler−/−* brains. Confocal images taken near the injection site, showed that similar to the WT slices, several labeled neurons positive for Satb2 could be identified in *reeler−/−* brain slices as well (Figs. 4b, d). Images acquired in the dorsal cortex showed that there was no general defect in tangential migration. However, although we could detect a multitude of migrating neurons, only 5.5% of them ($n=531$ migrating cells counted from *reeler−/−* brain slices taken from two separate litters) were positive for Satb2 (Figs. 4c, e).

Our data from the migration analysis agree with published data that *reeler* mutation does not affect tangential migration of interneurons in the cortex (Hevner et al., 2004). On the other hand, tangential migration of Satb2+ neurons in *reeler* brain slices is severely reduced compared to WT littermates (Fig. 5). Our results show that the reduction of Satb2 neurons in the subiculum of *reeler−/−* brains is not due to a general tangential migration defect but due to a severe reduction in the tangential migration of this specific population. These cells appear to be a subset of SVZ neurons that express *satb2*.

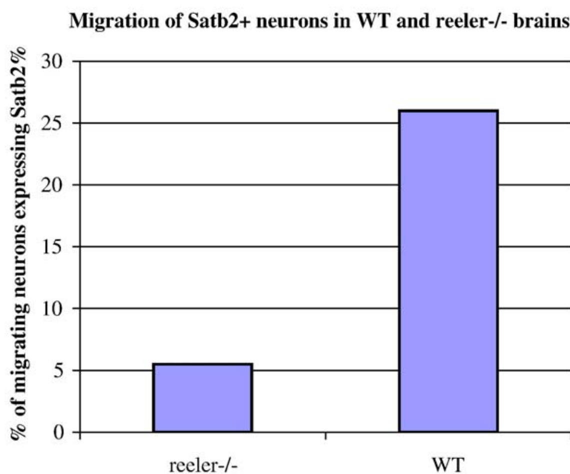

Fig. 5. Focal labeling of the SVZ in the lateral cortex with CMFDA results in a stream of neurons migrating towards the dorsal cortex in the WT as well as in the reeler brain slices. Staining for Satb2 showed that, in the WT E14.5 brain slices, 26% of the migrating neurons in the dorsal cortex ($n=230$ cells from WT slices taken from two independent litters) were positive for Satb2. In the reeler slices, however, only 5.5% of the migrating neurons were positive for Satb2 ($n=531$ cells from *reeler−/−* slices taken from two independent litters) suggesting that, in the absence of Reelin, tangential migration of Satb2+ neurons is severely reduced.

Satb2+ neurons are projection neurons derived from Emx1 lineage

Published data so far indicate that cortical projection neurons migrate to their final destination by radial migration, whereas the majority of cortical interneurons are born in the ganglionic eminences and go towards their final destination by tangential migration. Our results show that a subpopulation of tangentially migrating neurons in the IZ expresses *satb2*.

Although expression pattern analysis for *satb2* indicates that it is expressed exclusively in the cortex, we considered the possibility that a subset of interneurons born in the ganglionic eminences might up-regulate *satb2* once they enter into the cortex. In such a scenario, it is reasonable to argue that the Satb2+ tangentially migrating neurons we detected in the cortical IZ (26% of tangentially migrating neurons at E14.5) could actually be interneurons that up-regulated *satb2* in the cortex rather then cortically born projection neurons. To distinguish between these two possibilities, we examined whether any of the Satb2+ neurons were GABAergic interneurons.

We performed double immunolabeling using antibodies against GABA and Satb2, at sections prepared from E14.5 WT brains, a developmental stage in which the majority of subicular Satb2 neurons are born and migrate tangentially. Our results show that at this age none of the GABAergic neurons were positive for Satb2 (Fig. 6) suggesting that Satb2+ tangentially migrating cells are not interneurons. Furthermore, we confirmed that in fixed brains, GABAergic interneurons migrate at the lower part of the IZ whereas Satb2 immunoreactivity appears in the upper part of the IZ.

To further show that *satb2* is only expressed in neurons that are born in the cortical proliferative zone, we performed a lineage analysis of Satb2+ neurons based on a Cre-loxP approach. We crossed *Emx1IRESCre* mouse (Gorski et al., 2002) in which Cre recombinase was under the transcriptional control of the Emx1 locus to a *LacZ* ROSA 26 (R26R) reporter mouse in which the expression of β-gal can be activated by Cre (Soriano, 1999). It has been shown that the offspring of this crosses carrying both the *Emx1-Cre* and the reporter gene chromosomes, expresses β-gal in radial glia, Cajal–Retzius cells, glutamatergic neurons and astrocytes whereas GABAergic interneurons arise outside the Emx1-expressing lineage (Gorski et al., 2002). To find out if Satb2+ neurons are of dorsal telencephalic origin, we performed double immunohistochemistry in brain sections of P2 *Emx1IRESCre/R26R* animals using antibodies against Satb2 and β-gal. Our analysis was restricted to early postnatal stages because Satb2 expression in the brain is lost with age (Britanova et al., 2005). Double labeling for Satb2+ and β-gal in the subiculum and in the cortex demonstrated that virtually all of the Satb2+ neurons express β-gal at a detectable level (Fig. 6) suggesting that all Satb2+ neurons are projection neurons of dorsal telencephalic origin rather then being a subset of ventrally derived interneurons.

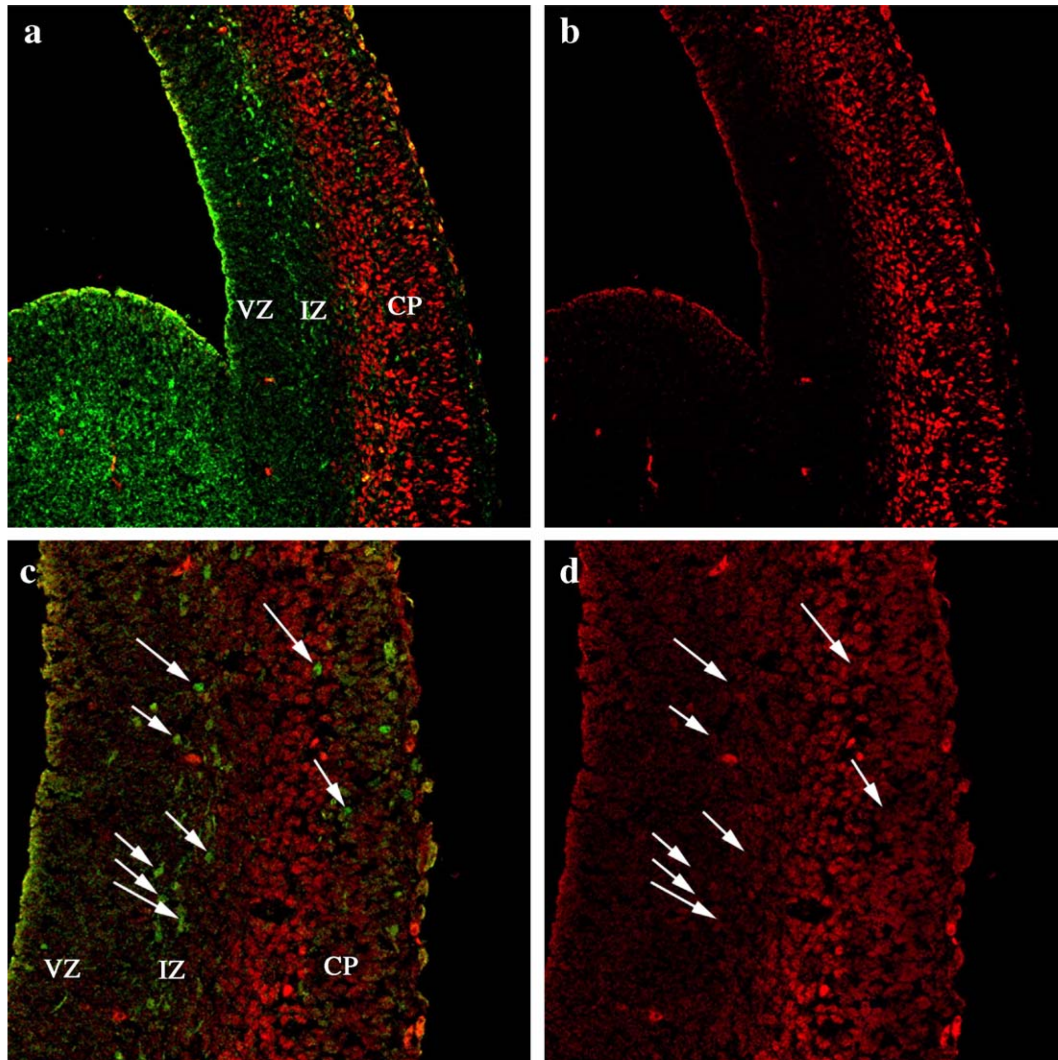

Fig. 6. GABAergic interneurons do not express Satb2. Characterization of tangentially migrating interneurons in the mouse cortex. (a and b) Low magnification of an E14.5 WT brain section labeled for GABA (green) and Satb2 (red). Note that the stream of GABAergic interneurons is at the lower IZ whereas Satb2 immunoreactivity is detected at the upper IZ. (c and d) High magnification of E14.5 WT brain section labeled for GABA (green) and Satb2 (red). Arrows in panels c and d indicate numerous tangentially migrating interneurons that are negative for Satb2. VZ: ventricular zone, IZ: intermediate zone, CP: cortical plate.

Finally, it has been suggested that oligodendrocytes born in the cortex from Emx1 lineage also migrate tangentially (Kessaris et al., 2006). To investigate if the tangentially migrating Satb2 cells we observed were oligodendrocytes, we performed double immunolabeling to see if any Satb2 were also positive for Olig1. As shown in Fig. 7, none of the Satb2 positive cells express Olig1. All our colocalization experiments indicate that Satb2 cells in the cortex are projection neurons.

Discussion

In the early 90s, it was believed that migration of cortical neurons is not only radial but also tangential (O'Rourke et al., 1995; Tan and Breen, 1993; Walsh and Cepko, 1988). Recent studies though, indicated that these tangentially migrating neurons were interneurons that were not born in the cortical proliferative zone, but were born instead in the ganglionic eminences. Thus, the current model states that different neuronal populations use different modes of migration (Marin and Rubenstein, 2003; Nadarajah et al., 2003). Excitatory cortical neurons migrate from the cortical germinal zone to the cortical layers by radial migration, whereas inhibitory interneurons are born in the germinal layers of the ganglionic eminences and migrate to the cortex by tangential migration.

Accumulating data suggest that radially migrating neurons in the developing cerebral cortex exhibit high affinity to the glia fibers at the initial steps of their migration and that this property is crucial for the inside out generation of the CP. A very important molecule in regulating the attachment of migrating neurons to glia fibers is Reelin. Recently, Reelin was suggested to serve as a signal (Hack et al., 2002) facilitating the detachment of neurons from glia fibers, followed by their translocation to the pia surface (Marin and Rubenstein, 2003 for review; Nadarajah et al., 2001). Interestingly, a series of time lapse imaging in brain slices showed that some SVZ neurons

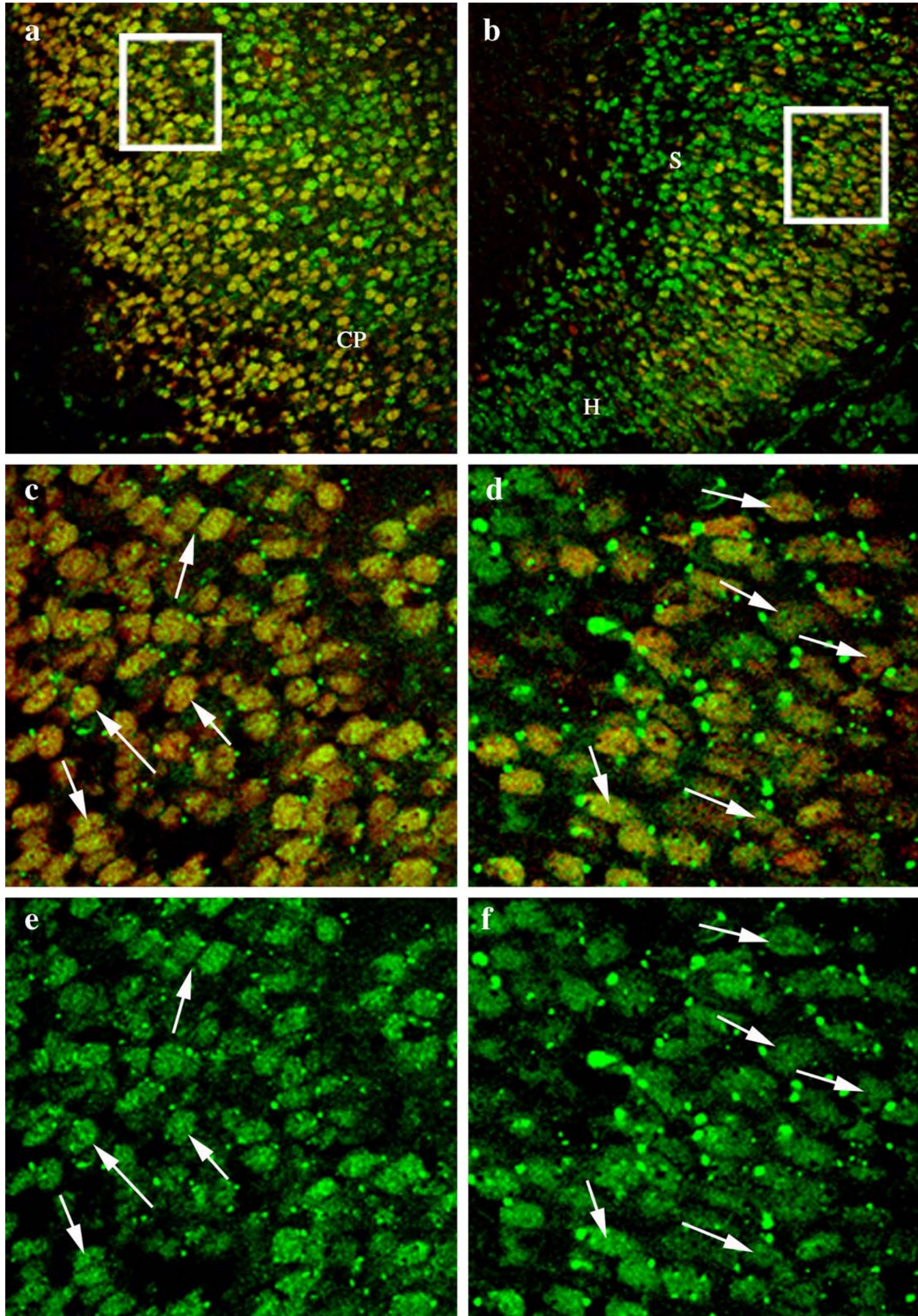

Fig. 7. Satb2 expressing cells originate from Emx1 lineage. Lineage analysis in brains of Emx1 IRESCre;R26R animals. Examination of sections from Emx1 IRESCre; R26R P2 brains labeled for β-gal (green) and Satb2 (red). Panels a and b are low magnification images; panels c and e are high magnification images of the cortex and panels d and f are high magnification images of the subiculum. In the lateral cortex (a and c) and in the subiculum (b and d), all Satb2 were positive for β-gal suggesting that Satb2 neurons were of cortical origin. Arrows indicate typical examples of double labeled cells. Panels e and f are single color images showing details of β-gal positive neurons. CP: cortical plate, H: hippocampus, S: subiculum.

form loose contacts with the glia fibers instead of interacting with them with high affinity (Kriegstein and Noctor, 2004; Noctor et al., 2004; Tabata and Nakajima, 2003). If indeed Reelin regulates the positioning of cortical projection neurons in the CP by signaling them to detach from the glia fibers then the behavior of some SVZ neurons (loose contact with glia fibers)

raises the question as to how (or if) their final positioning in the CP is regulated by Reelin.

In this report we provide evidence that not only ventrally derived interneurons migrate tangentially but also a subset of neurons born in the cortical proliferative zone can migrate tangentially as well. Furthermore, we showed that this mode of migration is disturbed in *reeler* brains. We initially compared the expression pattern of *satb2* (a marker of a subset of SVZ and some upper layer neurons) in WT and *reeler*−/− brains. In developing WT brains, Satb2 was expressed in the cortical SVZ and in the CP, while in the more mature cortex it appeared concentrated in upper layer neurons. In the lateral cortex of *reeler*−/− brains, the expression pattern of Satb2 followed the expected inversion pattern suggesting that the final positioning of Satb2 neurons in the CP depends on Reelin. However, a closer inspection of the expression pattern revealed a significant difference; the expression of Satb2 in the subiculum of *reeler*−/− brains was strongly reduced. Two explanations were possible for this phenotype: either Reelin has an instructive role in the specification of this particular neuronal type that is restricted in the subiculum and not in the medio-lateral cortex, or Satb2+ neurons reach their final destination at the subiculum using a novel mode of migration that is regulated by Reelin. Analyzing the expression pattern of *Nurr1* and *Sip1*, we concluded that although the subiculum in *reeler*−/− animals was reduced in size, it was properly specified, suggesting that the reduction in the expression of Satb2 could reflect a defect in the migration of these neurons.

Published data using time lapse imaging on brain slices have shown that the mode of migration of SVZ neurons is distinct. SVZ neurons do not display high affinity to the glia fibers in the initial steps of their migration (Noctor et al., 2004; Tanaka et al., 2003). These cells branch extensively and disperse tangentially while “jumping” between radial glial fibers (Noctor et al., 2004; Tanaka et al., 2003). We thus considered the possibility that these neurons could disperse tangentially in a wider area than previously thought and populate the subiculum. Thus, we performed migration assays to examine if Satb2+ neurons disperse tangentially. We confirmed our hypothesis and showed that a subset of Satb2+ neurons indeed disperse tangentially in the cortex. We then performed migration assays in *reeler*−/− organotypic brain slices and showed that this tangential dispersion was disrupted in *reeler* mutants, providing thus an explanation for the reduction of Satb2+ neurons from the subiculum. Finally, marker and lineage analysis showed that these neurons were born in the cortical proliferative zone and were projection neurons. Thus, in the absence of Reelin, a subset of Satb2+ neurons fails both to migrate tangentially and populate the subiculum.

Our results so far encourage us to update the existing model of neuronal migration of cortical projection neurons (Fig. 8). Young postmitotic neurons that migrate radially to the cortical plate are thought to do so by three distinct modes: translocation, locomotion and multipolar or branched migration (Figs. 8a, b, c, respectively) (Kriegstein and Noctor, 2004; Nadarajah and Parnavelas, 2002). Early in cortical development, the principal mode of neuronal migration appears to be translocation in which postmitotic neurons inherit the glia process and maneuver their somata towards the leading edge that is attached to the pia surface (Fig. 8a) (Nadarajah et al., 2001). Later in

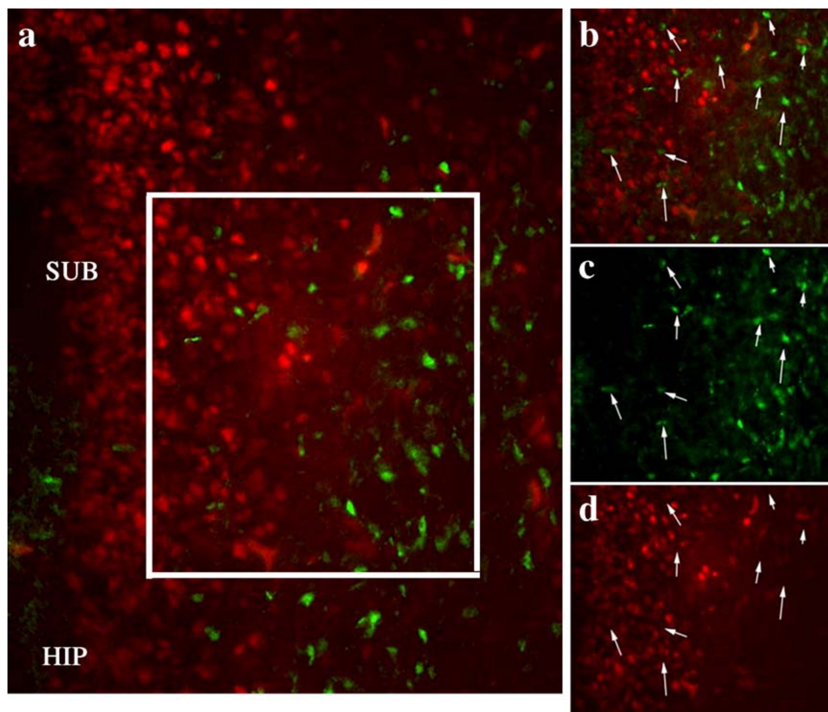

Fig. 8. Satb2 cells do not express oligodendrocytic marker. Double labeling of a P2 brain section for Satb2 (red) and Olig1 (green). (a) Low magnification, (b–d) high magnification of boxed area in panel a. Arrows in panels b–d indicate cells positive for Olig1. Note that none of the cells are double labeled for Satb2 and Olig1. SUB: subiculum, HIP: hippocampus.

development, the principal mode of radial migration is locomotion, in which postmitotic neurons are attached to radial glial fibers and migrate along them. The final step of this mode of migration involves the detachment of neurons from the glia fibers, resulting in their migration towards the pia surface via somal translocation (Fig. 8b) (Nadarajah et al., 2001). Time lapse imaging in brain slices, revealed that there is an additional mode of migration called multipolar. This mode of migration is a characteristic of SVZ neurons. In multipolar migration, neurons in the SVZ do not seem to attach to the glia fibers but branch dynamically at the onset of their migration (Fig. 8c). It has been further shown that these neurons can "jump" from one radial glia cell to another (Gupta et al., 2003; Noctor et al., 2004; Tabata and Nakajima, 2003). Our data show that in addition to what we already know from these studies, a subset of cortical projection neurons have the ability not only to jump between glia fibers but to migrate tangentially towards the forming subiculum. Although we did not provide evidence for a direct relation between these "jumping" multipolar neurons and the tangentially migrating Satb2+ neurons, it is conceivable that they are the same cells (Fig. 9).

Another important conclusion from our results is that only a subset of Satb2+ neurons seems to migrate tangentially. We do not know if this is due to a stochastic event or if these neurons belong to a specific subpopulation with a unique intrinsic program.

More intriguing is the regulation of tangential migration of cortical projection neurons by Reelin. We believe that two possible mechanisms can account for this phenomenon. The first mechanism would be that Reelin signals directly to these neurons. Thus, they might either receive a signal from Reelin halfway through their radial migration, destabilizing their attachment to radial glia and initiating their tangential migration towards the dorsal cortex, or they might be very sensitive to Reelin and loose their contact with the glia fibers early on (Fig. 8c). The second mechanism by which Reelin might affect the tangential migration of Satb2+ neurons could be an indirect one. It is possible that the inversion of the cortical layers (or additional architectural changes) in the *reeler* brains might result in the loss of guidance cues that would drive these neurons away from their site of birth. In such a scenario, the effect of Reelin to those neurons would be indirect.

It has been suggested that the cortical germinal layer is topographically similar to the differentiated cortical plate. This idea is supported by the fact that glutamatergic neurons simply ascend from the germinal layers towards the cortical plate aided by glia fibers (Rakic, 1988). We presented evidence, however, that a subpopulation of cortical projection neurons do migrate tangentially crossing the boundaries of newly formed areas. It is therefore likely that a direct relationship between the site of birth and the final destination is not apparent in all cortical neurons.

Materials and methods

In situ hybridization

Sectioning, in situ hybridization, washing and emulsion autoradiography were performed as described(Tarabykin et al., 2001). Two independent in situ analyses were performed for each stage on serial sections from WT and mutant littermates. Signals were compared at corresponding levels in WT and mutant brains that were processed in the same in situ hybridization experiment.

Animals and genotyping

Animal manipulations were carried out in accordance with German law and were approved by the Bezirksregierung Braunschweig. Heterozygous

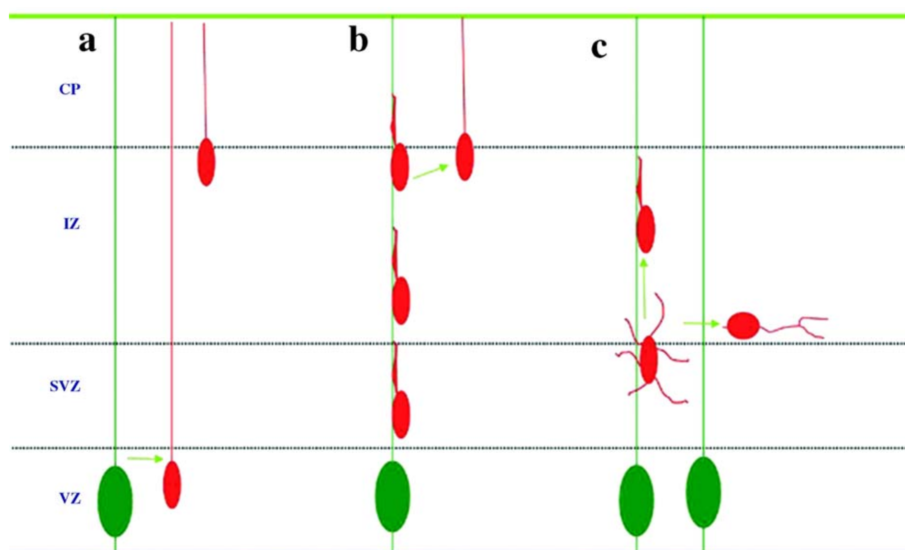

Fig. 9. An updated model on neuronal migration in the mammalian neocortex. (a) Early in development, cortical neurons migrate predominately via soma translocation. (b) Later in development, the majority of cortical projection neurons are attached to the radial glia and advance towards the CP via glia guided locomotion. In the final step of their migration, they detach from the glia fibers and translocate towards the surface. (c) In the cortical SVZ, cortical neurons branch dynamically and "jump" from one glia fiber to the other, a mode that has been described as multipolar or branched migration. We have shown that in addition to what has been described so far, cortical projection neurons migrate tangentially under the influence of Reelin over long distances.

reeler animals were crossed to obtain homozygous, heterozygous and wild type embryos in the same litter. The day of vaginal plug was considered embryonic day (E) 0.5. *Reeler* mice were obtained from Prof. Frotscher of Freiburg University. Genotyping has been performed as described(D'Arcangelo et al., 1996).

Organotypic cultures

Brain slices were prepared from embryonic brains as described previously (Alifragis et al., 2002). Briefly, brains embedded in 3% low melting point agarose (Sigma) were sectioned in ice cold ACSF, pH 7.4, at 300 μm using a Vibroslice (Leica Instruments). Coronal slices of embryonic brains were mounted onto porous nitrocellulose filters (0.45 μm; Millipore) and transferred into 12-well culture plates. The amount of medium added to each well was that required to cover the slices with a meniscus of fluid. The culture medium contained the following components: DMEM F-12 (Sigma), 5% heat inactivated fetal bovine serum (Gibco), 1× N-2 (Gibco), 100 μM L-glutamine, 2.4 g/L L-glucose and pen/strep (1:1000; Sigma). To follow the migration of neurons, slices of embryonic brains were cultured for 2 days. Cultured brain slices were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4), cryoprotected in 30% sucrose overnight, embedded in tissuetech and sectioned at 15 μm on a Leica cryostat.

Coating of tungsten particles with CMFDA

CMFDA (Molecular Probes) was dissolved in DMSO to yield a stock solution of 10 mM, and subsequently diluted in ethylene dichloride to yield a concentration of 1 mM. Fifty micrograms of tungsten particles (0.8 μm; Bio-Rad) was spread evenly as a thin layer on a glass microscope slide to which CMFDA was added. As the solvent evaporated, the precipitated dye was mixed well with the particles to ensure complete coating. The coated particles were stored at 4°C and applied with the aid of glass micropipettes.

Immunohistochemistry

Sections were blocked with 1% BSA (IgG free) and 0.1% Tween in phosphate-buffered saline (PBS) at room temperature for 1 h. After blocking, sections were incubated with primary antibodies overnight at 4°C. The primary antibodies used were a-Satb2 (rabbit), a-BrDU (monoclonal from SIGMA), a-β-gal (FITC conjugated from BD biosciences) and Olig1 (monoclonal, gift of Dr. J. Alberta; Arnett et al., 2004). For BrdU labeling, sections were treated with 2 N HCl at room temperature for 1 h, rinsed in 0.1 M Borate buffer and processed for immunohistochemistry. Following washes in PBT, the reaction was revealed using FITC-conjugated antibodies (Molecular Probes) at room temperature for 2 h. Sections were then washed, mounted with Citifluor and examined using a Zeiss dual channel confocal microscope. To reveal colocalization of CMFDA labeling with antibody staining, stacks of images were collected sequentially in the z-plane using Argon and Krypton lasers.

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