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Research Report

Abnormal laminar position and dendrite development of interneurons in the *reeler* forebrain

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ABSTRACT

The majority of cortical and hippocampal interneurons originate in the subcortical telencephalon and migrate tangentially into pallial regions before settling in various cortical layers. The molecular cues that regulate final positioning of specific interneurons in cortical structures have not yet been identified. The positioning of radially migrating principal neurons of the cortex and hippocampus depends upon Reelin, an extracellular protein expressed near the pial surface during embryonic development that is absent in *reeler* mutant mice. To determine whether the layer specification of interneurons, like that of principal neurons, requires Reelin, we crossed *reeler* with transgenic mice that contain Green Fluorescent Protein (GFP)-expressing Inhibitory Neurons (GINs). These neurons express basal forebrain markers Dlx1/2 in normal and *reeler* mice. In normal mice, GINs express Reelin and are localized to specific layers of the cortex and hippocampus. In *reeler* mutant mice, we show that GINs migrate normally into the pallium, but fail to acquire proper layer position. Double labeling experiments indicate that the neurochemical profile of these interneurons is not generally altered in *reeler* mice. However, the extension of their cellular processes is abnormal. Quantitative analysis of GINs in the cortex revealed that they are hypertrophic, bearing longer neuritic branches than normal. Thus, the lack of Reelin signaling results in abnormal positioning and altered morphology of forebrain interneurons.

1. Introduction

Neurons reach the dorsal forebrain by two modalities, radial and tangential migration. Principal neurons originate from the proliferation of precursors near the ventricular zone of forebrain structures and migrate radially towards the pia. Neurons born at the same time become positioned at the same level in the radial dimension, giving rise to cellular layers

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(Adgevine and Sidman, 1961). Radial migration of principal cortical neurons is regulated by the extracellular protein Reelin (D'Arcangelo et al., 1995). Its absence in the mutant mouse *reeler* causes layer disorganization in all cortical structures (D'Arcangelo et al., 1995, 1996 and reviewed by D'Arcangelo (2005); Lambert de Rouvroit and Goffinet (1998); Rice and Curran (2001). In the *reeler* cortex, principal neurons initiate their radial migration normally but fail to assemble into layers according to an inside-out mode of development. In the hippocampus, the pyramidal cell layer is split and the dentate gyrus disorganized. During embryonic corticogenesis, Cajal–Retzius cells express Reelin in the marginal zone of the developing neocortex and hippocampus (Alcantara et al., 1998; D'Arcangelo et al., 1995; Ogawa et al., 1995) As radially-migrating principal neurons encounter Reelin, signal transduction events mediated by the VLDLR and ApoER2 receptors and Dab1 result in their localization to appropriate cellular layers, the acquisition of proper orientation and dendrite outgrowth (reviewed by D'Arcangelo (2005); Rice and Curran (2001)).

Unlike principal neurons, GABAergic interneurons originate mostly in the basal telencephalon and invade the cerebral cortex, hippocampus, and olfactory bulb by a tangential route (reviewed by Anderson et al. (1999); Corbin et al. (2001); Marin and Rubenstein (2001)). Their migration into the pallium is inhibited by the disruption of specific basal forebrain homeodomain genes including Dlx1, Dlx2, Nkx2.1, Brn1, and Brn2 (Anderson et al., 1997; McEvilly et al., 2002). These transcription factors are required for interneuron specification and migration. Their migration into the dorsal forebrain is also regulated by class 3 Semaphorins and Neuregulin-1 through interaction with their specific receptors (Flames et al., 2004; Marin et al., 2001). Interneurons invade the pallium mostly via the subventricular and the marginal zone (Lavdas et al., 1999; Wichterle et al., 2001). However, it is not clear how the position of these cells is ultimately determined inside cortical structures. Interneurons form a complex population and express a variety of neurochemical markers (Flames and Marin, 2005; Markram et al., 2004). Specific subpopulations of interneurons, like principal neurons, appear to be destined to specific layers. In the transgenic mouse GIN (GFP-expressing Inhibitory Neurons), GFP is expressed in a subset of interneurons destined to specific layers of the cerebral cortex and hippocampus (here referred to as GINs) (Oliva et al., 2000). To determine whether laminar position and maturation of tangentially migrating interneurons are affected by Reelin, we crossed GIN transgenic with *reeler* mice, and examined the distribution and morphology of these interneurons in normal and mutant mice.

2. Results

2.1. *Forebrain GINs originate in the ganglionic eminences*

GFP expression in wild type GIN mice begins around postnatal day 7, and it becomes more intense during the following postnatal week and into adulthood. The distribution of neocortical GINs at all ages examined was limited to the upper layers of the cortex mostly II and III (Fig. 1A). In the hippocampus, few GINs were present in area CA1 and many more were seen in area CA3 (Fig. 1C). Their cell bodies are located either in the stratum oriens (SO), stratum pyramidale (SP), or stratum radiatum (SR) but never in the stratum lacunosum moleculare (SLM). Intense neuropil GFP fluorescence was seen in the SLM of area CA3, indicating that many hippocampal GINs project to this layer. Few GINs were also seen in the hilus of the dentate gyrus, but never in the granule cell layer (DGC) or in the outer marginal layer. This pattern of expression is consistent with that previously reported (Oliva et al., 2000).

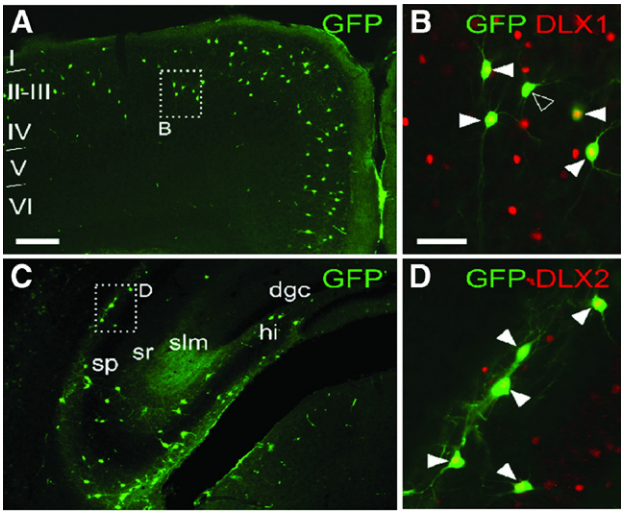

Fig. 1 – GFP-positive neurons in the cerebral cortex and hippocampus co-express Dlx1 and Dlx2. Coronal sections through the telencephalon showing GFP immunoreactivity in the neocortex (A) and hippocampus (C) of postnatal day 14 wild type mice. Panels B and D are higher magnification views of the boxed areas in panels A and C, respectively, showing co-localization of GFP with Dlx1 in the cortex (B) or Dlx2 in the hippocampus (D). Solid arrowheads show double labeled cells, whereas the open arrowhead indicates a cell expressing only GFP. hi = hilus; dgc = dentate granule cell layer; slm = stratum lacunosum moleculare; sr = stratum radiatum; sp = stratum pyramidale; I–VI, cortical layers I to VI. Scale bars: A, C 200 μm; B, D 50 μm.

GINs are a subpopulation of GABAergic and somatostatin-positive interneurons. Since the majority of forebrain interneurons originate from the ganglionic eminences and express the homeobox genes *Dlx1* and *Dlx2* Anderson et al., 1997, we sought to determine whether cortical and hippocampal GINs also express these basal forebrain markers. We conducted double-labeling immunofluorescence experiments in normal GIN brain sections using antibodies directed against the Dlx1 and Dlx2. This analysis revealed that 98% of GINs in the neocortex ($n=200$) and 95% of GINs in the CA1 region of the hippocampus ($n=200$) are also Dlx positive, expressing either Dlx1 or Dlx2 (Figs. 1B and D). A slightly lower percentage of double labeling was observed in the CA3 region of the hippocampus (83%, $n=50$), suggesting that some of the GINs in this area may not originate in the basal telencephalon.

2.2. *Abnormal laminar position and morphology of GINs in the reeler cerebral cortex*

The laminar position of principal neurons is disrupted in homozygous *reeler* mice lacking Reelin. To determine whether the position of forebrain interneurons is also affected by the lack of Reelin, we crossed heterozygous *reeler* mice with GIN transgenic mice. The progeny of this mating was interbred to generate wild type, heterozygous, and homozygous mutant *reeler* mice that express the GFP transgene in the GIN subpopulation of GABAergic interneurons. Mutant (*reelin* homozygous) and normal (heterozygous or wild type for *reelin*) littermates were sacrificed at postnatal ages, and GFP expression was analyzed by immunohistochemistry in floating sagittal brain sections. In wild type and heterozygous *reeler* mice, GINs were confined to the upper layers (layers II/III) of the neocortex (Figs. 2A and B). In *reeler* mutant mice, however, GINs can be seen in all layers of the neocortex (Fig. 2C). In addition, *reeler* GINs, especially those located in deep layers, appeared hypertrophic (Figs. 2F and G) compared to controls (Figs. 2D and E). Neurolucida reconstruction of GINs in the normal upper neocortex (Fig. 3A), or GINs located either in the upper (Fig. 3B) or lower (Fig. 3C) neocortex of *reeler* brains confirmed this observation. Quantitative analysis indicated that the number of cellular processes emanating from the GIN cell body was similar in wild type (5.4, SEM±0.56, $n=20$ neurons), heterozygous (5.8, SEM±0.23, $n=20$ neurons), and mutant *reeler* in the upper (6.15, SEM±0.39, $n=20$ neurons) or lower cortex (4.6, SEM±0.54, $n=10$ neurons) (Fig. 3D). However, the number of branch nodes and the total length of the processes were significantly increased in homozygous *reeler*, especially in the lower half of the cortex (Figs. 3E and F). GINs from the upper half of the *reeler* cortex exhibited a 1.5-fold increase in the number of branch nodes per neuron compared to wild type. The average number of branch nodes in each GIN was 16.65 in wild type (SEM±1.54), 17.8 in heterozygous (SEM± 1.35), and 25.5 in homozygous *reeler* mice (SEM±2.10). The difference between mutant and normal values was statistically significant ($P=0.001$). GINs from the lower half of the cortex exhibited a more pronounced difference from wild type, with a 2-fold increase in the number of branch nodes (mean=34.1, SEM±6.02, $P=0.00002$). The increase in neurite branching correlated very well with the increase in total length of the processes per neuron in *reeler* GINs. The total length of processes in mutant GINs in the upper layers was increased 1.5-fold compared to wild type neurons, whereas a 2.1-fold increase was observed in GINs located in the lower half of the cortex. The length of processes was 826.72 μm (SEM±102.23 μm) in wild type neurons, 895.19 μm (SEM±63.48 μm) in heterozygous neurons, 1303.08 μm (SEM± 102.23 μm) in upper layer mutant neurons, and 1749.64 μm (SEM±247.45) in lower layer mutant neurons. The difference between mutant and normal values was statistically significant both in the upper ($P=0.001$) and lower layers of the cortex ($P=0.00005$). A modest increase in the average length of the individual processes was also noted in *reeler* GINs compared to normal; however, it did not differ significantly among genotypes (not shown). Thus, the data indicate that the hypertrophy of *reeler* cortical GINs is mostly due to more extensive branching than normal.

2.3. *GIN ectopia in the reeler hippocampus*

We examined the position of GINs in the postnatal hippocampus of *reeler* and normal littermates. In the normal (wild type or heterozygous) hippocampus proper, GINs were seen

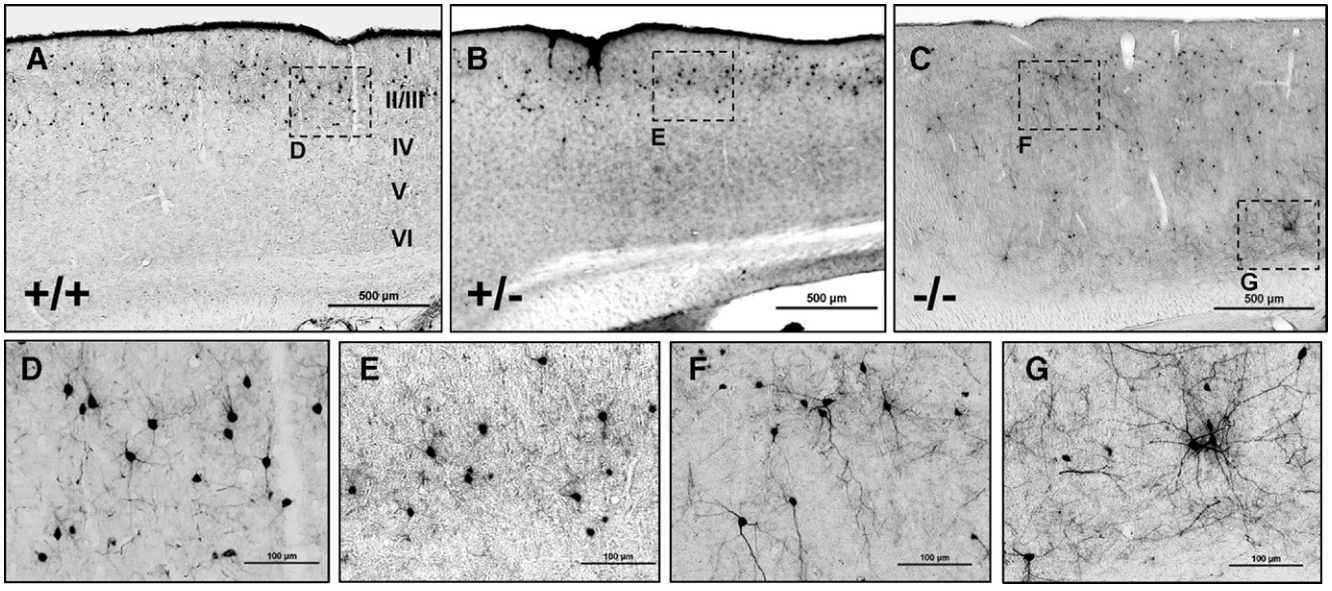

Fig. 2 – Ectopia and hypertrophy of GFP-positive interneurons in the *reeler* neocortex. Sagittal sections of postnatal neocortex in wild type (A), heterozygous (B), and homozygous *reeler* mice (C). GINs were identified by immunohistochemistry using anti-GFP antibodies. The mutant cortex contains hypertrophic GINs. Ectopic interneurons can also be seen in deep regions of the *reeler* cortex. Panels D–G represent higher magnifications of the corresponding boxed areas in panels A–C. Scale bars: A–C 500 μm; D–G 100 μm.

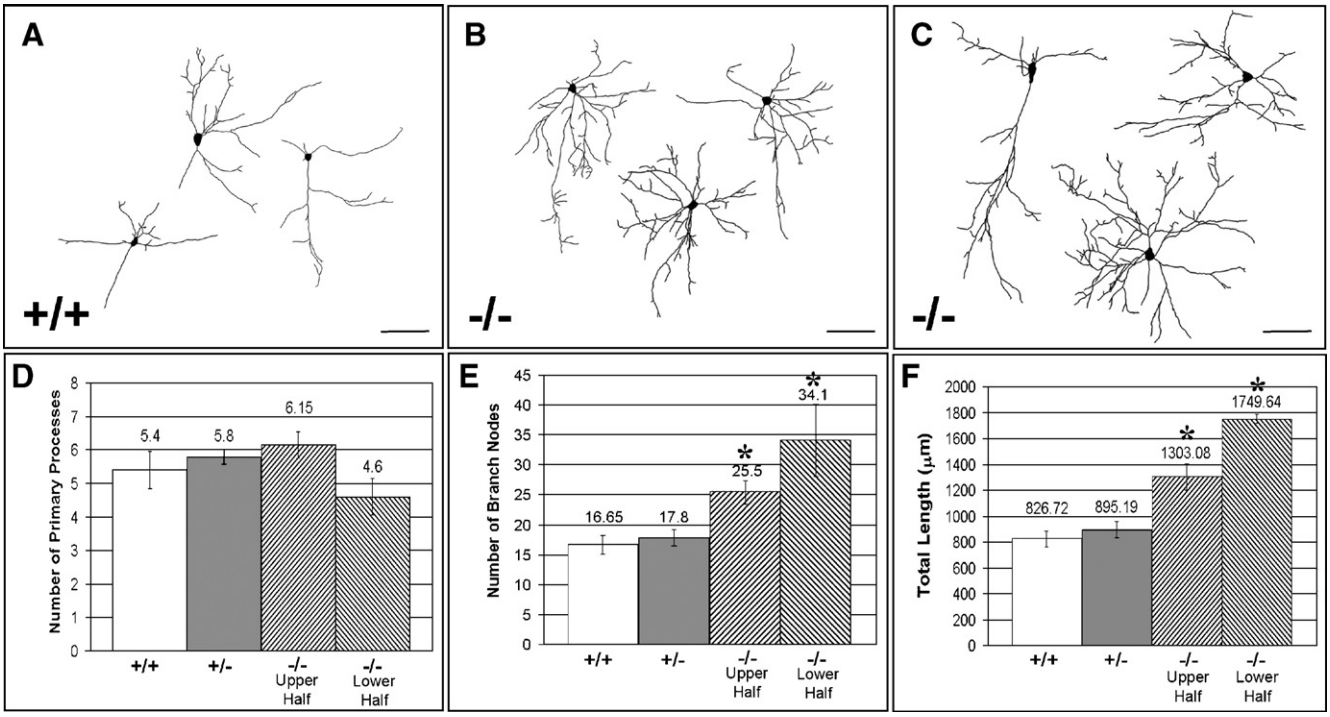

Fig. 3 – Analysis of GIN process extension in the *reeler* neocortex. Neurolucida reconstructions of representative GINs in the upper cortex of wild type (A) or mutant *reeler* mice (B), and in the lower cortex of mutant mice (C). The mutant neurons in both halves of the cortex appear hypertrophic compared to normal. Quantitative analysis of the number of primary processes (D), number of branches (E), and total length of the processes (F) in the indicated genotypes. Numbers on the plots represent mean values, bars indicate the standard error of the mean (SEM). Asterisks indicate values that are statistically different from wild type ($P \leq 0.001$). Scale bars: 20 μm.

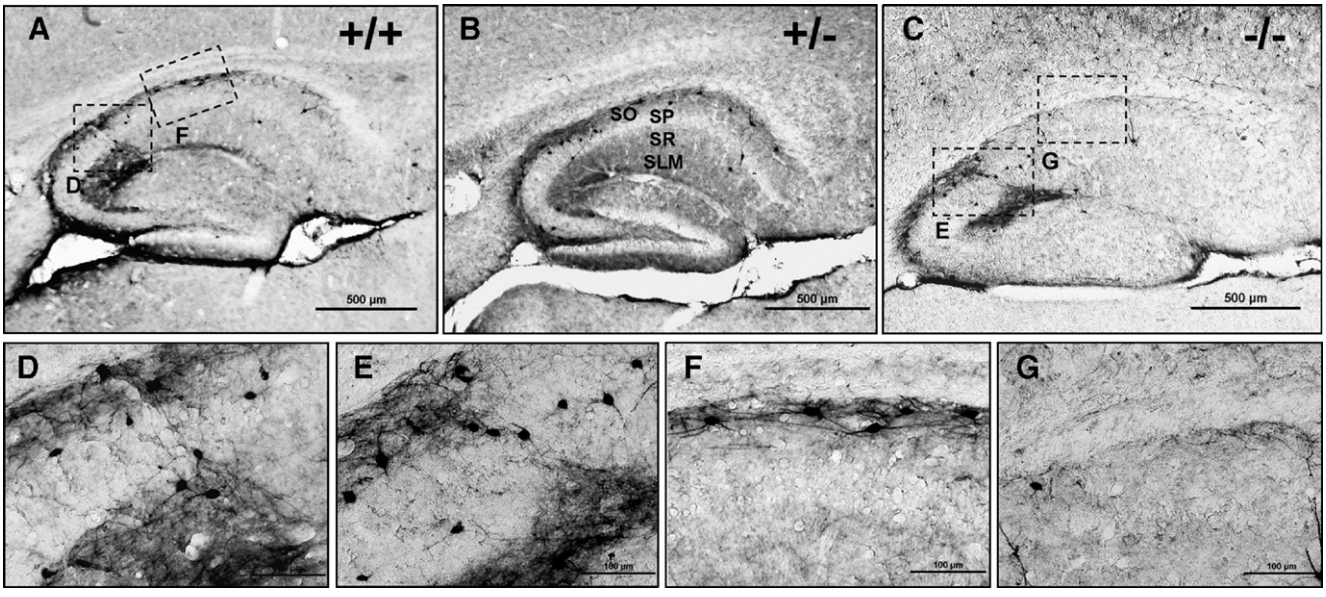

Fig. 4 – Ectopia of GFP-positive interneurons in the *reeler* hippocampus. Sagittal sections of postnatal hippocampus in wild type (A), heterozygous (B), and homozygous *reeler* hippocampus (C). GINs were identified by immunohistochemistry using anti-GFP antibodies. Panels D–G represent higher magnifications of the corresponding boxed areas in panels A and C. Panels D and E show the boundary of area CA3 in wild type and *reeler*, respectively. Panels F and G show the inner region of area CA1 in wild type and *reeler*, respectively. GINs are missing from the SO of area CA1 and are ectopically located in *reeler*. SO = stratum oriens; SP = stratum pyramidale; SR = stratum radiatum; SLM = stratum lacunosum moleculare. Scale bars: A–C 500 μm; D–G 100 μm.

mostly in the SO of area CA1 and CA3 and at the border between SR and SLM in CA3 (Figs. 4A and B). Their orientation, especially in SO, was mostly horizontal except for those GINs that appeared to delimit areas CA3 and CA1 which were mostly vertically oriented (Figs. 4D and F). In the normal dentate gyrus, few GINs were sometimes found in the hilus (not shown). As for the cerebral cortex, we found that GINs are ectopic in the *reeler* hippocampus. In the mutant, ectopic GINs were found dispersed throughout the split SP and the SLM of area CA1 and CA3 (Fig. 4C), and occasionally intermingled with the granular cell bodies of the dentate gyrus (not shown). Strikingly, the SO of area CA1 appeared depleted of GINs (Fig. 4G), whereas area CA3 appeared to contain a normal number of these interneurons (Fig. 4E). As in normal mice, *reeler* GINs accumulated along the border between areas CA1 and CA3 and arborized profusely in the SLM of area CA3.

2.4. *Neurochemical characteristics of GFP-expressing cells in normal and reeler brain*

We conducted immunohistochemical analysis with specific interneuron markers to identify the neurochemical phenotype of GINs in normal and *reeler* mice. As previously reported, normal GINs represent a subpopulation of somatostatin-expressing interneurons in the cerebral cortex and hippocampus (Oliva et al., 2000). We found that 88% of these cells co-express somatostatin in the normal cortex ($n=200$) and 48% in the hippocampus ($n=100$). Similarly, in the *reeler* brain, we detected a high degree of co-localization of somatostatin and GFP in the cortex (73%, $n=200$) and hippocampus (40%, $n=100$) (Figs. 5B and D). We also performed calretinin and calbindin immunostaining of normal and *reeler* sections. In both genetic backgrounds, we found a partial co-expression of calretinin and GFP in the cortex (27% in wild type and 21% in *reeler*, $n=200$), but essentially no co-expression in the hippocampus (<2% in both genotypes, $n=100$) (Figs. 5C and D). A small population of GINs also co-express calbindin in the cortex (11% in wild type and 6% in *reeler*, $n=200$) and hippocampus (8% in wild type and *reeler*, $n=100$) (Figs. 5E and F). Finally, we confirmed that GINs in *reeler* mice express Dlx2 in the cortex and hippocampus (data not shown), as for normal mice (Fig. 1). Although we measured a slight decrease in the percentage of co-expression of GFP and the analyzed markers in *reeler* cortical GINs, these cells still retained an overall expression profile similar to that of normal GINs. These data indicate that

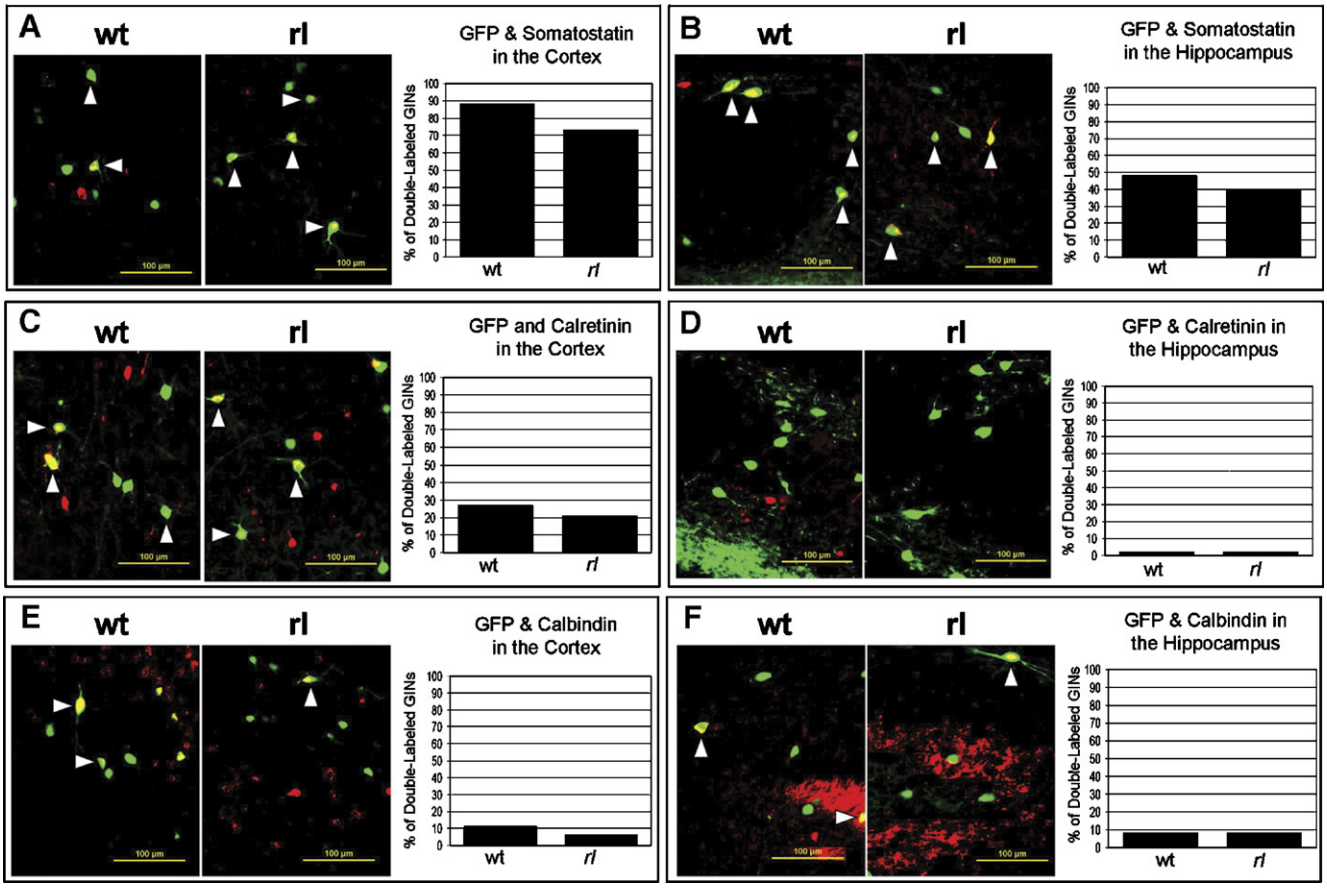

Fig. 5 – GFP-positive interneurons express similar markers in normal and *reeler* brain. GINs were identified by direct fluorescence (green), whereas somatostatin (A and B), calretinin (C and D), or calbindin (E and F) were detected by immunofluorescence using CY3-conjugated or AlexaFluor 594 antibodies (red). Neurons expressing both GFP and the markers appear yellow in the overlay images (arrowheads). Plots represent the percentage of GINs co-expressing the indicated marker. wt = wild type; rl = *reeler*. Scale bars: 100 µm.

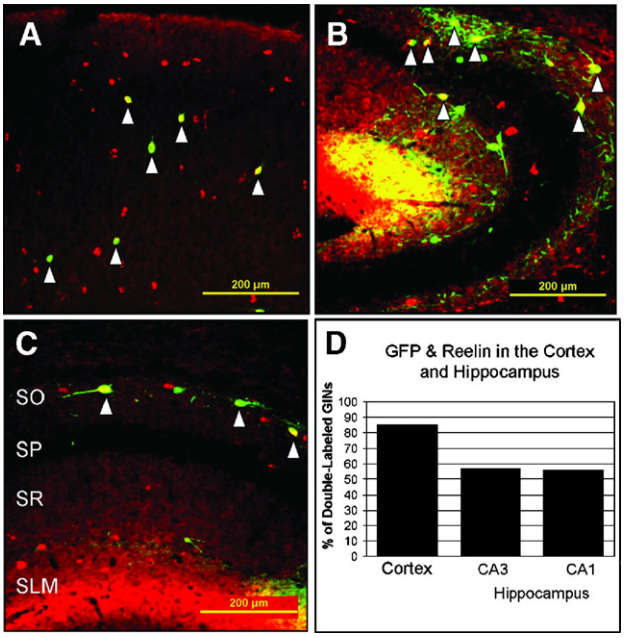

Fig. 6 – GFP-positive interneurons express Reelin in the forebrain. GINs were identified by direct fluorescence (green) whereas Reelin expression was detected by immunofluorescence using CY3-conjugated antibodies (red). Neurons expressing both GFP and Reelin (arrowheads) appear yellow in the overlay images of the neocortex (A), hippocampus area CA3 (B), or CA1 (C). Plots in panel D represent the percentage of GINs co-expressing Reelin. SO = stratum oriens; SP = stratum pyramidale; SR = stratum radiatum; SLM = stratum lacunosum moleculare. Scale bars: 200 µm.

the *reeler* mutation does not significantly alter the chemical phenotype of forebrain GINs.

2.5. *GINs express Reelin in the postnatal forebrain*

During embryonic development, cells that depend on Reelin for their layer positioning are often located adjacent to Reelin-expressing cells (Rice et al., 1998). Since the positioning of GINs is altered in *reeler* mice, we sought to analyze the spatial relationship between these cells and those expressing Reelin in the postnatal brain. Previous studies have shown that, in the postnatal brain, Reelin is expressed by a subset of GABAergic interneurons throughout the cerebral cortex and hippocampus (Alcantara et al., 1998; Pesold et al., 1998). In agreement with previous reports, we found that Reelin is expressed in all layers of the neocortex at postnatal day 15. A subset of Reelin-positive neurons also co-expressed GFP in the upper layers of the cortex and in the hippocampus. In the upper cortex, the majority of GINs (85%, $n=200$) co-expressed Reelin (Figs. 6A and D). In the postnatal hippocampus, Reelin is prominently expressed in the SLM by Cajal–Retzius cells, a residue of the embryonic expression pattern. However, cells expressing Reelin can also be seen in SO and, to a lesser extent, in other layers (Figs. 6B and C), consistent with a previous report (Alcantara et al., 1998). We found that a smaller fraction of GINs in hippocampal area CA1 and CA3 co-express Reelin (56% in area CA1 and 57% in area CA3, $n=50$ and 100, respectively) (Fig. 6D).

3. Discussion

In this study, we investigated whether basal forebrain-derived interneurons depend on Reelin for their laminar determination in the dorsal telencephalon. Given the enormous diversity of forebrain interneurons (Markram et al., 2004), it is difficult to ascertain laminar specificity based on general interneuron markers such as GAD67 or GABA. We have therefore narrowed our analysis to a subset of interneurons that display clear layer specificity in the neocortex as well as the hippocampus. We have taken advantage of the previously generated GIN transgenic mice, in which expression of GFP from the GAD67 promoter occurs only in a fraction of somatostatin-positive interneurons (Oliva et al., 2000). The GFP transgene provides the opportunity to study layer position and to examine in detail the growth of cellular processes.

We crossed the transgenic mouse on the *reeler* background to examine how the lack of Reelin affects the position and growth of GIN interneurons. We found that GINs are ectopic in the neocortex and hippocampus of *reeler* mice. Thus, these interneurons behave similarly to principal neurons, in that they require Reelin for proper laminar determination. Surprisingly, we also discovered that cortical *reeler* GINs are larger than normal, mostly due to a more extensive dendritic branching. This is contrary to the behavior of most hippocampal and dentate neurons, which grow reduced dendritic trees in the absence of Reelin or its signaling molecule Dab1 (Niu et al., 2004). Because of the high density of GIN processes in the hippocampus, we could not trace individual neurons and determine whether or not they are also abnormal in the *reeler* hippocampus. Therefore, it is presently unclear whether interneuron hypertrophy is limited to the *reeler* neocortex. It is also unclear at this time whether excessive dendrite branching is unique to GINs or whether it is a common feature of other inhibitory neurons in the *reeler* cortex. Given the importance of inhibitory neurons in the regulation of brain activity, this observation deserves further investigation.

The postnatal expression pattern of GFP in the GIN mouse prevented us from studying directly the migration of these interneurons in the *reeler* forebrain. Based on their Dlx1/2 immunoreactivity, we infer that they originated in the ganglionic eminences and that they entered the neocortex and hippocampus by tangential migration during late embryonic and perinatal ages. Despite their abnormal laminar position, the number of cortical and hippocampal GINs does not appear to be reduced in *reeler*, indicating that Reelin is not required for tangential migration.

It is not currently known what governs laminar specification of interneurons in the forebrain. Principal and inhibitory neurons that are born at the same time converge into similar layers despite their diverse origin and mode of migration. Therefore, it has been proposed that interneurons may follow molecular cues provided by principal neurons (Fairen et al., 1986; Hevner et al., 2004). Here, we showed that

Reelin is required for layer determination of GIN interneurons after they entered the forebrain. Since Reelin directly controls layer determination of principal neurons, we cannot exclude the possibility that the abnormal laminar positioning of interneuron in *reeler* is secondary to principal neuron ectopia.

We showed that the majority of GINs express Reelin. This is consistent with a previous report that demonstrated co-localization of Reelin with tangentially migrating neurons in slice cultures (Lavdas et al., 1999). Our findings are also consistent with the observation that, in Brn1/2 knock out mice, Reelin expression in layer 2–6, but not in layer 1, is abolished concurrently with an increase of Reelin immunoreactivity in the subcortical telencephalon (McEvilly et al., 2002). Reelin is expressed in a fraction of GABAergic neurons in the postnatal cortex and hippocampus in rodents (Alcantara et al., 1998; Pesold et al., 1999), primates (Rodriguez et al., 2002; Zecevic and Rakic, 2001), and humans (Meyer and Goffinet, 1998). With the exception of residual Cajal–Retzius cells, all Reelin-expressing neurons in the postnatal forebrain are indeed GAD67 immunoreactive. These Reelin-positive cells comprise phenotypically diverse interneuron subtypes, including multipolar, bitufted, and horizontal cells, which are distributed throughout all cortical layers. Here, we show that GINs represent a subset of Reelin-containing cells that are restricted to the upper layers of the cortex and that have mostly a bitufted or multipolar morphology. Since these interneurons are hypertrophic in the *reeler* cortex, it is possible that Reelin controls GIN cell growth and maturation through an autocrine mechanism.

We and others have shown that interneurons, including GINs, express Reelin in the postnatal forebrain. One possible function of Reelin at this later stage of development is to regulate neuronal maturation and plasticity. In the hippocampus, there is evidence that Reelin promotes axonal branching and synaptogenesis in the entorhinohippocampal projection (Borrell et al. 1999; Del Rio et al., 1997), and promotes dendrite outgrowth from hippocampal and dentate neurons in a Dab1-dependent manner (Niu et al., 2004). Reelin accumulates on dendritic spines of cortical neurons (Rodriguez et al., 2000) and the number of dendritic spines appears to be reduced in *reeler* heterozygous mice, which do not display cellular layer defects (Liu et al., 2001]). In addition, there are several data indicating that Reelin promotes synaptic function. Reelin affects long-term potentiation in hippocampal slices and promotes learning and memory through a NMDA receptor-mediated mechanism (Beffert et al., 2005; Weeber et al., 2002). A function in neuronal maturation and synaptic function may explain the persistence of Reelin and its signaling molecules in brain areas, such as the olfactory bulb and the hippocampus, where extensive neuronal plasticity takes place. Furthermore, it may provide a rationale for the proposed involvement of Reelin in cognitive disorders such as schizophrenia (Costa et al., 2002). Several reports have described a reduction of *Reelin* and *GAD67* mRNA expression in GABAergic neurons of schizophrenic tissue (Guidotti et al., 2000). Our findings should encourage the use of the GIN transgenic animal as a model to study the vulnerability of Reelin-positive interneurons to factors that may contribute to cognitive impairment.

4. Experimental procedures

4.1. *Generation and screening of GIN-reeler mice*

All mouse colonies are maintained in a fully AAALAC accredited facility at Baylor College of Medicine and treated according to an approved animal protocol. *Reeler* mice were obtained from The Jackson Laboratories (Bar Harbor, ME) and genotyped as previously described (Niu et al., 2004). GIN transgenic mice were produced and genotyped as previously described (Oliva et al., 2000) and are commercially available from The Jackson Laboratories.

2.2. *Tissue preparation*

Postnatal mice were perfused transcardially under anesthesia with 0.1 M Phosphate Buffer Saline (PBS) containing 4% paraformaldehyde. Cryoprotected brains were sectioned sagittally (50 μm) and collected as floating sections, or sectioned (20 μm) sagitally with a cryostat (Microm HM505E, Microm International; Walldorf, Germany) and placed on Superfrost Plus slides (Fisher, Pittsburgh, PA). Comparable mid-sagittal sections were selected from normal and mutant brains at the level of the fimbria. At least 3 mice per each genotype were examined.

4.3. *Immunohistochemistry and immunofluorescence*

Diaminobenzadine (DAB)-based immunohistochemistry was performed on 50 μm free-floating sections using Vectastain ABC reagent (Vector Laboratories, Burlingame, CA). Anti-GFP antibodies (G6539; Sigma, St. Louis, MO) were used at the 1:200 dilution. Sections were incubated overnight at 4 °C, then incubated with HRP-conjugated goat anti-mouse IgG antibodies (Jackson Immunoresearch Laboratories, West Grove, PA) for 1.5 h at room temperature. The slides were developed using a DAB Substrate Kit for Peroxidase (Vector Laboratories, Burlingame, CA). Positive neurons were visualized using an Olympus BH2 microscope.

For immunofluorescence, 20 μm slide sections or 40 μm floating sections were incubated with primary antibodies for 24–48 h at 4 °C, and then incubated with secondary antibody for 2 h at room temperature. Sections were coverslipped with Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Primary antibodies were: mouse monoclonal anti-GFP diluted 1:1000, rabbit polyclonal anti-Dlx1, diluted 1:10, or rabbit anti-Dlx2, diluted 1:500 (Eisenstat et al., 1999), mouse monoclonal anti-Reelin CR-50 (Ogawa et al., 1995) used at the 1:500 dilution, polyclonal anti-somatostatin (A566; Dako, Carpinteria, CA; 1:300 dilution), polyclonal anti-calretinin (#24445; ImmunoStar, Hudson, WI; 1:1000 dilution), and monoclonal anti-calbindin (C9848; Sigma, St. Louis, MO; 1:500 dilution). Cy3-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) and Alexa Fluor secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used at a 1:500 dilution. Fluorescent cells were observed on a Nikon TE200 inverted microscope, and images were captured using a Coolsnap Fx camera (RS Photometrics, Tucson, AZ) and

Metaview imaging software (Universal Imaging, West Chester, PA).

4.4. *Neurite analysis*

GINs labeled by anti-GFP immunohistochemistry in 50 μm floating sections from 2 mice per each genotype were traced using an Olympus-BH2 microscope equipped with a 100× lens connected to the Neurolucida 2000 4.03 imaging software (MicroBrightfield, Colchester, VT). At least 20 apparently intact neurons from the upper layers of the cortex, and 10 neurons from the bottom layers of *reeler* brains were selected from the middle of multiple sections based on the quality of the staining. The most intensely labeled neurons were selected for each genotype. All visible cellular processes arising from individual neurons were traced. Quantitative analysis of process length and number of branches was performed using the NeuroExplorer v. 3.0 (MicroBrightfield, Colchester, VT). Statistical analysis was conducted using the Student's *t* test.

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REFERENCES

Alcantara, S., Ruiz, M., D'Arcangelo, G., Ezan, F., de Lecea, L., Curran, T., Sotelo, C., Soriano, E., 1998. Regional and cellular patterns of *reelin* mRNA expression in the forebrain of the developing and adult mouse. J. Neurosci. 18, 7779–7799.

Anderson, S.A., Eisenstat, D.D., Shi, L., Rubenstein, J.L.R., 1997. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science 278, 474–476.

Anderson, S., Mione, M., Yun, K., Rubenstein, J.L.R., 1999. Differential origins of neocortical projection and local circuit neurons: role of Dlx genes in neocortical interneuronogenesis. Cereb. Cortex 9, 646–654.

Angevine, J.G., Sidman, R.L., 1961. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. Nature 192, 766–768.

Beffert, U., Weeber, E.J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J.D., Li, W.-P., Adelmann, G., Frotscher, M., Hammer, R.E., Herz, J., 2005. Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor ApoER2. Neuron 47, 567–579.

Borrell, V., del Rio, J.A., Alcantara, S., Derer, M., Martinez, A., D'Arcangelo, G., Nakajima, K., Mikoshiba, K., Derer, P., Curran, T., Soriano, E., 1999. Reelin regulates the development and synaptogenesis of the layer-specific entorhino-hippocampal connections. J. Neurosci. 19, 1345–1358.

Corbin, J.G., Nery, S., Fishell, G., 2001. Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. Nat. Neurosci. 4, 1177–1182 (Suppl.).

Costa, E., Chen, Y., Davis, J., Dong, E., Noh, J.S., Tremolizzo, L., Veldic, M., Grayson, D.R., Guidotti, A., 2002. REELIN and schizophrenia: a disease at the interface of the genome and the epigenome. Mol. Interv. 2, 47–57.

D'Arcangelo, G. 2005. The reeler mouse: anatomy of a mutant. In: Dhossche, D., Minor, C. (Eds.), GABA in Autism and Related Disorders. International Review of Neurobiology, vol. 17, Elsevier Press, USA, pp. 383, 417.

D'Arcangelo, G., Miao, G.G., Chen, S.C., Soares, H.D., Morgan, J.I., Curran, T., 1995. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature 374, 719–723.

D'Arcangelo, G., Miao, G.G., Curran, T., 1996. Detection of the *reelin* breakpoint in reeler mice. Mol. Brain Res. 39, 234–236.

Del Rio, J.A., Heimrich, B., Borrell, V., Forster, E., Drakew, A., Alcantara, S., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., Derer, P., Frotscher, M., Soriano, E., 1997. A role for Cajal–Retzius cells and reelin in the development of hippocampal connections. Nature 385, 70–74.

Eisenstat, D.D., Liu, J.K., Mione, M., Zhong, W., Yu, G., Anderson, S.A., Ghattas, I., Puelles, L., Rubenstein, J.L.R., 1999. DLX-1, DLX-2, and DLX-5 expression define distinct stages of basal forebrain differentiation. J. Comp. Neurol. 414, 217–237.

Fairen, A., Cobas, A., Fonseca, M., 1986. Times of generation of glutamic acid decarboxylase immunoreactive neurons in mouse somatosensory cortex. J. Comp. Neurol. 251, 67–83.

Flames, N., Marin, O., 2005. Developmental mechanisms underlying the generation of cortical interneuron diversity. Neuron 46, 377–381.

Flames, N., Long, J.E., Garratt, A.N., Fischer, T.M., Gassmann, M., Birchmeier, C., Lai, C., Rubenstein, J.L., Marin, O., 2004. Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. Neuron 44, 251–261.

Guidotti, A., Auta, J., Davis, J.M., DiGiorgi Gerevini, V., Dwivedi, Y., Grayson, D.R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., Costa, E., 2000. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch. Gen. Psychiatry 57, 1061–1069.

Hevner, R.F., Daza, R.A., Englund, C., Kohtz, J., Fink, A., 2004. Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. Neuroscience 124, 605–618.

Lambert de Rouvroit, C., Goffinet, A.M., 1998. The reeler mouse as a model of brain development. Adv. Anat. Embryol. Cell Biol. 150, 1–108.

Lavdas, A.A., Grigoriou, M., Pachnis, V., Parnavelas, J.G., 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J. Neurosci. 99, 7881–7888.

Liu, W.S., Pesold, C., Rodriguez, M.A., Carboni, G., Auta, J., Lacor, P., Larson, J., Condie, B.G., Guidotti, A., Costa, E., 2001. Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse. Proc. Natl. Acad. Sci. U. S. A. 98, 3477–3482.

Marin, O., Rubenstein, J.L.R., 2001. A long, remarkable journey: tangential migration in the telencephalon. Nat. Rev. 2, 1–11.

Marin, O., Yaron, A., Bagri, A., Tessier-Lavigne, M., Rubenstein, J.L.R., 2001. Sorting of striatal and cortical interneurons regulated by semaphorin–neuropilin interactions. Science 293, 872–875.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., Wu, C., 2004. Interneurons of the neocortical inhibitory system. Nat. Rev., Neurosci. 5, 793–807.

McEvilly, R.J., de Diaz, M.O., Schonemann, M.D., Hooshmand, F., Rosenfeld, M.G., 2002. Transcriptional regulation of cortical neuron migration by POU domain factors. Science 295, 1528–1532.

Meyer, G., Goffinet, A.M., 1998. Prenatal development of Reelin-immunoreactive neurons in the human neocortex. J. Comp. Neurol. 397, 29–40.

Niu, S., Renfro, A., Quattrocchi, C.C., Sheldon, M., D'Arcangelo, G.,

2004. Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway. Neuron 41, 71–84.

Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H., Mikoshiba, K., 1995. The reeler gene-associated antigen on Cajal–Retzius neurons is a crucial molecule for laminar organization of cortical neurons. Neuron 14, 899–912.

Oliva, A.A., Jiang, M., Lam, T., Smith, K.L., Swann, J.W., 2000. Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. J. Neurosci. 20, 3354–3368.

Pesold, C., Impagnatiello, F., Pisu, M.G., Uzunov, D.P., Costa, E., Guidotti, A., Caruncho, H.J., 1998. Reelin is preferentially expressed in neurons synthesizing g-aminobutyric acid in cortex and hippocampus of adult rats. Proc. Natl. Acad. Sci. U. S. A. 95, 3221–3226.

Pesold, C., Liu, W.S., Guidotti, A., Costa, E., Caruncho, H.J., 1999. Cortical bitufted, horizontal, and Martinotti cells preferentially express and secrete reelin into perineuronal nets, nonsynaptically modulating gene expression. Proc. Natl. Acad. Sci. U. S. A. 96, 3217–3222.

Rice, D.S., Curran, T., 2001. Role of the Reelin signaling pathway in central nervous system development. Ann. Rev. Neurosci. 24, 1005–1039.

Rice, D.S., Sheldon, M., D'Arcangelo, G., Nakajima, K., Goldowitz, D., Curran, T., 1998. *Disabled*-1 acts downstream of *Reelin* in a signaling pathway that controls laminar organization in the mammalian brain. Development 125, 3719–3729.

Rodriguez, M.A., Pesold, C., Liu, W.S., Khrino, V., Guidotti, A., Pappas, G.D., Costa, E., 2000. Colocalization of integrin receptors and reelin in dendritic spine postsynaptic densities of adult nonhuman primate cortex. Proc. Natl. Acad. Sci. U. S. A. 97, 3550–3555.

Rodriguez, M.A., Caruncho, H.J., Costa, E., Pesold, C., Liu, W.S., Guidotti, A., 2002. Patas monkey, glutamic acid decarboxylase-67 and reelin mRNA coexpression varies in a manner dependent on layers and cortical areas. J. Comp. Neurol. 451, 279–288.

Weeber, E.J., Beffert, U., Jones, C., Christian, J.M., Forster, E., Sweatt, J.D., Herz, J., 2002. Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. J. Biol. Chem. 277, 39944–39952.

Wichterle, H., Turnbull, D.H., Nery, S., Fishell, G., Alvarez-Buylla, A., 2001. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development 128, 3759–3771.

Zecevic, N., Rakic, P., 2001. Development of layer I neurons in the primate cerebral cortex. J. Neurosci. 21, 5607–5619.