THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Lamination of the cerebral cortex is disturbed in Gli3 mutant mice

Citation for published version:
Friedrichs, M, Larralde, O, Skutella, T & Theil, T 2008, 'Lamination of the cerebral cortex is disturbed in Gli3 mutant mice' Developmental Biology, vol. 318, no. 1, pp. 203-214. DOI: 10.1016/j.ydbio.2008.03.032

Digital Object Identifier (DOI):
10.1016/j.ydbio.2008.03.032

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Developmental Biology

Publisher Rights Statement:
Available under Open Access

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 05. Apr. 2019

Developmental Biology 318 (2008) 203–214

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Lamination of the cerebral cortex is disturbed in *Gli3* mutant mice

Melanie Friedrichs [a], Osmany Larralde [d], Thomas Skutella [c], Thomas Theil [a,b,c,d,*]

[a] Institute for Animal Developmental and Molecular Biology, Heinrich-Heine-University, D-40225 Düsseldorf, Germany
[b] Institute for Genetics, Heinrich-Heine-University, D-40225 Düsseldorf, Germany
[c] Centre for Regenerative Medicine, Anatomical Institute, Section Tissue Engineering, Eberhard-Karls-Universität Tübingen, Österbergstr. 3, D-72074 Tübingen, Germany
[d] Centres for Neuroscience Research and Integrative Physiology, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, UK

ARTICLE INFO

Article history:
Received for publication 26 July 2007
Revised 22 February 2008
Accepted 19 March 2008
Available online 28 March 2008

Keywords:
Gli3
Reelin
Cerebral cortex
Lamination

ABSTRACT

The layered organization of the cerebral cortex develops in an inside-out pattern, a process which is controlled by the secreted protein reelin. Here we report on cortical lamination in the *Gli3* hypomorphic mouse mutant Xt^J/*Pdn* which lacks the cortical hem, a major source of reelin$^+$ Cajal Retzius cells in the cerebral cortex. Unlike other previously described mouse mutants with hem defects, cortical lamination is disturbed in Xt^J/*Pdn* animals. Surprisingly, these layering defects occur in the presence of reelin$^+$ cells which are probably derived from an expanded Dbx1$^+$ progenitor pool in the mutant. However, while these reelin$^+$ neurons and also Calretinin$^+$ cells are initially evenly distributed over the cortical surface they form clusters later during development suggesting a novel role for *Gli3* in maintaining the proper arrangement of these cells in the marginal zone. Moreover, the radial glial network is disturbed in the regions of these clusters. In addition, the differentiation of subplate cells is affected which serve as a framework for developing a properly laminated cortex.

© 2008 Elsevier Inc. All rights reserved.

Introduction

The cerebral cortex as the main centre for all higher cognitive functions develops a layered structure which is essential for its function. This lamination develops in an inside-out fashion and requires the secreted glycoprotein reelin (Tissir and Goffinet, 2003). Lack of functional *reelin*, as in the *reeler* mouse mutant (Curran and D'Arcangelo, 1998; D'Arcangelo et al., 1995; Ogawa et al., 1995) and in human congenital lissencephaly patients (Hong et al., 2000) or interference with reelin signalling (Howell et al., 1997; Sheldon et al., 1997; Trommsdorff et al., 1999) result in inversion of cortical layers and in abnormally dispersed cells.

In contrast to the well understood role of reelin signalling, relatively little is known about the identity of the reelin$^+$ cell population directing cortical lamination. *Reelin* is expressed at high levels in CR cells, a major cell population in the MZ (D'Arcangelo et al., 1995; Meyer and Wahle, 1999; Ogawa et al., 1995). However, only indirect evidence supports a role for CR cells in controlling cortical layering (Ringstedt et al., 1998; Super et al., 2000). The understanding of such a role is further complicated by the existence of several reelin$^+$ cell populations with different sites of origins, migration routes, destination and molecular profiles. Some CR cells are generated from Dbx1$^+$ progenitor cells in the septum and in the ventral pallium at the pallial/subpallial boundary (PSB) which express *reelin* but not *p73* and preferentially populate the rostroventral and ventral cortex (Bielle et al., 2005). Consistently, ablation of *Dbx1* progenitor cells predominantly leads to cytoarchitectural defects in the lateral cortex (Bielle et al., 2005). In addition, the caudomedial wall of the telencephalon, including the cortical hem, is a major source of a CR cell population which is characterized by the expression of reelin, p73, Calretinin and glutamate (Meyer et al., 2002; Takiguchi-Hayashi et al., 2004). The role of these CR cells in cortical layering has recently been tested by genetic inactivation of *p73* and by ablation of the hem (Meyer et al., 2004; Yoshida et al., 2006). Surprisingly, both mutants show normal cortical lamination except for the caudal cortex, suggesting that hem derived CR cells are not required to control cortical layering and that other sources of reelin, particularly cortical plate (CP) interneurons, are sufficient to allow lamination to proceed (Yoshida et al., 2006).

The *Gli3* mouse mutant extra-toes (Xt^J) represents another mouse mutant with defective cortical hem development. This mutant shows severe defects in patterning the dorsal telencephalon (Fotaki et al., 2006; Grove et al., 1998; Kuschel et al., 2003; Theil et al., 1999; Tole et al., 2000a) and in preplate differentiation (Theil, 2005). In particular early cortical layering, i.e. the formation of the subplate (SP) and the marginal zone (MZ) is severely disrupted in these animals making it difficult to study cortical lamination. To circumvent these difficulties, we made use of the compound *Gli3* hypomorphic mouse mutant Xt^J/*Pdn* which shows much milder regionalization defects. This mutant lacks the cortical hem and consequently contains few cortical reelin$^+$/p73$^+$ CR cells but has

* Corresponding author. Centres for Neuroscience Research and Integrative Physiology, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, UK. Fax: +44 131 6506527.
E-mail address: thomas.theil@ed.ac.uk (T. Theil).

0012-1606/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.03.032

numerous reelin$^+$/p73$^-$ CR cells probably arising from an expanded Dbx1$^+$ progenitor pool. Despite the presence of these reelin$^+$ cells, however, cortical lamination is disturbed in the mutant. These reelin$^+$ cells form dense cell clusters and the radial glial scaffold is severely disturbed at the sites of these clusters. Our analysis therefore suggests a role for *Gli3* in regulating cortical lamination by maintaining an even distribution of CR neurons over the cortical surface.

Materials and methods

Mice

Xt^J and *Pdn* heterozygous animals were kept on a mixed C57Bl6/C3H and C3H/He background, respectively, and were interbred. Embryonic (E) day 0.5 was assumed to start at midday of the day of vaginal plug discovery. *Xt*^{J}/*Pdn* embryos were readily distinguished from heterozygous and wild-type embryos by forebrain and/or limb morphology (Kuschel et al., 2003). For each marker and each stage, 3–5 different, non-exencephalic embryos were analysed at rostral, medial and caudal levels of the developing cortex.

Explant culture of telencephalic tissue

The dorsal telencephalon or the neocortex of E10.5 wildtype and *Gli3* mutant embryos was dissected in HBSS and the surface ectoderm removed manually. Explants were cultured on Millicell-CM culture plate inserts (Millipore, #PICMORG50) in organ culture dishes. Culture medium was Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 20% fetal calf serum, 1× non-essential amino acids (GIBCO), 1 mM sodium pyruvate (GIBCO) and 1× streptomycin/penicillin (GIBCO). Tissue pieces were maintained under in vitro conditions for 48 h and then processed for in situ hybridization.

In situ hybridization and immunohistochemistry

Antisense RNA probes for *Bmp4* (Jones et al., 1991), *Conductin* (Lustig et al., 2002), *Cux2* (Zimmer et al., 2004), *Dbx1* (Yun et al., 2001), *Dlx2* (Bulfone et al., 1993), *ER81*

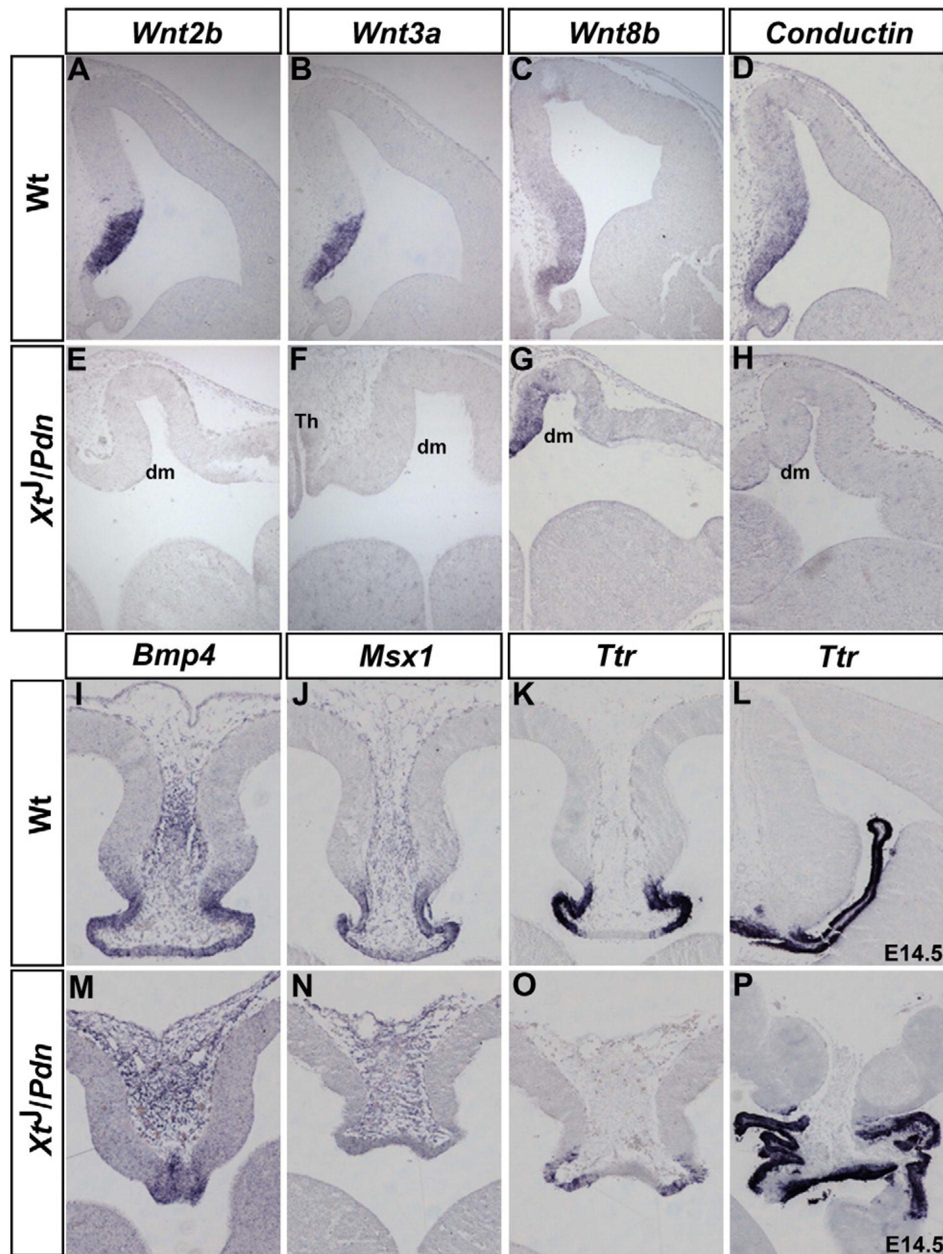

Fig. 1. Dorsal midline defects in *Xt*^{J}/*Pdn* embryos. In situ hybridization analysis on coronal sections through the brains of wild-type (A–D, I–L), and *Xt*^{J}/*Pdn* (E–H, M–P) embryos. (A–C, E–G) Cortical hem expression of *Wnt2b*, *Wnt3a* is absent in *Xt*^{J}/*Pdn* embryos while *Wnt8b* expression remains in the highly abnormal dorsomedial region. (D, H) *Xt*^{J}/*Pdn* mutants lack *Conductin* expression in the dorsomedial telencephalon. (I, J, M, N) *Bmp4* expression and signalling as indicated by *Msx1* expression is reduced in the dorsal telencephalon. (K, L, O, P) Delay of choroid plexus development in *Xt*^{J}/*Pdn* embryos. While only few cells express *Ttr* at reduced levels at E12.5, *Ttr* expression has recovered at E14.5. Note the disorganized structure of the choroid plexus. dm: dorsomedial telencephalon, Th: thalamus.

(Lin et al., 1998), *Msx1* (Hill et al., 1989), *Ngn2* (Gradwohl et al., 1996), *p73* (XM 131858; GenBank), *reelin* (D'Arcangelo et al., 1995), *RORβ* (Hevner et al., 2003a), *Sfrp2* (Kim et al., 2001), *Shh* (Echelard et al., 1993), *Sox5* (Lefebvre et al., 1998), *Ttr* (Duan et al., 1989), *Wnt2b* (Grove et al., 1998), *Wnt3a* (Roelink and Nusse, 1991) and *Wnt8b* (Richardson et al., 1999) were DIG labelled. In situ hybridization on tissue explants and on 12 μm serial paraffin sections of mouse embryos were performed as described (Kuschel et al., 2003; Theil, 2005).

Immunohistochemical analysis was performed as described previously (Theil, 2005) using the following antibodies: BrdU (1:20, Bio-Science), Calbindin (1:2000, Swant), Calretinin (1:2000, Chemicon), Calretinin (1:1000; Swant), CS56 (1:1000, Sigma), Foxg1 (1:50, Abcam), MAP2 (1:1000; Sigma), Nestin (1:100; DSHB), RC2 (DSHB; 1:50), reelin (G10; 1:200), Tbr1 (1:2500) (Englund et al., 2005).

For birth dating analysis of cortical lamination, pregnant females received a single, intraperitonal injection of BrdU (10 mg/ml) at E11.5, E13.5 or E15.5 and embryos were collected at P0. For BrdU-immunocytochemistry, slides were incubated in 0.1 M $Na_4B_4O_7$ after denaturing with 2 N HCl. For immunhistochemistry/RNA in situ hybridization, sections were washed after RNA detection for 24 h in PBS and then processed for immunohistochemistry as described above.

Results

Regionalization defects in the telencephalon of Xt^J/Pdn embryos

Previously, we reported that the early cortical layering, i.e. the formation of the preplate and its derivatives, the MZ and the SP, is severely affected in *extra-toes* (Xt^J) embryos (Theil, 2005) in which a deletion removes all *Gli3* sequences 3′ of the second zinc finger (Büscher et al., 1998). Due to the severity of the phenotype, however, it is difficult to analyze the proper lamination process in these animals. We therefore focussed on the *Gli3* hypomorphic mouse Xt^J/*Pdn* (Kuschel et al., 2003; Schimmang et al., 1994) in which the *Gli3* transcript levels are reduced due to the integration of a retrotransposon (Thien and Rüther, 1999). Before analyzing cortical lamination we started to define the extent to which early patterning of the telencephalon is affected in this mutant. Our previous whole mount in situ hybridization analysis of E12.5 forebrain development in these mutants had indicated the absence of *Wnt* gene expression in the dorsal telencephalic midline of these animals (Kuschel et al., 2003) which we could also confirm at E10.5 (data not shown). To analyze dorsal midline and in particular hem development more accurately, we performed a *Wnt* expression analysis on sectioned telencephalic tissue. While the expression of *Wnt3a* and *Wnt2b* was readily detectable in the wild-type cortical hem, the expression of these hem markers was absent from the Xt^J/*Pdn* mutant cortex (Figs. 1A, B, E, F). In contrast, *Wnt8b* expression which occurs in the hem and in the hippocampal anlage of wild-type embryos was still detected in dorsomedial structures of the E12.5 Xt^J/*Pdn* cortex (Figs. 1C, G). This residual *Wnt8b* expression, however, did not activate canonical Wnt signalling as indicated by the absence of *Conductin* expression (Figs. 1D, H).

In addition to several *Wnt* genes, the dorsal midline is also positive for several *Bmp* genes which are essential for development of the choroid plexus (Hebert et al., 2002). Interestingly, we detected weak expression of *Bmp4* in the dorsomedial telencephalon of Xt^J/*Pdn* embryos (Figs. 1I, M). We also observed weak expression of *Msx1* suggesting that Bmp signalling is weakly activated in the mutant (Figs. 1J, N). Consistent with this, *Ttr* a choroid plexus marker (Duan et al., 1989) showed a patchy and considerably weaker expression in the dorsal midline region of the E12.5 Xt^J/*Pdn* mutant (Figs. 1K, O). At E14.5, however, *Ttr* was strongly expressed in a highly dysmorphic choroid plexus (Figs. 1L, P). This analysis therefore suggests the absence of the cortical hem but residual Bmp signalling from the Xt^J/*Pdn* telencephalic roof coinciding with an abnormal development of the choroid plexus.

In addition to dorsal midline defects, the formation of the boundary between dorsal telencephalon and diencephalon is affected in Xt^J/Xt^J mutant (Theil et al., 1999) resulting in the juxtaposition of the neocortex and the eminentia thalamica and to a mixing of cells derived from both tissues (Fotaki et al., 2006). We therefore analyzed whether Xt^J/*Pdn* embryos show a similar defect. A morphological inspection of the telencephalon in E12.5, E14.5 and E18.5 embryos indicated a dysmorphic dorsomedial telencephalon but suggested a separation of dorsal telencephalon and diencephalon. To further analyze this we used various markers characteristic for specific subdivisions of the forebrain. In wildtype embryos, Foxg1 is expressed

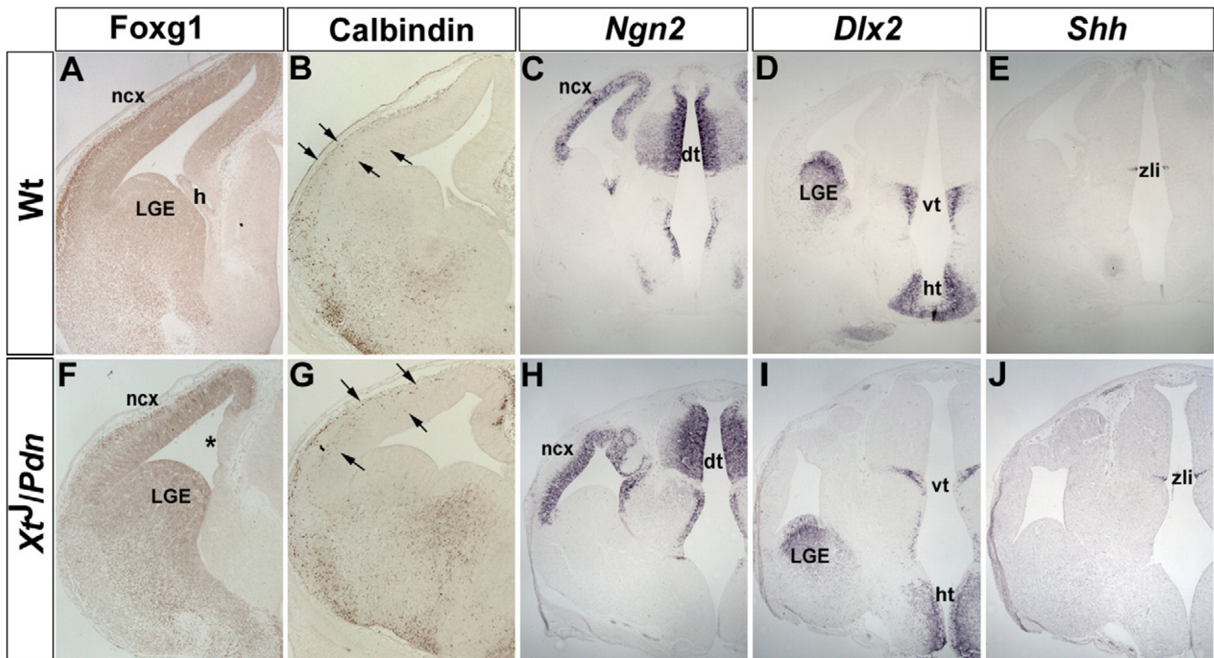

Fig. 2. Dorsal telencephalon and diencephalon are morphologically separated in Xt^J/*Pdn* embryos. Immunohistochemical and in situ hybridization analysis on E12.5 wildtype (A–E) and Xt^J/*Pdn* (F–J) using the indicated markers. (A, F) Foxg1 is expressed in the telencephalon except for the cortical hem (h) in wildtype and the highly abnormal dorsomedial region of the Xt^J/*Pdn* mutant embryo. (B, G) Calbindin stains interneurons originating in the LGE and migrating into the neocortex in both genotypes. *Ngn2* (C, H) and *Dlx2* (D, I) staining reveals the dorso/ventral subdivisions of the telencephalon and diencephalon. (E, J) *Shh* expression marks the zona limitans intrathalamica (zli).

by all telencephalic cells except for the cortical hem and for CR neurons (Hanashima et al., 2002; Tao and Lai, 1992). This expression pattern is maintained in the mutant where only the dorsomedial region is negative for Foxg1 (Figs. 2A, F). In contrast to *Xt*^J/*Xt*^J embryos, we did not notice the presence of Foxg1$^-$ cells within the *Xt*^{J}/*Pdn* neocortex. Similarly, Calbindin immunostaining did not reveal cell clusters in the neocortex (Figs. 2B, G) as described for *Xt*^{J}/*Xt*^{J} embryos (Fotaki et al., 2006). Finally, in situ hybridization for *Ngn2*, *Dlx2* and *Shh* indicated the presence of the major subdivisions of the *Xt*^{J}/*Pdn* telencephalon and diencephalon (Figs. 2C–E, H–J). These data suggest that the development of the dorsomedial telencephalon is severely affected in both, *Xt*^{J}/*Xt*^{J} and *Xt*^{J}/*Pdn* embryos, but that the latter show a morphological and molecular separation of telencephalon and diencephalon.

The Xt^J*/Pdn cortex shows a severe reduction in hem derived CR cells*

Our previous analysis showed that preplate development and differentiation are severely affected in *Xt*^{J}/*Xt*^{J} embryos (Theil, 2005). We therefore analyzed this process in *Xt*^{J}/*Pdn* embryos. The transcription factor Tbr1 is expressed in the preplate and is essential for its differentiation (Hevner et al., 2001). Immunohistochemical analysis revealed a continuous band of Tbr1$^+$ cells at the outer part of the developing cortex in E12.5 and E14.5 wild-type and in *Xt*^{J}/*Pdn* embryos although the mutant preplate appeared undulated dorsomedially (Figs. 3A, F, K, P). Similarly, the expression of MAP2 which labels preplate neurons and the SP/MZ at E12.5 and E14.5, respectively, was unaffected in the E12.5 *Xt*^{J}/*Pdn* cortex but the mutant SP and MZ appeared more diffuse at E14.5. In addition, several MAP2$^+$ cell clusters were detected in the medial neocortex (Figs. 3B, G, L, Q).

We next used in situ hybridization analysis to gain insights into the cellular composition of the preplate and its derivatives. Hem derived CR cells express *reelin*, *p73* and Calretinin (Alcantara et al., 1998; del Rio et al., 1995; Hevner et al., 2003b; Meyer et al., 2004, 1999, 2002; Ogawa et al., 1995; Soda et al., 2003). This analysis showed a single layer of *reelin* expressing cells at the entire wild-type E12.5 and E14.5 cortical surface (Figs. 3C, M). While in E12.5 *Xt*^{J}/*Pdn* embryos the layer of reelin expressing cells appeared relatively normal (Fig. 3H), *reelin* expressing cells formed small aggregates in the dorsomedial E14.5 mutant neocortex (Fig. 3R) which are reminiscent of the large clusters of *reelin* expressing cells in the *Xt*^{J}/*Xt*^{J} cortex (Theil, 2005). In addition, *reelin* expression was detected at low levels in two rows of cells within the wild-type E14.5 neocortex which correspond to migrating interneurons and which have recently been suggested to play an important role in cortical lamination (Alcantara et al., 2006; Yoshida et al., 2006). Distinct rows of *reelin* expressing neurons were only detected in the mutant lateral neocortex while more medial regions showed an

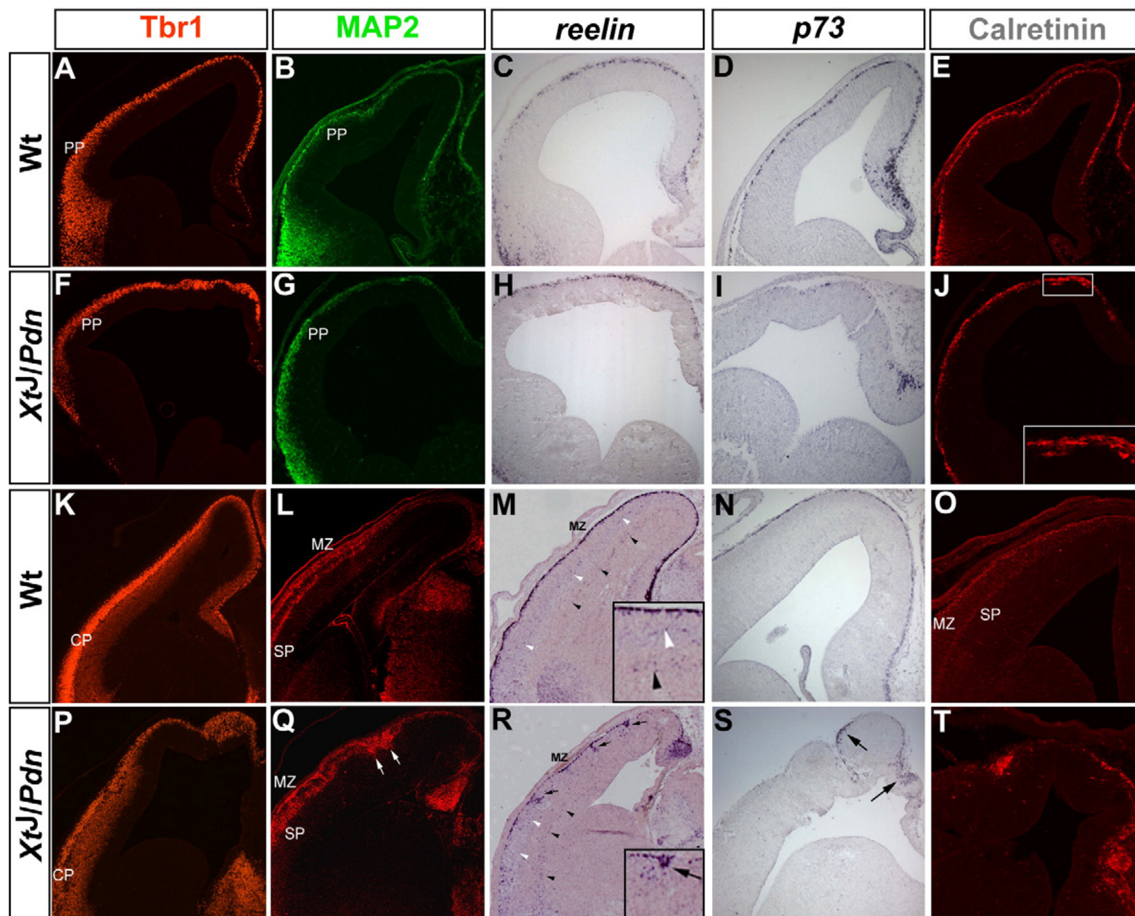

Fig. 3. Development of the preplate and its differentiation in *Xt*^{J}/*Pdn* embryos. Immunohistochemical and in situ hybridization analysis of wild-type (A–E, K–O) and *Xt*^{J}/*Pdn* embryos (F–J, P–T) on coronal sections through E12.5 (A–J) and E14.5 (K–T) forebrains. (A, F) High Tbr1 expression levels mark preplate neurons and the cortical plate in wild-type embryos. (F, P) While the Tbr1 expression domain at E12.5 appears thinner dorsomedially, E14.5 *Xt*^{J}/*Pdn* embryos lack Tbr1 expression in dorsomedial regions. (B, L) Anti-MAP2 immunostaining characterizing preplate neurons (B) and the MZ and SP at E14.5 (L). MAP2 expression is unaffected in E12.5 *Xt*^{J}/*Pdn* embryos but appears disorganized at E14.5. (C, D, M, N) *reelin* and *p73* expression in a single row of cells in the wild-type telencephalon. (H, R) *reelin* expressing cells are less densely packed and form small clusters (arrows) in the E14.5 mutant cortex. (I, S) Nearly complete lack of *p73* expression in the neocortex of *Xt*^{J}/*Pdn* embryos. (E) Calretinin$^+$ cells form a single, continuous row of cells on the E12.5 basal cortical surface. (J) Calretinin$^+$ cells in the mutant cortex. The inset shows the formation of a multilayered structure. (O) Calretinin labels the MZ and SP. (T) Calretinin$^+$ cells cluster in the mutant.

uneven distribution of *reelin* in the CP. In contrast to the less severely affected *reelin* expression, *p73* expression was drastically altered in *Xt*^J^/*Pdn* embryos. Like reelin$^+$ cells, *p73* expressing cells covered the cortical surface of wild-type embryos (Figs. 3D, N) whereas E12.5 Xt^J/*Pdn* embryos had only a few *p73* expressing cells in the neocortical MZ and a cluster of cells in the dorsomedial telencephalon. A similar distribution of *p73* expressing cells was observed in E14.5 Xt^J/*Pdn* embryos (Fig. 3S). Taken together with the altered *reelin* expression this finding suggests a severe reduction in the hem derived reelin$^+$/p73$^+$ CR subpopulation and a concomitant increase in a reelin$^+$/p73$^-$ CR cell population similar to our previous observations in Xt^J/Xt^J embryos (Theil, 2005). Finally, immunohistochemical analysis of Calretinin expression, a marker for CR and pioneer neurons, showed few abnormalities in E12.5 Xt^J/*Pdn* embryos. As in wild-type embryos, Calretinin$^+$ neurons formed a single layer except for the dorsomedial telencephalon where several layers were detected (Figs. 3E, J). In contrast, at E14.5, Calretinin$^+$ neurons formed dense clusters dorsomedially but were absent in the lateral MZ similar to *p73* expression (Figs. 3O, T). This analysis suggests that the formation of the preplate is not affected in Xt^J/*Pdn* embryos, but that the MZ shows a marked reduction of hem derived reelin$^+$/p73$^+$/Calretinin$^+$ CR neurons and a clustering of reelin$^+$ and Calretinin$^+$ cells.

Interestingly, similar alterations in the cellular composition of the MZ were observed in newborn mutants. In wild-type newborn animals, strong *reelin* expression was detected in Cajal Retzius cells within the MZ (Fig. 4A). An additional weak expression was detected in the lower cortical plate at the level of layer IV/V (Yoshida et al., 2006). In Xt^J/*Pdn* animals we could identify fewer *reelin* expressing cells in the MZ (Fig. 4B). Also, a band of *reelin* expressing cells was observed in the lateral CP but not in the medial neocortex where these cells showed a strong dispersal (Fig. 4B). In contrast to the numerous *reelin* expressing cells in the MZ, few *p73*$^+$ cells were found in the Xt^J/*Pdn* neocortical MZ while groups of *p73* expressing cells were found in the highly dysmorphic dorsomedial cortex (Figs. 4C, D). Finally, Calretinin$^+$ cells were detected in the wildtype MZ and SP while Calretinin expression was absent from the mutant MZ and SP (Figs. 4E, F). The ectopic Calretinin expression in the lateral cortical MZ corresponds to an ectopic nerve bundle (T.T., unpublished data). Taken together these data indicate that hem derived CR cells (p73$^+$; reelin$^+$; Calretinin$^+$) are nearly absent while reelin$^+$/p73$^-$ cells are found in the medial and lateral cortex.

Neocortical lamination in Xt^J/Pdn embryos

Given these alterations in the MZ and its importance for the cortical lamination process (Super et al., 1998) we used immunohistochemistry and in situ hybridization analysis to characterize the developing neocortical layers in Xt^J/*Pdn* animals. As these pups die shortly after birth this analysis was confined to the P0 stage. The transcription factors Cux2, RORβ, and ER81 distinguish emerging layers II/III, IV and V, respectively (Figs. 5A, C, E). In the Xt^J/*Pdn* mutants, we could identify regions in the medial (Cux2) or lateral neocortex (RORβ, and ER81) which show a layered expression of these markers, though cells expressing these genes are more dispersed within these domains. In addition, we also identified regions where the cortical layering is severely disturbed. In these areas, the *Cux2*, *RORβ* and *ER81* expression domains are undulated and discontinuous. Cells expressing these genes were even found close to the ventricular surface (Figs. 5B, D, F). Finally, the Tbr1 transcription factor shows high level expression in the SP, layer VI and CR neurons and weaker expression levels in layer II/III neurons

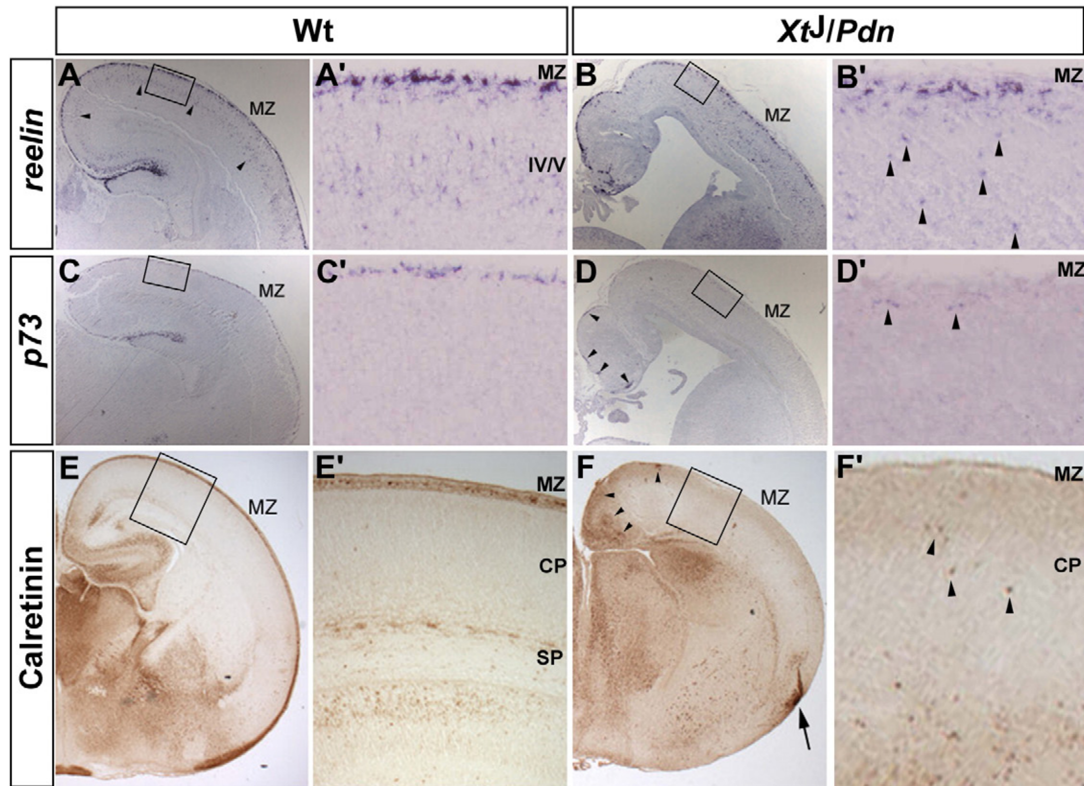

Fig. 4. Development of the MZ and subplate is affected in Xt^J/*Pdn* embryos. Coronal sections through the brains of newborn wild-type (A, C, E) and Xt^J/*Pdn* mutant animals (B, D, F). (A′, B′ C′, D′, E and F′) Higher magnifications of the boxed areas in A, B, C, D, E and F. (A, B) Fewer *reelin* expressing cells in the MZ are found in the Xt^J/*Pdn* brains. *Reelin*$^+$ cells are not so densely packed as in wild-type embryos. Also note the more dispersed distribution of weakly *reelin* expressing cells in layer IV/V (arrowheads). (C, D) *p73* expression is strongly reduced in the mutant MZ except for the dorsomedial most area (arrowheads). (E, F) The Xt^J/*Pdn* neocortex lacks Calretinin staining in the MZ. The arrow in F marks an ectopic cluster of Calretinin positive fibers.

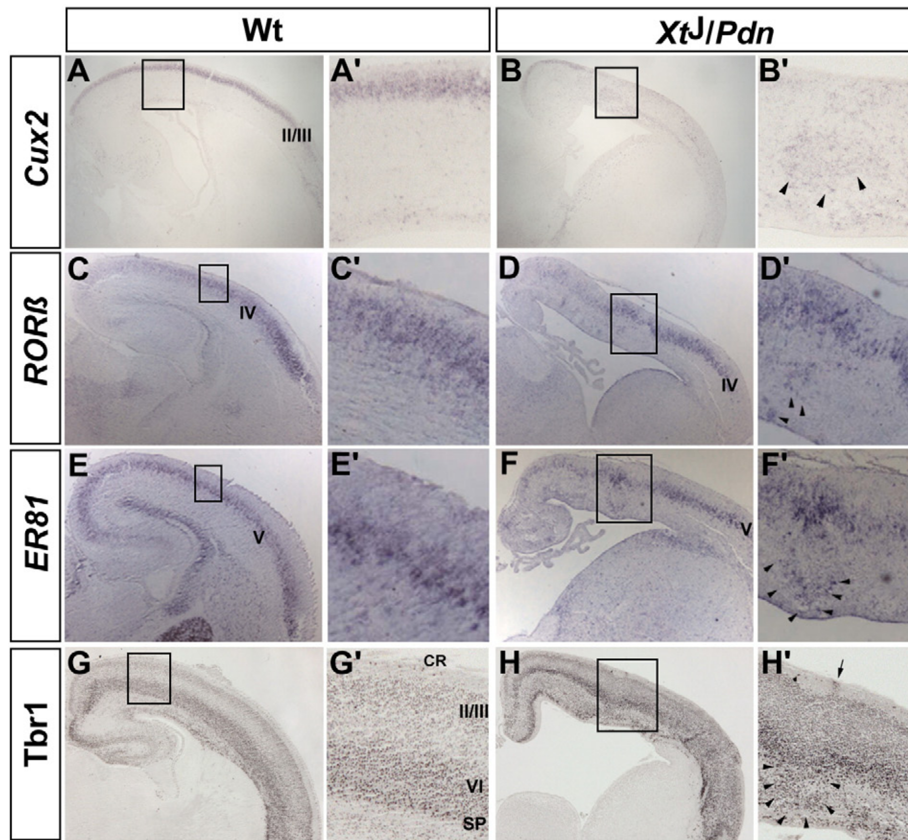

Fig. 5. Cortical lamination defects in *Xt*[J]/*Pdn* embryos. In situ hybridization and immunofluorescence analysis on coronal sections of newborn wild-type (A, C, E, G) and *Xt*[J]/*Pdn* animals (B, D, F, H). (A, C, E) *Cux2*, *RORβ*, and *ER81* expression mark cortical laminae II/III, IV, and V of wild-type embryos, respectively. (B, D, F) In *Xt*[J]/*Pdn* embryos, the expression of these markers is more diffuse and even occurs close to the ventricular surface (see insets B′, D′ and F′). (G, H) Tbr1 is strongly expressed in layer VI, SP and CR neurons and at weaker levels in layer II/III of wildtype brains. In the mutant, Tbr1^{+} cells are positioned close to the ventricular surface (arrowheads). Ectopic Tbr1 staining was also found in the MZ (arrow).

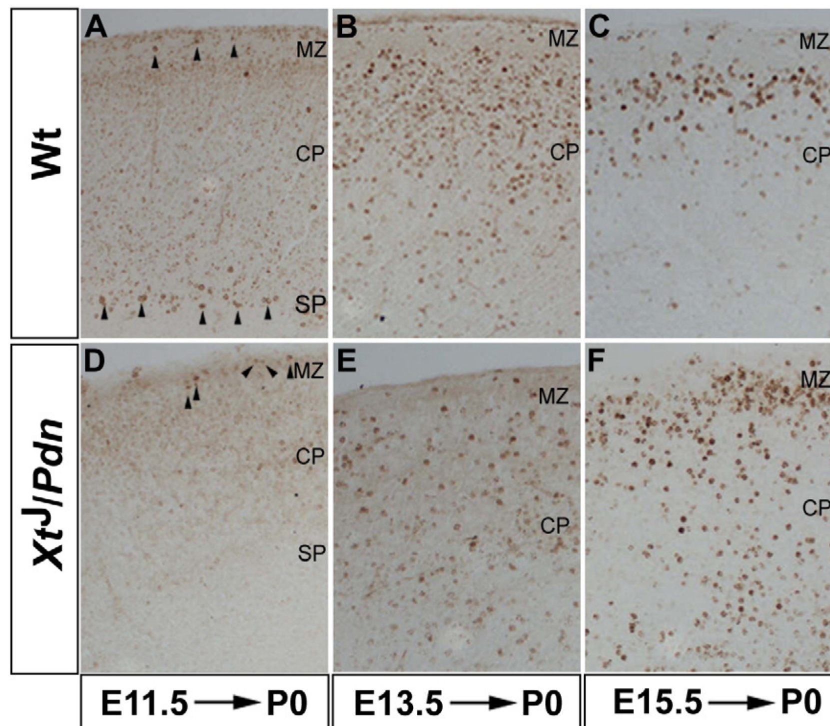

Fig. 6. BrdU birthdating analysis of cortical neurons. (A–F) Coronal sections at P0 stained with an anti-BrdU antibody after BrdU administration at E11.5 (A, D), E13.5 (B, E) and E15.5 (C, F). (A, D) In wild-type embryos, administration of BrdU at E11.5 labels MZ and SP neurons (arrowheads), whereas only few or no neurons are BrdU labelled in the MZ (arrowheads) and SP of *Xt*[J]/*Pdn* animals, respectively. (B, C; E, F) BrdU^{+} neurons are found in the lower and upper cortex of wild-type animals after labelling at E13.5 (B) and E15.5 (C), respectively. (E, F) In *Xt*[J]/*Pdn* newborns, BrdU labelled neurons occupy all cortical layers.

(Fig. 5G). Similar to the other markers, Tbr1^{+} cells were also found in an abnormal position close to the ventricular surface (Fig. 5H). In addition, we did not observe Tbr1 staining in the MZ characteristic of CR neurons. These results suggest that the layered neocortical organization is disturbed in the mutant.

To further analyze cortical lamination we performed a birthdating analysis of cortical neurons. To this end, we injected pregnant mice with BrdU at E11.5, E13.5 and E15.5 and examined the distribution of labeled neurons at P0 to determine the migration of cortical neurons. After labeling preplate neurons at E11.5, we detected BrdU^{+} cells in the MZ and the SP of wild-type pups (Fig. 6A). In contrast, only few neurons were labeled in the MZ of *Xt*[J]*/Pdn* newborns consistent with the lack of Calretinin^{+} neurons and the reduced numbers of *p73* and *reelin* expressing cells. Also, no labeling was observed in the SP (Fig. 6D). Furthermore, wild-type neurons labeled by injection at E13.5 and E15.5 predominately migrated to layers IV/V and II/III of the P0 cortex, respectively (Figs. 6B, C). Similar to our findings on lamina specific gene expression, however, BrdU^{+} neurons showed a more dispersed distribution and in some regions did not migrate to their prospective layers but settled throughout the entire CP in the newborn *Xt*[J]*/Pdn* neocortex (Figs. 6E, F). Thus, the BrdU birthdating analysis further confirms the lamination defects in the *Gli3* mutant cortex.

Clusters of reelin^{+} neurons disrupt CP organization

Next, we started to analyze causes for the cortical lamination defects in *Xt*[J]*/Pdn* embryos. As *reelin* is required for neocortical organization (D'Arcangelo et al., 1995; Ogawa et al., 1995; Rice and Curran, 2001), the altered distribution of reelin^{+} cells and in

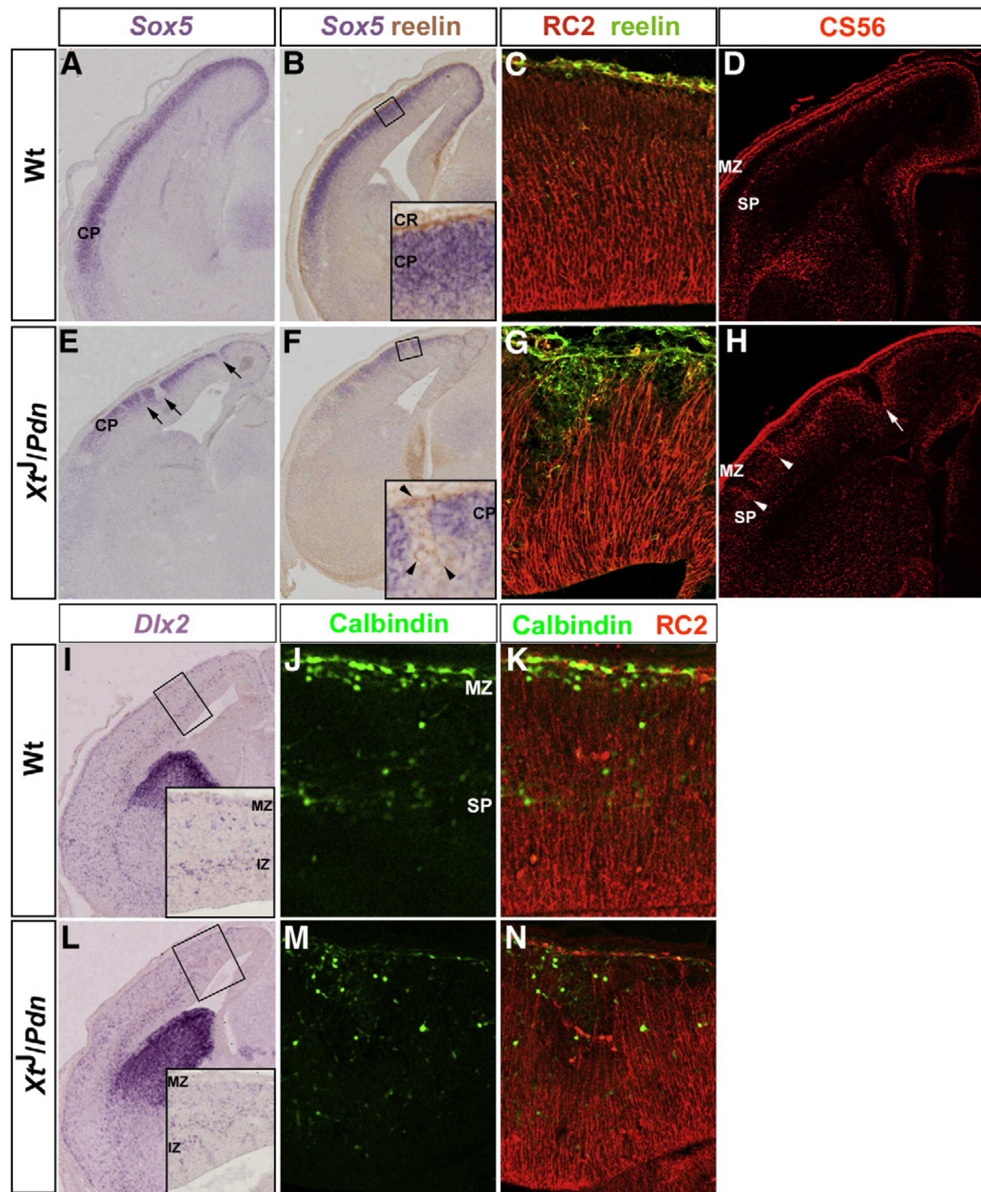

Fig. 7. Ectopic reelin[+] clusters disrupt the organization of the CP. Coronal sections through the brains of E14.5 wild-type (A–D, I–K) and *Xt*[J]*/Pdn* (E–H, L–N) embryos. (A, E) The *Sox5* expression domain exhibits gaps in the mutant CP (arrows). Also note the reduced expression levels in the medial neocortex and the complete absence of *Sox5* expression in the lateral neocortex. (B, F) In situ hybridization (*Sox5*) combined with immunohistochemical analysis (reelin) shows that clusters of reelin^{+} neurons are located within the *Sox5* negative areas. (C, G) Immunostaining for RC2 reveals a shortening of radial glial fibers at the sites of reelin[+] clusters. Between the two reelin[+] clusters, radial glial cells extend their processes to the pial surface. (D, H) CS56 staining reveals the MZ and SP. The arrow in (H) marks the SP underneath a cluster, arrowheads CS56^{+} cells in the CP. (I, J, K) *Dlx2* and Calbindin expression mark migrating interneurons in the wildtype neocortex. (L, M, N) The interneuron migratory routes appear more diffuse in *Xt*[J]*/Pdn* embryos. Note the absence of *Dlx2* or Calretinin clusters.

particular the clustering of these cells in the Xt^J/Pdn mutant may interfere with proper cortical layering. To examine this possibility we first analyzed the generation of the CP in the developing mutant cortex. *Sox5* marks CP neurons in the E14.5 medial and lateral neocortex of wild-type embryos (Fig. 7A). While *Sox5* expression is nearly completely abolished in the lateral neocortex of Xt^J/Pdn embryos the Sox5 expression domain shows several gaps in more medial cortical areas (Fig. 7E) which correspond to the ectopic *reelin* clusters in the mutant CP (Fig. 7F). Moreover, even single ectopic $reelin^+$ cells in the CP are surrounded by a ring of *Sox5* expressing cells (data not shown). This complementary expression patterns suggests an exclusion of *Sox5* expressing CP neurons from the $reelin^+$ territories.

To further analyze whether the $reelin^+$ cell aggregates might interfere with the cortical lamination process we examined the formation of the radial glial scaffold which is essential for guiding migrating cortical neurons (Rakic, 2003). In wild-type embryos and in most parts of the mutant cortex, radial glial cells extend fibers from the ventricular to the pial cortical surface (Figs. 7C, G). However, in regions immediately underlying the $reelin^+$ cell clusters, the radial glial scaffold appears to be disrupted in the mutant (Fig. 7G). In these areas, radial glial fibers are severely shortened and do not reach the pial surface but end within the CP suggesting that the exclusion of *Sox5* expressing CP neurons from the reelin clusters is caused by this shortening of the radial glial fibers.

As the $reelin^+$ aggregates appear to be smaller than the actual gaps in the CP (Fig. 7F) we investigated the possibility that SP cells might be part of these clusters. The SP as well as the MZ is labelled by MAP2 and CS56. Interestingly, $MAP2^+$ cells form clusters in the E14.5 Xt^J/Pdn neocortex (Fig. 3Q). In contrast, immunofluorescence analysis with CS56 revealed two separate rows of cells corresponding to the MZ and the SP (Figs. 7D, H). Interestingly, the SP cells surround the lower end of a bulge located in the medial neocortex. However, the CS56 staining appeared more diffuse and we occasionally observed groups of $CS56^+$ cells in the CP.

A clustering of $reelin^+$ cells has been observed in transgenic mice overexpressing BDNF under the control of the nestin enhancer (Ringstedt et al., 1998). These $reelin^+$ aggregates form as a consequence of a segregation from clusters of GABAergic interneurons (Alcantara et al., 2006). This analysis prompted us to investigate the distribution of interneurons which are derived from the ventral telencephalon, enter the cortex by tangential migration and are marked by *Dlx2*, *Gad67* and Calbindin expression (Anderson et al., 2001; Ang et al., 2003; Nery et al., 2002). In situ hybridization for *Dlx2* and *Gad67* revealed migrating interneurons on their migratory routes in the MZ and in the intermediate zone (IZ) of the E14.5 wild-type neocortex (Fig. 7I and data not shown). In the Xt^J/Pdn neocortex, these interneurons are more diffusely distributed, but do not cluster in the MZ (Fig. 7L). Immunofluorescence analysis for Calbindin showed a similar pattern (Figs. 7J, K, M, N) suggesting that the $reelin^+$ aggregates form independently of potential defects in interneuron development.

As the radial glia scaffold is disrupted at the sites of $reelin^+$ clusters we finally investigated whether this disruption might cause the lamination defects in Xt^J/Pdn embryos. To this end, we performed double immunofluorescence staining for Nestin which marks the radial glia scaffold and for Tbr1 to reveal lamina organization in newborn animals. In wild-type P0 pups, the layer specific distribution of $Tbr1^+$ neurons coincides with radial glial extensions from the ventricular to the pial surface (Figs. 8A, C). In the *Gli3* mutant P0

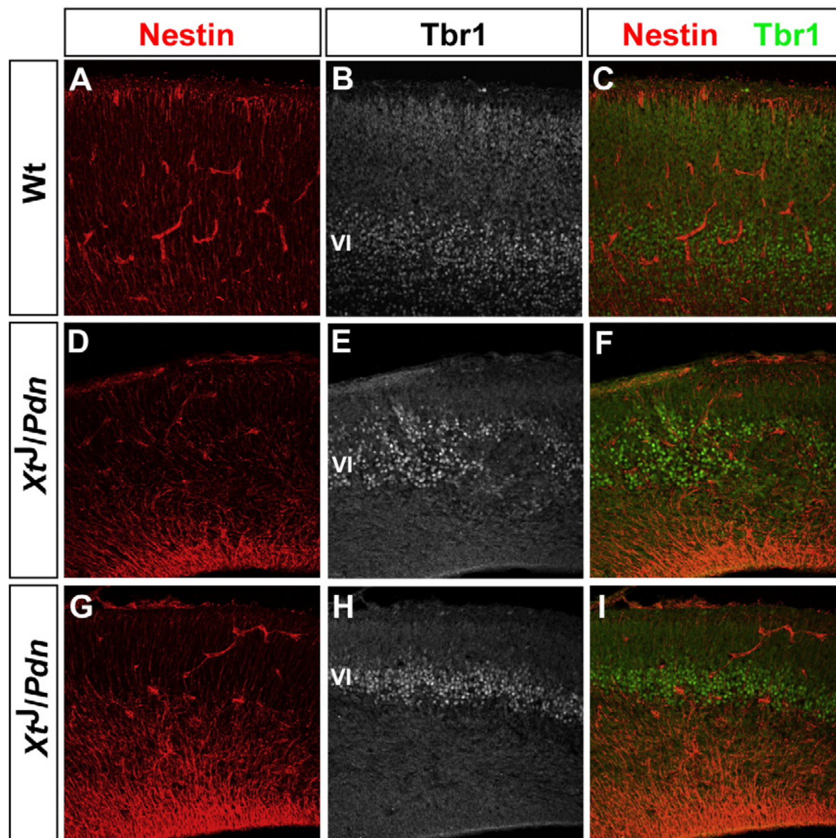

Fig. 8. A disorganized radial glial network correlates with lamination defects in the *Gli3* mutant neocortex. Immunofluorescence analysis on coronal sections through the brains of wildtype (A–C) and Xt^J/Pdn (D–I) P0 animals with the indicated antibodies. (A–C) Radial glia scaffold and distribution of $Tbr1^+$ neurons in layer VI (high level expression) and in layer II/III (low level expression). (D–F) The layer specific distribution of $Tbr1^+$ layer VI neurons is severely disturbed in regions with a disorganized radial glial network. (G–I) In regions where radial glia extensions reach the pial surface positioning of $Tbr1^+$ neurons is slightly more diffuse than in wild-type.

neocortex, however, regions where the laminar organization of Tbr1$^+$ layer VI neurons is severely disturbed correspond to areas with a dramatic disorganization of the glial scaffold (Figs. 8D–F). In contrast, in regions where the radial glia reach the pial surface Tbr1$^+$ layer VI neurons show a layered though more diffuse organization than in the wild-type cortex (Figs. 8G–I). This analysis therefore suggests that the disruption of the radial glial scaffold which is present already early in development causes at least some of the lamination defects in the *Gli3* mutant.

Expansion of Dbx1 expression in Gli3 mutants

Despite the absence of the cortical hem *reelin* expressing cells are present in the *Gli3* mutant cortex (Fig. 3R and Theil, 2005). To address the potential origin of these cells, we analyzed *Dbx1* expression in *Gli3* mutants. Recently, reelin$^+$ cells have been reported to originate from Dbx1$^+$ progenitor cells in the septum and in the ventral pallium (VP) (Bielle et al., 2005) raising the possibility that the *reelin*$^+$/*p73*$^-$ cells in the *Gli3* mutant originate from such progenitors. As in *Gli3* mutants the rostral most dorsal telencephalon expresses ventral telencephalic marker genes (Kuschel et al., 2003; Tole et al., 2000b) we could not investigate whether *Dbx1* expression in the septum is affected by the *Gli3* mutation. However, we observed a widespread, though patchy expression of *Dbx1* in the VZ of both Xt^J/Xt^J and Xt^J/Pdn embryos while *Dbx1* expression is confined to progenitor cells residing immediately at the PSB of E12.5 wild-type embryos (Figs. 9A–C). To investigate whether this ectopic *Dbx1* expression represents an expansion of the VP we performed in situ hybridization for *Sfrp2* and *Tgfα* which are co-expressed with *Dbx1* in the VP (Assimacopoulos et al., 2003; Kim et al., 2001). Interestingly, *Sfrp2* is ectopically expressed in groups of cells within the Xt^J/Xt^J neocortex while its expression remains confined to the VP region of Xt^J/Pdn embryos (Figs. 9D–F). In contrast, *Tgfα* expression expands into the neocortex of both mutants (Figs. 9G–I) suggesting that several VP markers are ectopically expressed in the *Gli3* mutant neocortex though to different extents.

The ectopic *Dbx1* expression might also suggest that an increased *Dbx1*$^+$ progenitor pool may give rise to the *reelin*$^+$ but p73$^-$ cells. To begin to address this hypothesis we employed an explant culture assay using wildtype E10.5 telencephalic tissue. This time point corresponds to the start of CR cell emigration from the cortical hem when only few CR cells have reached the neocortex (Muzio and Mallamaci, 2005). In a control experiment, we first tested whether the complete dorsal telencephalon of wildtype embryos can give rise to CR neurons under these conditions using in situ hybridization for *reelin* and *p73*. Indeed, we could detect two stripes of *reelin* expression in the centre of the explants and strong *reelin* expression at the lateral margins of the explants but only a few *reelin* expressing cells in the centres of the two telencephalic hemispheres which corresponds to neocortical tissue ($n=4$) (Fig. 10A). Also, *p73* expression is confined to the midline regions of the explant ($n=4$) (Fig. 10E). In the next set of experiments, we dissected just neocortical tissue excluding dorsal midline and VP tissue and analyzed the formation of *reelin* expressing cells after 48 h in vitro culture. In line with a previous report (Muzio and Mallamaci, 2005), neocortical tissue from wildtype embryos did not give rise to *reelin* or *p73* expressing cells ($n=8$ for both markers) (Figs. 10B, E). However, when we cultivated neocortical tissue from either Xt^J/Xt^J embryos or Xt^J/Pdn embryos which, in contrast to the wildtype explant, expresses *Dbx1* we observed strong *reelin* but not *p73* expression in the explants ($n=6$ for both mutants and for both markers) (Figs. 10C, D, G, H). In combination with the ectopic *Dbx1*

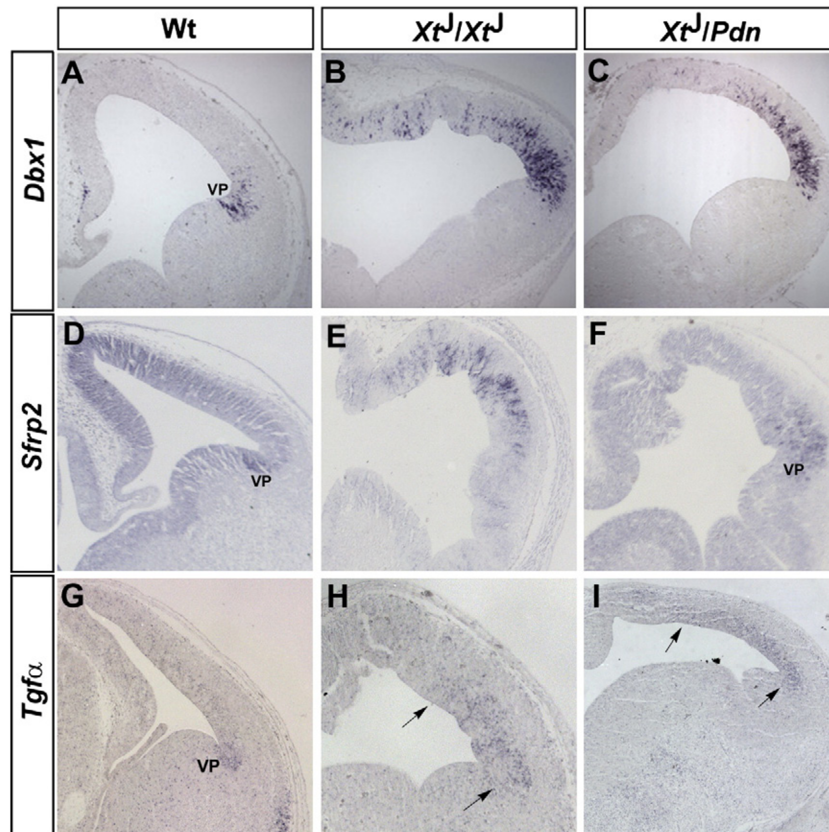

Fig. 9. Ectopic *Dbx1* expression in the *Gli3* mutant neocortex. Coronal sections through the brains of E12.5 wild-type (A, D, G), Xt^J/Xt^J (B, E, H), and Xt^J/Pdn (C, F, I) embryos. (A, D, G) *Dbx1*, *Sfrp2* and *Tgfα* expression in wild-type neocortex are confined to the VP area at the dorsal/ventral telencephalic boundary. (B, C) *Dbx1* is ectopically expressed in the VZ of the *Gli3* mutant neocortex. (E, F) *Sfrp2* is ectopically expressed in the Xt^J/Xt^J neocortex but not in the Xt^J/Pdn mutant. (H, I) Expanded *Tgfα* expression domain in the *Gli3* mutant neocortex as indicated by arrows.

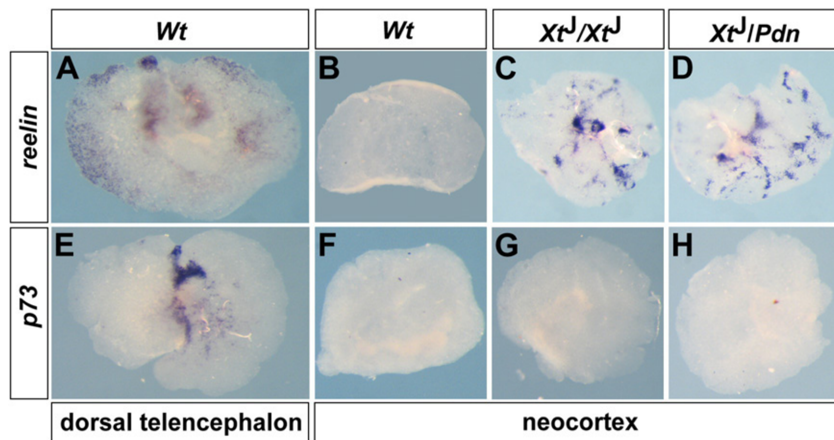

Fig. 10. *Gli3* mutant neocortex gives rise to reelin+/p73− cells in explant cultures. Explant cultures of E10.5 whole dorsal telencephalon (A, E) or neocortex (B–D, F–H) were hybridized with the indicated probes after 48 h of vitro culture. (A, E) Dorsal telencephalic explants give rise to *reelin* expressing cells in the centre of the explant and at its rostral and lateral margins. Note the region between the central reelin stripes and the margins has fewer *reelin*+ cells. (A) *p73* expressing cells are only formed in the centre of the explant (E). (B–D) Wildtype neocortical explants do not form *reelin* expressing cells (B) while *Gli3* mutant explants show widespread *reelin* expression (C, D). (F–H) Neocortical explants from all genotypes lack *p73* expression.

expression, this result suggests that the ectopic Dbx1+ progenitors may give rise to reelin+ neurons in the *Gli3* mutants.

Discussion

Regionalization defects in the Gli3 compound heterozygous mutant XtJ/Pdn

XtJ/XtJ embryos were previously shown to have severe defects in the regionalization of the telencephalon which are also present in *XtJ/Pdn* embryos but in a milder form. In the latter mutant, the expression of ventral telencephalic markers in dorsal locations occurs in a smaller domain and is restricted to the rostral most telencephalon (Kuschel et al., 2003). Dorsomedial structures are highly defective showing a morphological absence of the hippocampus and an overgrowth of choroid plexus tissue consistent with an altered balance between Bmp and Wnt signalling. In contrast to *XtJ/XtJ* embryos (Fotaki et al., 2006) the telencephalon and diencephalon are not fused and we could not find evidence for mixing of cells from both tissues. In addition to these findings, our analysis revealed a novel regionalization defect in *Gli3* mutants, namely an expansion of the VP into the lateral and dorsal pallium. This expansion may result from a lack of *Emx1* expression (Kuschel et al., 2003; Theil et al., 1999) as has been suggested previously (Medina et al., 2004; Puelles et al., 2000) or from a reduced *Lhx2* expression (Mangale et al., 2008). Alternatively, *Gli3* could play a general role in controlling *Dbx1* expression as ectopic *Dbx1* transcription was also found in the *XtJ/XtJ* spinal cord (Persson et al., 2002). Irrespective of the exact mechanism, these data indicate that the *XtJ/Pdn* telencephalon has similar but milder regionalization defects than *XtJ/XtJ* embryos consistent with *Pdn* being a hypomorphic *Gli3* allele. Our analysis also shows that except for this VP expansion the *XtJ/Pdn* neocortex is largely unaffected allowing us to investigate *Gli3* functions in layering.

XtJ/Pdn mice show cortical lamination defects

Our analysis of cortical layering indicates a lamination phenotype in the *XtJ/Pdn* neocortex. The expression of several layer specific markers including the layer IV/V expression of *reelin* indicate a stronger dispersal of cortical neurons throughout the neocortex. This analysis also revealed areas with strong layering defects where cortical neurons were even positioned close to the ventricular surface. Given the mildly affected cortical lamination in hem ablated animals the finding of layering defects in the *XtJ/Pdn* neocortex comes as a surprise especially as significant numbers of *reelin*+ cells are present in the *Gli3* mutant MZ. Their molecular profile (reelin+ p73− Calretinin−) and the fact that, unlike wildtype neocortical tissue, explants from mutant neocortex gives rise to reelin+ but not p73+ cells in an in vitro culture assay strongly suggests that these cells derive from the expanded Dbx1+ progenitor pool in the mutant. The generation of these reelin+ cells in the *XtJ/Pdn* neocortex but not in the hem ablated animals is likely to reflect differences in the timing of hem loss and concomitant changes in Wnt mediated patterning of the dorsal telencephalon and/or differences in patterning the VP (see above). Irrespective of the mechanism, these additional reelin+ cells are not sufficient to drive radial migration of cortical neurons. Therefore, additional signalling pathways and their interaction with reelin signalling may underlie cortical lamination (Meyer et al., 2004; Yoshida et al., 2006). Furthermore, the reelin+/p73−/Calretinin− cell population might functionally differ from hem derived reelin+ CR cells and might not be able to fully compensate for the loss of the latter cells (Bielle et al., 2005; Meyer et al., 2004). Collaboratively, these findings point at intrinsic functional differences between CR cell subpopulations. Such differences may be important for the establishment of different lamination patterns in distinct cortical regions.

Gli3 functions in cortical lamination

Except for a difference in timing, the *Gli3* mutation and hem ablation both lead to a loss of the cortical hem but have strikingly different effects on layering suggesting hitherto unknown roles for *Gli3* in lamination. The most striking observation of this manuscript relates to the rearrangement of reelin+ and Calretinin+ cells which initially show an even distribution over the cortical surface but cluster later in *XtJ/Pdn* embryos. These clusters could mechanically block access to the upper CP. Also, migrating neurons could be differentially exposed to reelin signals consistent with a recent report linking regular spaced clusters of CR cells in the immature presubicular cortex with the formation of vertical arrays of CP neurons (Nishikawa et al., 2002). More importantly, however, the radial glial network which serves as a guidance structure for migrating cortical neurons (Rakic, 2003) is severely disturbed in the vicinity of the reelin+ cell clusters. A severe shortening of these processes and their detachment from the pial surface may lead to a failure to guide migrating neurons to the upper CP. Indeed, regions in the P0 *XtJ/Pdn* neocortex with the most severe lamination defects correlate with sites where the radial glial scaffold is severely disturbed. At present it is unknown whether the clustering of neurons in the MZ precedes the disorganization of the

radial glial scaffold or vice versa. However, as the disturbance of the radial glial network is only found locally while *Gli3* is expressed throughout the VZ it seems more likely that the formation of MZ clusters is the primary cause of the lamination phenotype.

The formation of these clusters could involve a role for *Gli3* in controlling the adhesive properties of neurons which are an important determinant in establishing cortical layers and are known to affect the spreading and distribution of reelin^{+} and other MZ cell types (Borrell and Marin, 2006; Paredes et al., 2006). The formation of these clusters could also involve changes in adhesion as CR cells express specific cell adhesion molecules (Seki and Arai, 1991; Tsuru et al., 1996). Similarly, loss of Gli function in the spinal cord results in neuronal dispersal in the developing spinal cord (Bai et al., 2004; Lei et al., 2004; Wijgerde et al., 2002). As the molecules which control the adhesion of CP neurons and/or CR cells are currently unknown future work will have to address the identity of such factors.

Acknowledgments

We would like to thank Drs. Vassiliki Fotaki, Magdalena Götz, John Mason, David Price and Andrea Wizenmann for critically reading the manuscript. We are grateful to Drs. Jürgen Behrens, Tom Curran, Andre Goffinet, Robert Hevner, Véronique Lefebvre, Andy MacMahon, John Mason and John Rubenstein for providing antibodies and probes for in situ hybridization. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (TH 770/6-1).

References

Alcantara, S., Pozas, E., Ibanez, C.F., Soriano, E., 2006. BDNF-modulated spatial organization of Cajal-Retzius and GABAergic neurons in the marginal zone plays a role in the development of cortical organization. Cereb. Cortex 16, 487–499.

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., Marin, O., Horn, C., Jennings, K., Rubenstein, J.L., 2001. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128, 353–363.

Ang Jr., E.S., Haydar, T.F., Gluncic, V., Rakic, P., 2003. Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. J. Neurosci. 23, 5805–5815.

Assimacopoulos, S., Grove, E.A., Ragsdale, C.W., 2003. Identification of a Pax6-dependent epidermal growth factor family signaling source at the lateral edge of the embryonic cerebral cortex. J. Neurosci. 23, 6399–6403.

Bai, C.B., Stephen, D., Joyner, A.L., 2004. All mouse ventral spinal cord patterning by hedgehog is Gli dependent and involves an activator function of Gli3. Dev. Cell 6, 103–115.

Bielle, F., Griveau, A., Narboux-Neme, N., Vigneau, S., Sigrist, M., Arber, S., Wassef, M., Pierani, A., 2005. Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. Nat. Neurosci. 8, 1002–1012.

Borrell, V., Marin, O., 2006. Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. Nat. Neurosci. 9, 1284–1293.

Bulfone, A., Puelles, L., Porteus, M.H., Frohman, M.A., Martin, G.R., Rubenstein, J.L., 1993. Spatially restricted expression of Dlx-1, Dlx-2 (Tes-1), Gbx-2, and Wnt-3 in the embryonic day 12.5 mouse forebrain defines potential transverse and longitudinal segmental boundaries. J. Neurosci. 13, 3155–3172.

Büscher, D., Grotewold, L., Rüther, U., 1998. The Xt-J allele generates a Gli3 fusion transcript. Mamm. Genome 9, 676–678.

Curran, T., D'Arcangelo, G., 1998. Role of reelin in the control of brain development. Brain Res. Brain Res. Rev. 26, 285–294.

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.

del Rio, J.A., Martinez, A., Fonseca, M., Auladell, C., Soriano, E., 1995. Glutamate-like immunoreactivity and fate of Cajal-Retzius cells in the murine cortex as identified with calretinin antibody. Cereb. Cortex 5, 13–21.

Duan, W., Cole, T., Schreiber, G., 1989. Cloning and nucleotide sequencing of transthyretin (prealbumin) cDNA from rat choroid plexus and liver. Nucleic Acids Res. 17, 3979.

Echelard, Y., Epstein, D.J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J.A., McMahon, A.P., 1993. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 75, 1417–1430.

Englund, C., Fink, A., Lau, C., Pham, D., Daza, R.A., Bulfone, A., Kowalczyk, T., Hevner, R.F., 2005. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J. Neurosci. 25, 247–251.

Fotaki, V., Yu, T., Zaki, P.A., Mason, J.O., Price, D.J., 2006. Abnormal positioning of diencephalic cell types in neocortical tissue in the dorsal telencephalon of mice lacking functional Gli3. J. Neurosci. 26, 9282–9292.

Gradwohl, G., Fode, C., Guillemot, F., 1996. Restricted expression of a novel murine atonal-related bHLH protein in undifferentiated neural precursors. Dev. Biol. 180, 227–241.

Grove, E.A., Tole, S., Limon, J., Yip, L., Ragsdale, C.W., 1998. The hem of the embryonic cerebral cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-deficient mice. Development 125, 2315–2325.

Hanashima, C., Shen, L., Li, S.C., Lai, E., 2002. Brain factor-1 controls the proliferation and differentiation of neocortical progenitor cells through independent mechanisms. J. Neurosci. 22, 6526–6536.

Hebert, J.M., Mishina, Y., McConnell, S.K., 2002. BMP signaling is required locally to pattern the dorsal telencephalic midline. Neuron 35, 1029–1041.

Hevner, R.F., Daza, R.A., Rubenstein, J.L., Stunnenberg, H., Olavarria, J.F., Englund, C., 2003a. Beyond laminar fate: toward a molecular classification of cortical projection/pyramidal neurons. Dev. Neurosci. 25, 139–151.

Hevner, R.F., Neogi, T., Englund, C., Daza, R.A., Fink, A., 2003b. Cajal-Retzius cells in the mouse: transcription factors, neurotransmitters, and birthdays suggest a pallial origin. Brain Res. Dev. Brain Res. 141, 39–53.

Hevner, R.F., Shi, L., Justice, N., Hsueh, Y., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A.M., Campagnoni, A.T., Rubenstein, J.L., 2001. Tbr1 regulates differentiation of the preplate and layer 6. Neuron 29, 353–366.

Hill, R.E., Jones, P.F., Rees, A.R., Sime, C.M., Justice, M.J., Copeland, N.G., Jenkins, N.A., Graham, E., Davidson, D.R., 1989. A new family of mouse homeo box-containing genes: molecular structure, chromosomal location, and developmental expression of Hox-7.1. Genes Dev. 3, 26–37.

Hong, S.E., Shugart, Y.Y., Huang, D.T., Shahwan, S.A., Grant, P.E., Hourihane, J.O., Martin, N.D., Walsh, C.A., 2000. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat. Genet. 26, 93–96.

Howell, B.W., Hawkes, R., Soriano, P., Cooper, J.A., 1997. Neuronal position in the developing brain is regulated by mouse disabled-1. Nature 389, 733–737.

Jones, C.M., Lyons, K.M., Hogan, B.L., 1991. Involvement of Bone Morphogenetic Protein-4 (BMP-4) and Vgr-1 in morphogenesis and neurogenesis in the mouse. Development 111, 531–542.

Kim, A.S., Anderson, S.A., Rubenstein, J.L.R., Lowenstein, D.H., Pleasure, S.J., 2001. Pax-6 regulates expression of SFRP-2 and Wnt-7b in the developing CNS. J. Neurosci. 21, 132RC-.

Kuschel, S., Rüther, U., Theil, T., 2003. A disrupted balance between Bmp/Wnt and Fgf signaling underlies the ventralization of the Gli3 mutant telencephalon. Dev. Biol. 260, 484–495.

Lefebvre, V., Li, P., de Crombrugghe, B., 1998. A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. EMBO J. 17, 5718–5733.

Lei, Q., Zelman, A.K., Kuang, E., Li, S., Matise, M.P., 2004. Transduction of graded Hedgehog signaling by a combination of Gli2 and Gli3 activator functions in the developing spinal cord. Development 131, 3593–3604.

Lin, J.H., Saito, T., Anderson, D.J., Lance-Jones, C., Jessell, T.M., Arber, S., 1998. Functionally related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. Cell 95, 393–407.

Lustig, B., Jerchow, B., Sachs, M., Weiler, S., Pietsch, T., Karsten, U., van de Wetering, M., Clevers, H., Schlag, P.M., Birchmeier, W., Behrens, J., 2002. Negative feedback loop of Wnt signaling through upregulation of Conductin/Axin2 in colorectal and liver tumors. Mol. Cell. Biol. 22, 1184–1193.

Mangale, V.S., Hirokawa, K.E., Satyaki, P.R., Gokulchandran, N., Chikbire, S., Subramanian, L., Shetty, A.S., Martynoga, B., Paul, J., Mai, M.V., Li, Y., Flanagan, L.A., Tole, S., Monuki, E.S., 2008. Lhx2 selector activity specifies cortical identity and suppresses hippocampal organizer fate. Science 319, 304–309.

Medina, L., Legaz, I., Gonzalez, G., De Castro, F., Rubenstein, J.L., Puelles, L., 2004. Expression of Dbx1, Neurogenin 2, Semaphorin 5A, Cadherin 8, and Emx1 distinguish ventral and lateral pallial histogenetic divisions in the developing mouse claustroamygdaloid complex. J. Comp. Neurol. 474, 504–523.

Meyer, G., Wahle, P., 1999. The paleocortical ventricle is the origin of reelin-expressing neurons in the marginal zone of the foetal human neocortex. Eur. J. Neurosci. 11, 3937–3944.

Meyer, G., Goffinet, A.M., Fairen, A., 1999. What is a Cajal-Retzius cell? A reassessment of a classical cell type based on recent observations in the developing neocortex. Cereb. Cortex 9, 765–775.

Meyer, G., Perez-Garcia, C.G., Abraham, H., Caput, D., 2002. Expression of p73 and Reelin in the developing human cortex. J. Neurosci. 22, 4973–4986.

Meyer, G., Cabrera Socorro, A., Perez Garcia, C.G., Martinez Millan, L., Walker, N., Caput, D., 2004. Developmental roles of p73 in Cajal-Retzius cells and cortical patterning. J. Neurosci. 24, 9878–9887.

Muzio, L., Mallamaci, A., 2005. Foxg1 confines Cajal-Retzius neuronogenesis and hippocampal morphogenesis to the dorsomedial pallium. J. Neurosci. 25, 4435–4441.

Nery, S., Fishell, G., Corbin, J.G., 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat. Neurosci. 5, 1279–1287.

Nishikawa, S., Goto, S., Hamasaki, T., Yamada, K., Ushio, Y., 2002. Involvement of reelin and Cajal-Retzius cells in the developmental formation of vertical columnar structures in the cerebral cortex: evidence from the study of mouse presubicular cortex. Cereb. Cortex 12, 1024–1030.

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.

Paredes, M.F., Li, G., Berger, O., Baraban, S.C., Pleasure, S.J., 2006. Stromal-derived factor-1 (CXCL12) regulates laminar position of Cajal-Retzius cells in normal and dysplastic brains. J. Neurosci. 26, 9404–9412.
Persson, M., Stamataki, D., te Welscher, P., Andersson, E., Bose, J., Ruther, U., Ericson, J., Briscoe, J., 2002. Dorsal–ventral patterning of the spinal cord requires Gli3 transcriptional repressor activity. Genes Dev. 16, 2865–2878.
Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., Keleher, J., Smiga, S., Rubenstein, J.L., 2000. Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. J. Comp. Neurol. 424, 409–438.
Rakic, P., 2003. Elusive radial glial cells: historical and evolutionary perspective. Glia 43, 19–32.
Rice, D.S., Curran, T., 2001. Role of the reelin signaling pathway in central nervous system development. Annu. Rev. Neurosci. 24, 1005–1039.
Richardson, M., Redmond, D., Watson, C.J., Mason, J.O., 1999. Mouse Wnt8B is expressed in the developing forebrain and maps to chromosome 19. Mamm. Genome 10, 923–925.
Ringstedt, T., Linnarsson, S., Wagner, J., Lendahl, U., Kokaia, Z., Arenas, E., Ernfors, P., Ibanez, C.F., 1998. BDNF regulates reelin expression and Cajal-Retzius cell development in the cerebral cortex. Neuron 21, 305–315.
Roelink, H., Nusse, R., 1991. Expression of two members of the Wnt family during mouse development–restricted temporal and spatial patterns in the developing neural tube. Genes Dev. 5, 381–388.
Schimmang, T., Oda, S.I., Ruther, U., 1994. The mouse mutant Polydactyly Nagoya (Pdn) defines a novel allele of the zinc finger gene Gli3. Mamm. Genome 5, 384–386.
Seki, T., Arai, Y., 1991. Expression of highly polysialylated NCAM in the neocortex and piriform cortex of the developing and the adult rat. Anat. Embryol. (Berl) 184, 395–401.
Sheldon, M., Rice, D.S., D'Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., Howell, B.W., Cooper, J.A., Goldowitz, D., Curran, T., 1997. Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. Nature 389, 730–733.
Soda, T., Nakashima, R., Watanabe, D., Nakajima, K., Pastan, I., Nakanishi, S., 2003. Segregation and coactivation of developing neocortical layer 1 neurons. J. Neurosci. 23, 6272–6279.
Super, H., Soriano, E., Uylings, H.B., 1998. The functions of the preplate in development and evolution of the neocortex and hippocampus. Brain Res. Brain Res. Rev. 27, 40–64.
Super, H., Del Rio, J.A., Martinez, A., Perez-Sust, P., Soriano, E., 2000. Disruption of neuronal migration and radial glia in the developing cerebral cortex following ablation of Cajal-Retzius cells. Cereb. Cortex 10, 602–613.
Takiguchi-Hayashi, K., Sekiguchi, M., Ashigaki, S., Takamatsu, M., Hasegawa, H., Suzuki-Migishima, R., Yokoyama, M., Nakanishi, S., Tanabe, Y., 2004. Generation of reelin-positive marginal zone cells from the caudomedial wall of telencephalic vesicles. J. Neurosci. 24, 2286–2295.
Tao, W., Lai, E., 1992. Telencephalon-restricted expression of BF-1, a new member of the HNF-3/fork head gene family, in the developing rat brain. Neuron 8, 957–966.
Theil, T., 2005. Gli3 is required for the specification and differentiation of preplate neurons. Dev. Biol. 286, 559–571.
Theil, T., Alvarez-Bolado, G., Walter, A., Rüther, U., 1999. Gli3 is required for Emx gene expression during dorsal telencephalon development. Development 126, 3561–3571.
Thien, H., Rüther, U., 1999. The mouse mutation Pdn (Polydactyly Nagoya) is caused by the integration of a retrotransposon into the Gli3 gene. Mamm. Genome 10, 205–209.
Tissir, F., Goffinet, A.M., 2003. Reelin and brain development. Nat. Rev. Neurosci. 4, 496–505.
Tole, S., Goudreau, G., Assimacopoulos, S., Grove, E.A., 2000a. Emx2 is required for growth of the hippocampus but not for hippocampal field specification. J. Neurosci. 20, 2618–2625.
Tole, S., Ragsdale, C.W., Grove, E.A., 2000b. Dorsoventral patterning of the telencephalon is disrupted in the mouse mutant extra-toes(J). Dev. Biol. 217, 254–265.
Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R.E., Richardson, J.A., Herz, J., 1999. Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell 97, 689–701.
Tsuru, A., Mizuguchi, M., Uyemura, K., Takashima, S., 1996. Immunohistochemical expression of cell adhesion molecule L1 during development of the human brain. Early Hum. Dev. 45, 93–101.
Wijgerde, M., McMahon, J.A., Rule, M., McMahon, A.P., 2002. A direct requirement for Hedgehog signaling for normal specification of all ventral progenitor domains in the presumptive mammalian spinal cord 10.1101/gad.1025702. Genes Dev. 16, 2849–2864.
Yoshida, M., Assimacopoulos, S., Jones, K.R., Grove, E.A., 2006. Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. Development 133, 537–545.
Yun, K., Potter, S., Rubenstein, J.L., 2001. Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. Development 128, 193–205.
Zimmer, C., Tiveron, M.C., Bodmer, R., Cremer, H., 2004. Dynamics of Cux2 expression suggests that an early pool of SVZ precursors is fated to become upper cortical layer neurons. Cereb. Cortex 14, 1408–1420.