Strabismus regulates asymmetric cell divisions and cell fate determination in the mouse brain

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Introduction

The planar cell polarity (PCP) pathway maintains cell polarity in the plane of epithelial tissues in Drosophila melanogaster embryos through the complex interplay of several core molecular components, including Frizzled, Dishevelled, Strabismus (Stbm), and Prickle (Tree et al., 2002). The same proteins also regulate polarized cell intercalation during gastrulation and neurulation in vertebrate embryos and polarization of inner ear cells in mammals (Sokol, 2000; Kibar et al., 2001; Jessen et al., 2002; Montcouquiol et al., 2003; Torban et al., 2004). In many cases, the PCP pathway has been proposed to modulate the cytoskeleton and influence cell morphology rather than cell fates (Wolff and Rubin, 1998). Nevertheless, some PCP components are essential for asymmetric cell division (ACD) of Drosophila sensory organ precursors (SOPs; Gho and Schweisguth, 1998; Bellaiche et al., 2004). Specifically, the transmembrane protein Stbm promotes the anterior cortical localization of partner of inscuteable (Pins), an activator of G protein signaling, which is required for proper orientation of the mitotic spindle and SOP daughter cell identity (Bellaiche et al., 2004). Although SOP divisions represent a highly specialized system, these observations suggest that the PCP pathway might influence cell fate determination during asymmetric division of other progenitor cells, as defined by unequal inheritance of fates between daughter cells and asymmetric distribution of specific proteins that may control this process. Given the potential importance of ACD in cell fate determination in the vertebrate brain, we investigated the possible involvement of PCP signals in regulating mammalian neurogenesis.

The development of the complex cytoarchitecture of the mammalian brain is thought to depend on the balance between symmetric and asymmetric divisions of neural progenitors (NPs) occupying the ventricular zone (VZ; Chenn and McConnell, 1995; Kosodo et al., 2004; Noctor et al., 2004; Gotz and Huttner, 2005). Vertical cleavage planes that are perpendicular to the ventricular surface usually result in symmetric divisions, whereas horizontally shifted cleavage planes may lead to asymmetry (Chenn and McConnell, 1995; Haydar et al., 2003; Kosodo et al., 2004; Gotz and Huttner, 2005). The latter were hypothesized to play a role in the specification of neuronal fates through
Results and discussion

To investigate a possible role of conserved PCP machinery in regulating vertebrate neurogenesis, we examined Lp/Lp (Loop-tail) mice that carry a point mutation in Vangl2 (Kibar et al., 2001). This gene encodes a mammalian homologue of Stbm, a specific component of the PCP pathway in Drosophila (Wolff and Rubin, 1998; Montcouquiol et al., 2003). We constructed a mutated Stbm/Vangl2 cDNA carrying the Lp/Lp mutation that converts a serine to asparagine (S464N). The corresponding StbmS464N protein delocalized from the plasma membrane and failed to inhibit convergent extension movements in Xenopus laevis embryos, supporting the proposed loss of function phenotype of Lp/Lp mice (Fig. S1). Furthermore, although Vangl2 protein was broadly expressed in neuroepithelium of wild-type embryos, it was undetectable in Lp/Lp mice (Fig. S1, A–D), which is consistent with a previously reported loss of protein stability (Torban et al., 2007). Embryonic day (E) 15.5 cerebral cortices from Lp/Lp mice were relatively similar to those of Lp/+ and +/+ mice in both gross morphology (Fig. S2) and a number of progenitors (RC2; Fig. 1, A and B) and revealed only a slight reduction in the neuronal layer (βIII-tubulin or TuJ1; Fig. 1, C and D). However, the number of Reelin-positive Cajal-Retzius cells, the earliest born neurons (Chae et al., 2004), was significantly increased in Lp/Lp cortices as compared with wild-type cortices (Fig. 1, E–G; and Table I). The total number of DAPI-positive nuclei in the marginal layer was largely unaffected, indicating that the increase is not caused by compaction of cells in the mutant cortex (Table I). These observations suggest precocious differentiation of Lp/Lp NPs into early-born neurons.

Premature differentiation of Lp/Lp NPs during corticogenesis would be expected to gradually diminish both the pool of progenitors and their derivatives: late-born neurons and glia. Consistent with this hypothesis, E18.5 cerebral cortices stained with hematoxylin/eosin and the TuJ1 antibody revealed a significant reduction in the overall size of the neocortex and a decrease of the neuronal population in Lp/Lp embryos as compared with Lp/+ and wild-type embryos (Fig. 2, A and B). In the E18.5 Lp/Lp brain, we observed a reduction in glial fibrillary acidic protein (GFAP)–positive astrocytes and RC2-positive and Nestin-positive radial glia progenitors in Lp/Lp cortices as compared with Lp/+ and wild-type cortices (Fig. 2, C, D, F, and G; and not shown).

Table I. Frequency of Reelin-positive cells in the E15.5 marginal zone

<table>
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<tr>
<th>Cell number</th>
<th>+/+</th>
<th>Lp/Lp</th>
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<tr>
<td>Reelin positive</td>
<td>5.8 ± 1.3</td>
<td>15.8 ± 1.9</td>
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<tr>
<td>DAPI positive</td>
<td>61.2 ± 8.3</td>
<td>72.2 ± 7.4</td>
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Reelin-positive cells were scored over four 110-µm fields per E15.5 embryo (+/+, n = 2; lp/lp, n = 3). DAPI-positive cells were determined over six 100-µm fields per genotype. The number of cells ± SD is given.

unequal inheritance of localized determinants (Betschinger and Knoblich, 2004; Gotz and Huttner, 2005). A disruption in the number of asymmetric divisions may deplete the progenitor population, leading to reduced brain size (Bond et al., 2002) and precocious neuronal differentiation (Sanada and Tsai, 2005). Therefore, factors regulating mitotic spindle orientation are expected to maintain the pool of NPs and regulate the sequential differentiation of cortical neurons and glia (Qian et al., 2000; Shen et al., 2006). Although a conserved Pins/G protein–dependent mechanism was found to regulate mitotic spindle orientation in mammalian VZ progenitors (Sanada and Tsai, 2005; Konno et al., 2008), the involvement of PCP signals in this process has yet to be examined.
cells with activated caspase 3 at both E14.5 and E17.5 (Fig. S2, G–J; and not depicted). Together, these findings suggest that Lp/Lp progenitors prematurely differentiate into early neuronal lineages at the expense of later born neurons and glia.

To study the intrinsic differentiation potential of Lp/Lp NPs, we established in vitro cultures and analyzed cell differentiation using conventional techniques (Chandran and Caldwell, 2004). NP cultures were derived from the developing cerebellum (E18.5) or cerebral cortex (E14.5) of Lp/Lp embryos as well as from Lp/+ and +/+ littermates. When maintained as undifferentiated neurospheres in the presence of basic FGF (bFGF)

Figure 2. **Precocious differentiation of NPs in Lp/Lp mice.** (A–H) Coronal sections of Lp/+ cortices at E18.5 are shown. They are reduced in size and show premature depletion of progenitors. Histological analysis of E18.5 neocortex, hematoxylin/eosin staining, and the corresponding TuJ1 staining (right) are shown for wild-type and Lp/+ (A) and Lp/Lp (B) cortices. The respective positions of the pial layer (P), marginal zone (MZ; layer I), cortical plate (CP; layers II–VI), sub-VZ (SVZ), and VZ are shown. The corresponding regions examined in C–H are also shown. The astrocytes marked by GFAP (C and F), radial glia progenitors marked by RC2 (D and G), and late-born outer layer neurons marked by Brn-1 (E and H) were all significantly reduced in Lp/Lp mice. Brackets in E and H represent the relative size of the Brn-1 layer. Bars, 50 µm.
and EGF, all precursors showed similar expression levels of progenitor markers (Fig. S3 A), including Nestin and Mash1 and the radial glia markers BLBP and Glast (Conti et al., 2005). All cell lines further showed a low expression level of Dlx2, a marker of transiently amplifying neuronal progenitors (Fig. S3), and did not express the neuronal marker TuJ1 or the astrocytic marker GFAP (not depicted). Furthermore, in the presence of bFGF and EGF, the NP lines grew as neurospheres at similar rates independent of the genotype, indicating that self-renewal was not affected by the mutation (Fig. S3 B). After withdrawal of bFGF and EGF from the medium, culturing under adherent conditions resulted in neuronal differentiation, with TuJ1-expressing neurons appearing after 2 d. A greater number of TuJ1-expressing neurons was observed for Lp/Lp NP cultures (Fig. 3, A–C). To determine the potential of these cultures to subsequently generate glial lineages, the culture medium was complemented with 2% FCS after 3 d of differentiation, and the number of astrocytes was determined after an additional 4 d in culture. Lp/Lp progenitors from both the cortex and the cerebellum produced significantly fewer astrocytes than Lp/+ or wild-type progenitors (Fig. 3, D–F). These findings are consistent with the precocious differentiation observed in the Lp/Lp brain and suggest that Vangl2 suppresses progenitor differentiation and promotes cell fate diversity.

One mechanism for suppressing premature overt differentiation is ACD that generates both a self-renewing progenitor and a committed precursor. Although our data are consistent with the idea that the PCP pathway regulates asymmetric divisions of vertebrate NP cells, the direct evidence supporting this hypothesis has been lacking. In Drosophila SOPs, Stbm activity was shown to maintain the asymmetric localization of Pins (Bellaiche et al., 2004), a regulator of G protein signaling, which is required for mitotic spindle orientation (Betschinger and Knoblich, 2004). A knockdown of a mouse homologue of Pins caused precocious differentiation of NPs both in vitro and in vivo (Sanada and Tsai, 2005), indicating its importance for proper spindle positioning in neural fate decisions. Furthermore, Leu-Gly-Asn–enriched protein (LGN), one of the two functionally conserved mammalian homologues of Pins (Du and Macara, 2004), is asymmetrically distributed in dividing NPs (Fuja et al., 2004). Thus, we decided to evaluate whether the PCP pathway influences mitotic spindle orientation in vertebrate NPs by monitoring subcellular localization of LGN during neuronal differentiation.

LGN localization was evaluated in cultures of dividing NPs isolated from wild-type, heterozygous, and mutant mouse embryonic brains after 24 h of differentiation in the bFGF/B27 medium (Fig. 4, A–F; Table II; Qian et al., 2000). Under these conditions, Lp/Lp NPs had a reduced number of mitoses after 3 d of culture as compared with Lp/+ NPs (Fig. S3, C–E) but revealed increased TuJ1 expression consistent with enhanced neuronal differentiation (Fig. S3, F and C). Cultures were additionally treated with nocodazole to synchronize mitotic NPs for analysis of endogenous LGN asymmetry during each phase of the cell cycle. This permitted enrichment of mitotic progenitors without any apparent disruption in LGN distribution. Asymmetric LGN was detectable in a subcortical crescent in over a third of wild-type cells at prophase (Fig. 4, A–C) and prometaphase/metaphase, when it was found adjacent to the spindle poles (Fig. 4, D–F; and Table II). By anaphase, few asymmetries were visible (6.9% ± 3.3% of wild type), as LGN localized primarily to the cell center or midbody (n = 123; unpublished data). This indicates that spindle orientation is likely determined early within the cell cycle before separation of daughter chromosomes at anaphase. In two independent Lp/Lp cultures, the frequency of cells with asymmetrically distributed LGN was significantly decreased at both prophase and prometaphase/metaphase (Fig. 4, C and F; and Table II). These findings indicate that the Stbm/Vangl2 function in maintaining spindle orientation is conserved in neuronal precursors from Drosophila to mammals.

At the next stage of analysis, VZ cells were immunostained for the microtubule-binding nuclear mitotic apparatus
protein (NuMa), a putative target of LGN (Du and Macara, 2004) during ACD (Siller et al., 2006). NuMa is thought to determine spindle orientation between metaphase and anaphase by anchoring astral microtubules to the cell membrane. To maximize detection of asymmetric divisions that produce a progenitor cell and a neuron, we examined developing cortices at E14.5 near the peak in occurrence of this type of cell division (Haydar et al., 2003). Mitotic progenitors were identified in cortical sections by costaining for phosphohistone H3 (Fig. 5, A–G) and the centrosomal marker centrosome staining and excluded from further studies, whereas those showing a significant deviation were scored as asymmetric (Figs. 5, D–F, and 6). Cleavage orientation was defined by the angle between the line segregating daughter chromosomes and the ventricular surface (Chenn and McConnell, 1995). In prior studies, cleavage planes approaching 90° (vertical; Fig. 5 H) usually led to symmetric fates, whereas orientations closer to 0° (horizontal; Fig. 5 J) were more likely to be asymmetric, producing both a neuron and progenitor (Chenn and McConnell, 1995).

To directly assess whether asymmetric cleavage planes were disrupted in Lp/Lp mice, we examined developing cortices at E14.5, which is consistent with Vangl2 regulating ACD of VZ progenitors rather than influencing cell proliferation in general. Additionally, E14.5 Lp/Lp cortices showed no significant changes in the apical distribution of ZO1, nonphosphorylated β-catenin, and atypical PKC (Fig. 5, A and B; not depicted; Gotz and Huttner, 2005). By late corticogenesis, BrdU incorporation and apical markers became significantly reduced in Lp/Lp mice (Fig. S2 and not depicted), which is consistent with Vangl2 regulating ACD of VZ progenitors.

These findings demonstrate the conservation of a molecular mechanism regulating ACD from fly to mouse embryos and provide the first evidence that the PCP pathway, commonly

**Table II.** Frequency of LGN asymmetries in cerebellar NPs in vitro

<table>
<thead>
<tr>
<th>Cell cycle phase</th>
<th>Wild type</th>
<th>Lp/+</th>
<th>Lp/lp1</th>
<th>Lp/lp2</th>
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<tbody>
<tr>
<td>Prophase</td>
<td>38.9 ± 3.4</td>
<td>36.5 ± 5.8</td>
<td>19.5 ± 5.7</td>
<td>21.8 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>(n = 207)</td>
<td>(n = 228)</td>
<td>(n = 257)</td>
<td>(n = 294)</td>
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<tr>
<td>Prometaphase</td>
<td>33.6 ± 5.0</td>
<td>33.6 ± 6.8</td>
<td>24.3 ± 6.9</td>
<td>22.3 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>(n = 331)</td>
<td>(n = 351)</td>
<td>(n = 416)</td>
<td>(n = 445)</td>
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</table>

Determined over four independent experiments. Percentage ± SD is given.
known to affect cell polarity and morphogenesis, plays a critical role in vertebrate neural fate decisions. Similar to what is observed in Drosophila SOP cells, Vangl2 may orient the mitotic spindle in a manner that promotes ACD (Bellaiche et al., 2004). Indeed, asymmetric cleavages within mouse cortical VZ progenitors must bypass apically positioned determinants (Chenn and McConnell, 1995; Kosodo et al., 2004; Lee et al., 2006), indicating the importance of mitotic spindle regulation in vertebrate neural fate decisions. This spindle control appears to depend on a conserved LGN–NuMa–Gαi complex, where subcortical LGN may anchor spindle poles to the cell membrane through coordinate interaction with both aster microtubule-associated NuMa and membrane-associated Gαi (Du and Macara, 2004). In murine VZ cortical progenitors, this complex may capture mitotic spindle poles symmetrically to promote similar daughter fates (Konno et al., 2008) or asymmetrically for cleavage planes that promote different daughter cell fates (Sanada and Tsai, 2005).

Consistent with a role in ACD, inhibition of G protein activity decreased asymmetric VZ cleavage planes and increased precocious neuronal differentiation in the mouse cortex (Sanada and Tsai, 2005). Similarly, in Lp/Lp embryos, the reduced asymmetry in distribution of LGN in vitro or NuMa in vivo was associated with precocious neuronal differentiation and depletion of the progenitor population. We propose that Lp/Lp NPs undergo an increased frequency of symmetric neurogenic

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**Figure 5.** Asymmetric divisions of NPs in the developing cortex. (A and B) Coronal cryosections of E14.5 Lp/Lp (n = 3) and Lp/+ (n = 3) forebrains were stained for phosphohistone H3 (PH3) to identify dividing VZ progenitors. Apical surfaces facing the lateral ventricle were analyzed and shown with their apical surfaces oriented down. Asymmetric (C–C′ and E–E′) and symmetric (D–D′ and F–F′) centrosomal localization of NuMa at prometaphase (C–D) and anaphase (E–F). Metaphase to anaphase NuMa asymmetries are summarized in G. Images shown are of Lp/+ (C) and Lp/Lp (D–F) cortices. Arrows indicate centrosomes. (H–J) Cleavage plane orientation with respect to the apical surface was scored as described in Materials and methods and is presented in three broad categories: 60–90°, 30–60°, and 0–30°. Representative images are shown. Boxed regions indicate telophase nuclei represented graphically. (K) Comparison of cleavage plane orientation during anaphase/telophase of cortical cell divisions in Lp/+ (n = 172) and Lp/Lp (n = 112) embryos (three for each genotype). Statistical significance was determined by a standard two-tailed Student’s t test (**, P < 0.01). Error bars indicate mean ± SD. Bars: (B) 25 µm; (D′, F′, and J) 5 µm.
Table III. Orientation of cleavage planes in VZ NPs in vivo

<table>
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<tr>
<th>Angle of division</th>
<th>Lp+/+ (n = 172)</th>
<th>Lp/Lp (n = 112)</th>
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<tbody>
<tr>
<td>60–90°</td>
<td>50.3 ± 3.0</td>
<td>65.0 ± 2.2</td>
</tr>
<tr>
<td>30–60°</td>
<td>34.3 ± 2.5</td>
<td>27.3 ± 4.7</td>
</tr>
<tr>
<td>0–30°</td>
<td>15.5 ± 2.4</td>
<td>7.8 ± 4.4</td>
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Determined over three independent E14.5 mice per genotype. Percentage ± SD of total divisions is given.

divisions that normally occur during late stages of cortical development (Haydar et al., 2003). These results support a conserved role for Vangl2 in promoting ACD to preserve the pool of progenitors needed to complete multiple rounds of neurogenesis. Although it is likely that this function of Vangl2 is accomplished through its interactions with Dlg, LGN, and NuMa (Bellaïche et al., 2004; Du and Macara, 2004), this role may be permissive rather than instructive, as Vangl2 protein does not appear to be localized in embryonic brain cells at E12.5 (Fig. S1 B). Further studies are needed to identify direct molecular targets and upstream modulators of Vangl2 to understand the complex regulation of cell renewal and differentiation in the developing cortex.

Materials and methods

Mouse embryos
Lp/lp mice of the Lp/Lp/Le stock were provided by D. Sassoon (Mount Sinai School of Medicine, New York, NY) and were maintained as described previously (Montcourtil et al., 2003). For histology, E18.5 embryos (Lp/lp, n = 4; Lp+/+, n = 2) were fixed, embedded in paraffin, sectioned at 10–12 µm, and stained with hematoxylin/eosin.

NP culture
NPs were dissociated from E14.5 forebrain (cortical hemispheres) or E18.5 cerebella using 0.25% trypsin and/or gentle trituration. Single cells were seeded at a density of 10^5 cells/cm^2 in DME/F12 media containing 2% FCS was added to the medium. For astrocyte (GFAP) differentiation, 2% FCS was added to the medium. Cells were fixed and stained after 2 d. DAPI-positive cells were scored for TuJ1 staining at 40× magnification in 4 (cerebellar) or 10 (cortical) fields per experimental group (~25–50 cells per field). For astrocyte (GFAP) differentiation, 2% FCS was added to the medium after 3 d, and cells were stained on day 7. Scoring was performed as described for TuJ1 (with 50–100 cells per field). For analysis of ACD, NPs (4.5 × 10^5 cells/cm^2) were cultured 1–7 d in a defined medium (N2-ST: DME/F12/N2, 2% B27 supplement [Invitrogen], and 2% B27 supplement [Invitrogen]). Neuropheres were passaged using Versene (Invitrogen) and/or gentle trituration and resuspended at a density of 1.25 × 10^5 cells/cm^2 without B27. All experiments were performed with NPs that were dissociated from passage 3–5 neurospheres.

Differentiation of dispersed NPs was on coverslips coated with poly-l-ornithine (Sigma-Aldrich) and laminin (Invitrogen). For sequential neurosphere differentiation, NPs were cultured without bFGF/EGF. Cells were fixed and stained after 2 d. DAPI-positive cells were scored for TuJ1 staining at 40× magnification in 4 (cerebellar) or 10 (cortical) fields per experimental group (~25–50 cells per field). For astrocyte (GFAP) differentiation, 2% FCS was added to the medium after 3 d, and cells were stained on day 7. Scoring was performed as described for TuJ1 (with 50–100 cells per field). For analysis of ACD, NPs (4.5 × 10^5 cells/cm^2) were cultured 1–7 d in a defined medium (N2-ST: DME/F12/N2, 2% B27 supplement [Invitrogen], and 2% B27 supplement [Invitrogen]). Neuropheres were passaged using Versene (Invitrogen) and/or gentle trituration and resuspended at a density of 1.25 × 10^5 cells/cm^2 without B27. All experiments were performed with NPs that were dissociated from passage 3–5 neurospheres.

Immunofluorescence
Immunofluorescence experiments of the telencephalic hemispheres were performed at similar points along the anterior posterior axis. Brain tissue from E18.5 Lp/lp (n = 4, Lp+/+, n = 2) and +/+ (n = 2) mouse embryos was embedded in O.C.T. (Tissue-Tek; Sakura Finetek) and cooled to −20 °C. Sections were cut at 8 µm and mounted in Prolong Gold Antifade medium (Invitrogen) and observed using a confocal microscope (Carl Zeiss, Inc.). Images were obtained using 5, 10, 20, 40, and 63× oil objectives with the Apotome attachment (Carl Zeiss, Inc.) at room temperature with fixed samples mounted in Vectashield mounting medium. Basic γ adjustments were performed using either Axiovision software and/or Photoshop (Adobe). Cell images were obtained using 5, 10, 20, 40, and 63× oil objectives with the Apomat attachment (Carl Zeiss, Inc.). Composite images (Fig. S1, A and B) involved overlaying multiple individual fields from a single section in Photoshop. For cleavage plane orientation, anaphase and telophase progenitors were identified in cortical sections contained with γ-tubulin and DAPI, and the angle between the line segregating daughter chromosomes and the ventricular surface was determined using the AxioVision imaging software. For analysis, division angles were grouped into bins at either 10 or 30° increments. Means and standard deviations were generated using Excel. Statistical significance was determined by a standard two-tailed Student's t test.

RT-PCR analysis of NPs
RNA was extracted (RNeasy; Qiagen) from cerebellum (passage 4) or cortical (passage 3) neurospheres for cDNA synthesis with Superscript II reverse transcriptase (Invitrogen). PCR conditions and primer sequences for Dlx2, Bmi1, Mash1, and Glast (scl1a3) were as described previously (Conti et al., 2005). Additional primer sequences were Nestin (forward, 5′-AGGACAAAGAAAAAGAGAGTTG-3′; reverse, 5′-TTCTCAGATGAGGAGTCAGA3′) and GADPH (forward, 5′-TTCACCA-CCATGGAAAGAGC-3′; reverse, 5′-GCCATGGACCTTGTCGCTAA-3′).

Online supplemental material
Fig. S1 shows Vangl2 expression, subcellular localization, and functional activity. Fig. S2 shows characterization of cell proliferation and apoptosis in Lp/lp cortices. Fig. S3 shows gene expression and growth properties of Lp/lp NPs. Western blots show specificity of the LGN antibody. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200807073/DC1.

We thank D. Sassoon and M. Kelley for the LGN antibody and pcDNA-hLGN plasmid, M. Mlodzik for pCS2+CFP-XStbm (provided by M. Mlodzik, Mount Sinai School of Medicine, New York, NY) and pCS2+CFP-XStbmS464N was generated by Pu-directed mutagenesis as described previously (Brott and Sokol, 2005) using the primer 5′-GGCAAAAGACGTGACCCTGTTAAGAAGGAAACCCGTCATC- CAAACG-3′ and confirmed by sequencing. For Western analysis, anti-GFP (Life: BD) detected XStbm proteins, and anti–β-tubulin (Biogenex) controlled protein loading.

Immunofluorescence experiments of the telencephalic hemispheres were performed at similar points along the anterior posterior axis. Brain tissue from E18.5 Lp/lp (n = 4, Lp+/+, n = 2) and +/+ (n = 2) mouse embryos was embedded in O.C.T. (Tissue-Tek; Sakura Finetek) and cooled to −20 °C. Sections were cut at 8 µm and mounted in Prolong Gold Antifade medium (Invitrogen) and observed using a confocal microscope (Carl Zeiss, Inc.). Images were obtained using 5, 10, 20, 40, and 63× oil objectives with the Apomat attachment (Carl Zeiss, Inc.) at room temperature with fixed samples mounted in Vectashield mounting medium. Basic γ adjustments were performed using either Axiovision software and/or Photoshop (Adobe). Cell images were obtained using 5, 10, 20, 40, and 63× oil objectives with the Apomat attachment (Carl Zeiss, Inc.). Composite images (Fig. S1, A and B) involved overlaying multiple individual fields from a single section in Photoshop. For cleavage plane orientation, anaphase and telophase progenitors were identified in cortical sections contained with γ-tubulin and DAPI, and the angle between the line segregating daughter chromosomes and the ventricular surface was determined using the AxioVision imaging software. For analysis, division angles were grouped into bins at either 10 or 30° increments. Means and standard deviations were generated using Excel. Statistical significance was determined by a standard two-tailed Student's t test.

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We thank D. Sassoon and M. Kelley for Lp+/+ mice, S. Lanier and J. Blumer for the LGN antibody and pcDNA-hLGN plasmid, M. Mlodzik for pCS2+CFP-XStbm, and D. Von正面nor and M. Montcourtil for the Vangl2 antibody. We also thank J. Hebert and R. Krauss for critical comments on the manuscript.


References


