A new subtype of progenitor cell in the mouse embryonic neocortex

Xiaoqun Wang1,2, Jin-Wu Tsai1,2, Bridget LaMonica1–3 & Arnold R Kriegstein1,2

A hallmark of mammalian brain evolution is cortical expansion, which reflects an increase in the number of cortical neurons established by the progenitor cell subtypes present and the number of their neurogenic divisions. Recent studies have revealed a new class of radial glia–like (oRG) progenitor cells in the human brain, which reside in the outer subventricular zone. Expansion of the subventricular zone and appearance of oRG cells may have been essential evolutionary steps leading from lissencephalic to gyrencephalic neocortex. Here we show that oRG-like progenitor cells are present in the mouse embryonic neocortex. They arise from interkinetic divisions of radial glia and undergo self-renewing asymmetric divisions to generate neurons. Moreover, mouse oRG cells undergo mitotic somal translocation whereby centrosome movement into the basal process during interphase precedes nuclear translocation. Our finding of oRG cells in the developing rodent brain fills a gap in our understanding of neocortical expansion.

One of the most notable features in the evolution of the neocortex is the increase in neuron number that reaches its peak in the human brain1–4. Although the laminar organization of the cortex is relatively similar in all mammals, an expansion in cortical surface area underlies the transformation from smooth cortex to the highly folded primate neocortex, and the associated alteration of cortical architecture that is the substrate for the ‘higher’ cortical functions that distinguish Homo sapiens from other mammalian species5. This transition underscores the importance of understanding the process of neurogenesis in the developing neocortex.

Recent studies have identified two subtypes of neuronal progenitor cell in the developing rodent embryonic neocortex: radial glia and intermediate or basal progenitors6–11. Neuroepithelial cells located in the apical-most region, the ventricular zone, transform to radial glia cells at the onset of neurogenesis. In addition to their well-characterized function as a scaffold supporting neuronal migration12,13, radial glia constitute the main population of neural progenitor cells in the developing mammalian neocortex13–15. Radial glia show interkinetic nuclear migration (INM) and proliferate extensively at the luminal (that is, apical) surface of the ventricular zone16,17. The nuclei of newborn radial glia cells move away from the apical surface toward the basal lamina during the G1 phase of the cell cycle, undergo S phase at a basal location, and return to the apical surface during G2 to undergo mitosis at the ventricular lumen6,10,13. Thus, INM is responsible for the pseudostratified appearance of the ventricular zone. Notably, by moving interphase nuclei of radial glia cells away from the apical surface during G1, INM reserves the apical space for mitosis and thereby may promote an expansion of radial glia cell number18,19. During the peak phase of neurogenesis, around embryonic day 13 to 18 (E13–E18) in mice, radial glia cells predominantly undergo asymmetric division to self-renew while simultaneously giving rise either to a neuron or to an intermediate progenitor cell, the latter of which subsequently divides symmetrically to produce two neurons. Intermediate progenitor cells seem to lack apical–basal polarity9,11,20.

An evolutionary increase in size and functional complexity of the cerebral cortex has culminated in the modern human brain, which diverged from a rodent lineage ~100 million years ago4,18,21–23. Recent studies suggest that the development of oRG cells and their transit-amplifying daughter cells (that is, intermediate progenitor–like cells) may be the cellular mechanism underlying expansion in primate corticogenesis24. Beads coated with the lipophilic dye Dil applied to the pial surface of fixed fetal human cortical tissue have revealed oRG cells with radial glia–like morphology but lacking apical processes, and time-lapse imaging of fluorescently labeled human fetal brain slices shows that oRG cells can self-renew and produce neuronal precursors24. Unlike radial glia cells, oRG cells show distinctive mitotic somal translocation behavior instead of INM. It has been suggested that the outer subventricular zone (OSVZ) may be a primate-specific feature and a hallmark of primate corticogenesis18,25. But recent studies have shown that OSVZ progenitors (that is, oRG cells) also exist in a non-primate species with a gyrencephalic brain, the ferret26,27, which raises the question of whether oRG cells exist in lissencephalic species such as rodent, even though they have no cytoarchitectonically distinct OSVZ. Although the radial glia cells and intermediate progenitor cells of the ventricular zone and SVZ, respectively, are responsible for generating most cortical neurons in rodent6,10, extra sites of progenitor cell activity have been suggested including the subplate (the first layer of cortical neurons produced in the mammalian cerebral cortex), the cortical plate (future gray matter), the marginal zone and the extra-ventricular zone28–30, which prompted us to ask whether oRG-like cells exist in the developing mouse neocortex. Furthermore, the origin of this distinct cell type remains unknown because of the

1Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California San Francisco, San Francisco, California, USA. 2Department of Neurology, University of California San Francisco, San Francisco, California, USA. 3Neuroscience Graduate Program, University of California San Francisco, San Francisco, California, USA. Correspondence should be addressed to X.W. (wangx2@stemcell.ucsf.edu) or A.R.K. (kriegsteina@stemcell.ucsf.edu).

Received 9 September 2010; accepted 17 March 2011; published online 10 April 2011; doi:10.1038/nn.2807
Figure 1 oRG cells in the developing mouse neocortex. (a) Labeling of radial glia and oRG-like cells (white arrows) with adeno-GFP. Note the oRG-like cell (box 1) that has a long basal process (open arrowheads) but no apical process. Red arrowhead, presumed oRG daughter cell. High-magnification images are shown at right (1 and 2). IZ, intermediate zone; VZ, ventricular zone. Scale bars, 50 μm (left) and 15 μm (right). (b) Representative oRG-like cell (arrow); open arrowheads indicate the basal process. CP, cortical plate. Scale bar, 25 μm. (c) Phosphovimentin (green) labels oRG cells in mitosis. The basal process has varicosities characteristic of M phase oRG cells. The oRG-like cells stain with radial glial progenitor markers Pax6 (blue) and Sox2 (red). Arrows indicate triple-positive oRG-like cells; open arrowheads indicate basal process. Scale bars, 50 μm (left) and 10 μm (right). (d) Quantification of the percentage of mitotic, basal process bearing oRG-like cells identified as P-vim+Pax6+Sox2+ by immunostaining in the VZ (92.95 ± 5.90%) and superficial SVZ plus IZ (7.05 ± 3.70%) (total 78 cells from six mice). (e) P-vim+ (green) oRG cells at E16.5 stain for Sox2 (red) but are Tbr2− (blue; an intermediate progenitor marker). High-magnification images of the representative outlined cell are shown to the right. Arrows indicate oRG-like cells co-stained for P-vim (green) and Sox2 (red); open arrowheads indicate the basal process. Scale bars, 50 μm (left) and 10 μm (right). Error bars, s.e.m.

Results

oRG-like cells exist in mouse neocortex

Recent studies have shown that radial glia–like cells referred to as oRG abundantly populate the OSVZ of the fetal human brain and account for between 40% and 75% of all proliferating cells.24 They are also present in the cortex of the ferret, a carnivore that also has an expanded gyrencephalic brain.26-27 These findings raise the question of whether OSVZ progenitors are specific to gyrencephalic species or whether they also exist in lissencephalic mammals. To examine this possibility, we introduced green fluorescent protein (GFP)-expressing adenoovirus (adeno-GFP) into the lateral ventricle of developing neocortex of E12.5 mouse embryos by in utero injection.10 Searching for GFP+ cells with oRG morphology 2 d after infection, we observed GFP+ monopolar cells located in the superficial or outer region of the SVZ (Fig. 1a). Similar to oRG cells in human fetal brain,24,26,27 the mouse cells had a long basal process but not an apical process (Fig. 1) so that they do not make contact with the ventricle (Fig. 1a). The morphology of these cells suggested that oRG-like cells might exist in the outer SVZ of the rodent brain. Moreover, in sparsely labeled slices we occasionally observed pairs of GFP+ cells (4 out of 25) close to each other in the region between the SVZ and intermediate zone, where one cell often had a long basal process and the adjacent cell was rounded, suggesting that the oRG-like cell might have divided (Fig. 1a).

We used immunohistochemistry to examine whether cells with oRG-like morphology also expressed the transcription factors Pax6 and Sox2, neural stem or progenitor cell markers that are expressed by human and ferret oRG cells.24,26-27 We also studied phosphovimentin (P-vim), which marks the cytoplasm of neural progenitor cells in M phase of the cell cycle and helps to reveal their morphology. We observed Pax6+Sox2+P-vim+ cells located in the intermediate zone and the region adjacent to the SVZ (Fig. 1c). Among triple-positive cells, 93% were located in the ventricular zone and only 7% were located in the superficial SVZ (Fig. 1d). Most of the triple-positive cells (67 out of 78, at E16) had a basal process revealed by the P-vim antibody (Fig. 1c,e). Furthermore, P-vim+ cells with a basal process always expressed the radial glia cell markers Sox2 and Pax6 (67 out of 67, E16), indicating that the monopolar cells are progenitor cells. Tbr2 (T-box brain 2) is a T-domain transcription factor that is specifically expressed by intermediate progenitor cells during development. Intermediate progenitor cells are transit-amplifying progenitors arising from radial glia that can be characterized by expression of Tbr2 and by concurrent downregulation of Pax6. Intermediate progenitor cells divide symmetrically within the ventricular zone or SVZ and generate a strictly neuronal population. We used Sox2, Tbr2 and P-vim expression to further classify the cortical progenitors we observed. All of the P-vim+ cells with oRG-like morphology in the outer region of the SVZ were Sox2−Tbr2+ (Fig. 1c; total of 46 cells from six mice), indicating that the oRG-like cells in the mouse resembled radial glia cells and not intermediate progenitors.

To examine the divisions of oRG-like cells directly, we used a low-titer GFP-expressing retrovirus (retro-GFP) to transfec t only dividing cells. We injected the retrovirus into the lateral ventricle of E12.5 mouse embryos by in utero injection as described.10,11 Observations of division of the GFP-labeled oRG-like cells with long
basal but no apical process confirmed that they were actively dividing progenitor cells (Fig. 1b). Moreover, a low density of dividing progenitors located in the intermediate zone and outer SVZ was revealed by phospho-histone H3 immunostaining with Sox2 and Pax6 co-labeling (Supplementary Fig. 1). These results indicate that the developing mouse cortex contains a subtype of actively dividing progenitor cell that maintains process contact with the pia but not the ventricle. On the basis of their oRG-like morphology and expression of molecular markers characteristic of radial glia cells, we tentatively identified these cells as mouse oRG cells to set them apart from traditional radial glia in the ventricular zone and intermediate progenitor cells in the SVZ.

Mouse oRG cells undergo mitotic somal translocation

INM is a characteristic feature of neuroepithelial and radial glia cells. The apical anchoring of the centrosome within the ventricular endfoot serves as the cellular mechanism for INM by maintaining cell polarity, orienting the microtubule minus ends apically to direct dynein motor protein migration and asymmetric division of radial glia cells. Recent studies in human fetal brain show that oRG cells show a distinctive behavior before mitosis, referred to as mitotic somal translocation (MST), in which the cell body moves rapidly along the basal fiber. The similar morphological and molecular characteristics of mouse and human oRG cells prompted us to ask whether mouse oRG cells behave similarly. To test this, we first developed an assay to specifically visualize oRG cells in the mouse (Fig. 2a).

Figure 2 oRG cells undergo mitotic somal translocation. (a) Experimental procedure for time lapse. (b) oRG-like cells undergo mitotic somal translocation before mitosis (see Supplementary Movie 1). Arrows indicate oRG-like cells (white) and a non-oRG daughter (red). Asterisks indicate the characteristic swelling in the proximal basal process. Dashed line indicates the cleavage plane. Scale bar, 20 μm; time stamp, h.min. (c) Representative image showing method for measuring mitotic somal translocation distances. Average distance, 23.56 ± 1.56 μm (from 114 time-lapse sequences). (d) Dual-labeled oRG cell (box, cell 1) 1 d after electroporation in utero at E13.5. Right, high-magnification images of mitotic cell behavior from the outlined regions. IZ, intermediate zone; VZ, ventricular zone. Scale bars, 50 μm (left) and 10 μm (right). (e) Three-dimensional illustration of oRG cell distribution pattern in E16 brain. Yellow rings indicate the locations of mouse oRG cells. Blue, Pax6; green, P-vim; red, Sox2. (f–i) Quantification of the percentage of Pax6+/P-vim+/Sox2+ cells located in the outer region of ventricular zone and SVZ versus total triple-positive cells located in the entire developing neocortex. *P < 0.05; **P < 0.005; ***P < 0.001; error bars, s.e.m.

We found that GFP retrovirus–labeled oRG cells spontaneously divided (Fig. 2b and Supplementary Movie 1) and showed the same distinctive behaviors as human oRG cells. The cell body moved rapidly along the basal process, led by a swelling within the process (Fig. 2b). The pattern of somal translocation was reminiscent of behavior observed in migrating neurons. The duration of oRG cell body translocation was usually less than 1 h, which is similar to the duration observed in human oRG cells. However, the translocation distance of oRG cell bodies was around 25 μm (Fig. 2c), which is shorter than that of human oRG cells, which average 57 μm (ref. 24). Of ~114 oRG cell divisions we observed, all but 2 cells divided with a horizontal cleavage plane (parallel to the ventricular surface). This plane of division ensures that the basal daughter cell inherits the basal fiber and maintains oRG morphology while the apical daughter does not, consistent with asymmetric oRG cell division (Fig. 2b). The two cells that did not divide horizontally gave rise to two similar daughter cells, both of which had oRG morphology and basal fibers (Supplementary Fig. 2).

The mitotic somal translocation of oRG cells contrasts with the INM of radial glia cells, in which the nucleus moves apically and mitosis occurs at the ventricular surface (Supplementary Fig. 3a and Supplementary Movie 2), and with the mitotic behavior of intermediate progenitor cells, which divide in place without nuclear translocation (Supplementary Fig. 3b and Supplementary Movie 3). To explore this further, we used a plasmid encoding human histone H2B, a protein that enables sensitive analysis of the nucleus in living mammalian cells, fused with the red fluorescent protein DsRed-Express (DsRed-H2B) and electroporated this with the plasmid pCAG-GFP, encoding GFP that diffuses throughout cells and thereby reveals their morphology. These constructs were electroporated into the developing mouse neocortex at E13.5 (Fig. 2d). We were
oRG cells distribute sparsely in developing neocortex

We next asked how numerous are oRG cells during mouse neocortical development and where are they located. To address these questions we mapped the location of Pax6\(^+\) P-vim\(^+\) Sox2\(^+\) triple-positive cells with basal processes at four embryonic ages (E12, E14, E16 and E18) in the rostro-caudal and medio-lateral axes (Fig. 2e–i and Supplementary Fig. 4). We compared the distribution of oRG cells located in the superficial SVZ and intermediate zone versus total triple-positive cells located in the entire developing neocortex (Fig. 2e–i and Supplementary Fig. 4b–d). At E12, the SVZ was present throughout the ventro-medial extent of the cortical wall, although no cortical plate was detected medially. At this age we observed oRG cells (cells that were Pax6\(^+\) P-vim\(^+\) Sox2\(^+\) with basal process) located in the superficial SVZ and inner intermediate zone (Fig. 2f and Supplementary Fig. 4b). oRG cells represented 5.35 ± 1.25\% (n = 200), 7.64 ± 2.06\% (n = 157) and 4.31 ± 1.80\% (n = 209) of mitotic progenitors in, respectively, the rostral, intermediate and caudal cortex. More oRG cells were observed in the dorso-lateral (3.65 ± 1.70\%, n = 411) than dorso-medial cortex (2.24 ± 2.15\%, n = 402). At E14, the proportions of oRG cells increased compared to E12, with 6.09 ± 1.65\% (n = 115) in rostral cortex and to 2.04 ± 0.51\% (n = 49) in caudal cortex (Fig. 2i and Supplementary Fig. 4d).

At all stages, a similar distribution pattern of oRG cells was observed, with a lateral to medial developmental gradient (Fig. 2f–i). At E12, the proportion of oRG cells in lateral cortex comprised 3.65 ± 1.70\% (15 out of 411) of all progenitors and in the medial cortex 2.24 ± 2.15\% (9 out of 402) (Fig. 2f). At E14, lateral oRG cells accounted for 5.06 ± 0.85\% (18 out of 356) compared to 3.58 ± 1.24\% of medial oRG cells (12 out of 335) (Fig. 2g). At E16, oRG cells showed a more lateral (10.06 ± 1.54\%, 32 out of 318) than medial (5.03 ± 1.10\% 15 out of 298) location (Fig. 2h). At E18, we still detected 6.11 ± 1.78\% (8 out of 131) oRG cells laterally, but a marked reduction medially to 2.03 ± 1.02\% (6 out of 296) (Fig. 2i). These results suggest that oRG cells are relatively rare at all ages, but show a general lateral to medial spatial gradient with the density of oRG cells highest in intermediate cortex, less in rostral cortex and lowest in caudal cortex at all stages of embryonic neocortical development.

oRG cells generate neurons

We next examined whether oRG cells generate neurons. Taking advantage of the basal processes of oRG cells, we developed a new assay using in \textit{utero} pial surface injection of adeno-GFP to retrogradely label oRG cells close to the cortical plate (Fig. 3a) and started long-term (days long) time-lapse imaging 2 d after injection. Successfully labeled oRG cells were identified by their monopolar morphology (Supplementary Fig. 5a). We observed asymmetric, self-renewing, oRG cell divisions, in which the apical daughter cell acquired neuronal morphology over the subsequent ~40 h, including a leading process oriented toward the pia (Fig. 3b and Supplementary Movie 4). After acquiring a short trailing process,
the bipolar daughter cells migrated radially to the cortical plate with speeds (0.11 ± 0.02 μm min\(^{-1}\)) similar to those of neighboring migrating neurons, identified by their morphology (0.12 ± 0.03 μm min\(^{-1}\))\(^{31}\).

To further explore whether oRG cells produce neurons, we monitored oRG cell divisions in real time and then determined daughter cell fate 12 or more hours later by immunostaining with cell type–specific markers. A commitment to the neuronal lineage was assessed by the expression of NeuN or βIII-tubulin (Tuj1). In most of the oRG cell divisions (13 out of 17), the apical daughter cell expressed NeuN or Tuj1 (Fig. 3c,d and Supplementary Fig. 5b). In all cases, the basal daughter cells inherited the basal fiber and expressed Pax6 (Fig. 3c,d). In four cases, daughter cells were unlabeled, possibly owing to limitation of antibody penetration. These results demonstrate that oRG cells divide asymmetrically to self-renew and give rise to neurons.

**oRG cells originate from radial glia cells**

We previously hypothesized that human oRG cells originate in the ventricular zone and use mitotic somal translocation to move into the SVZ24. However, owing to technical limitations this has not been demonstrated in the human fetal brain. Having identified oRG cells in the developing mouse neocortex, we next examined their origin. We used retro-GFP to label radial glia cells in E11.5 embryos by *in utero* intraventricular injection as described10,11. At E13.5 we removed the embryos and prepared organotypic brain slice cultures for time-lapse imaging 11,17,33.

To examine centrosome behavior, we introduced plasmid DsRedex-fused to centrin, a central component of the centrosome, into the developing neocortex of E13.5 mouse embryos by *in utero* electroporation17,33. Six dividing oRG cells from independent experiments proceeded through mitosis in the outer SVZ and reached the two-cell stage. In all cases, we found movement of the centrosome into a varicosity in the basal process at interphase, followed by movement of the nucleus in M phase (Fig. 5a and Supplementary Movie 7), suggesting that the centrosome is involved in the maintenance of oRG cell polarity.

**Distinct centrosome dynamics of oRG cells**

Given that the centrosome is required for the maintenance of radial glia cell progenitors in the ventricular zone16,17, we explored the cellular mechanism of oRG self-renewing asymmetric division by analyzing the distribution and dynamics of centrosome behavior in oRG cells. We electroporated pCAG-YFP plasmid, encoding yellow fluorescent protein, or injected retro-GFP to reveal cell morphology.

We previously hypothesized that human oRG cells originate in the ventricular zone and use mitotic somal translocation and divides; white and red arrowheads follow the two daughter cells after oRG division (see Supplementary Movie 6). Scale bar, 10 μm. (b) Lineage tree of radial glia and oRG cell divisions. Radial glia cells can divide asymmetrically to self-renew and generate oRG cells. Both progenitors can divide again to self-renew and generate daughter cells including neurons (N) and intermediate progenitors (IP).
Our data indicate that mitotic somal translocation is one of the defining features of oRG cells in mouse as well as in human, with the centrosome situated in a varicosity in the basal process. This feature is reminiscent of centrosome behavior in migrating neurons in which the centrosome also moves into a varicosity in the leading process, followed by the saltatory movement of the nucleus.

We hypothesized that a subcellular mechanism of centrosome positioning regulates mitotic somal translocation (Fig. 5a and Supplementary Movie 7). To study centrosome behavior in all three subtypes of progenitors in mouse neocortex, we monitored centrosome positioning and behavior in radial glia and intermediate progenitor cells. The positioning of the centrosome in the endfoot at the ventricular zone surface helps explain why radial glia cells go through interkinetic nuclear migration. In all seven dividing radial glia cells with centrosome labeling, the centrosome divides, and one daughter centrosome migrates back to the ventricular endfoot of the self-renewed radial glia cell, while the other daughter centrosome migrates away from the ventricular zone with the non-radial glia daughter cell as previously reported (Fig. 5b and Supplementary Movie 8). Furthermore, we found that centrosomes remain within the intermediate progenitor cell body throughout the cell cycle of intermediate progenitor cells (n = 7); consistent with the observation that intermediate progenitor cells remain stationary when they divide (Fig. 5c and Supplementary Movie 9). Taken together, these data strongly suggest that specific centrosome positioning underlies mitotic somal translocation.

**DISCUSSION**

Our finding of mitotic oRG cells provides a new lineage for neurogenesis in the rodent cortex. Real-time imaging data show that OSVZ progenitors are generated directly from radial glia cells in the ventricular zone, and daughter oRG cells migrate away to the superficial SVZ by mitotic somal translocation. Because the number of oRG cells is very low, the contribution of oRG cells to neurogenesis and cortical layer formation in rodents is small. This may help explain why genetic mutations causing strong microcephaly phenotypes in human (ASPM mutations, for example) do not necessarily cause the same severe phenotype in rodents. Moreover, although mouse oRG cells share two defining features (namely, morphology and mitotic somal translocation) with human oRG cells, time-lapse imaging reveals an important difference between those two cells: namely that mouse oRG cells undergo self-renewing asymmetric division to generate neurons directly, whereas human oRG cells generate transit-amplifying cells (that is, intermediate progenitor cells) that in turn generate neurons.

Our data also demonstrate that during mitotic somal translocation, the oRG centrosome moves into a varicosity in the basal process at interphase, and the nucleus follows before mitosis. In this way oRG cell mitosis shares features of both neuronal migration and the G2 phase of interkinetic nuclear migration characteristic of neuroepithelial cell division. Given that centrosomes anchored in the ventricular (apical) endfeet of radial glia cells are a defining feature of the asymmetric division of radial glia cells, these data imply an important role of the centrosome in establishing oRG cell polarity. oRG cells go through asymmetric division with the cleavage plane perpendicular to the apico-basal axis, whereas radial glia cells undergo asymmetric division with a cleavage plane parallel to the apico-basal axis, implying that it is not cleavage plane orientation but possibly centrosome positioning that determines asymmetric division of oRG cells. Lack of...
apical polarity proteins (such as Par3) in human and ferret OSVZ progenitors raises the question of whether the asymmetric inheritance of mother versus daughter centrosome is necessary for ORG cell asymmetric division and fate specification, as it is in radial glia cells. The results presented here suggest that a specific new subtype of progenitor cell exists in the superficial SVZ of mouse neocortex. Expression of cell fate markers, morphological features and mitotic behavior link this progenitor with an OSVZ-like progenitor cell, the ORG cell, recently described in the developing human and ferret neocortex. Progenitors in this region of developing mouse cortex were observed a decade ago but had not been well characterized. It has been suggested that OSVZ-like cells may be primate specific and contribute to expansion of the neocortex in human and gyrencephalic species. However, the observation of ORG-like cells in mouse, a lissencephalic species, suggests that ORG cells are not unique to gyrencephalic brain development. The appearance of ORG cells in rodents may nonetheless foreshadow human cortical expansion. One may speculate that ORG-like cells arose in an ancestral rodent-like animal about 100 million years ago and selective pressures led to an expansion in their numbers in the lineages leading to primates. Features such as an increase in the number of progenitor cell cycles and the appearance of an intervening transit-amplifying type of cell may have provided further mechanisms to increase neuron number during neocortical evolution. It would be interesting to study the cortical development of more species with gyrencephalic or lissencephalic brains to obtain a more complete understanding of how progenitor cell behavior contributes to the architecture of the developing neocortex.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We thank A. Alvarez-Buylla, D. Lim, C. Harwell, W.P. Ge and L. Fuenteaiba for comments on the manuscript and members of the Kriegstein laboratory for discussions. We thank W. Walantu and Y.Y. Wang for mouse surgery and technical support and D.V. Hansen and J.H. Lui for ideas and discussion. We thank E. Gage (Salk Institute) for GFP-retrovirus reagents. This work was supported by grants from the Bernard Osher Foundation and the US National Institute of Neurological Disorders and Stroke (to A.R.K.)

AUTHOR CONTRIBUTIONS

X.W. conceived the project and carried out most of the experiments. J.-W.T. helped on some of the time-lapse imaging experiments and B.L. helped on the immunohistochemistry staining procedure. X.-W. analyzed data, interpreted results and wrote the manuscript. A.R.K., as the principal investigator, provided conceptual and technical guidance for all aspects of the project. All authors edited the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Plasmids and in utero electroporation. DsRedex cDNAs were obtained by PCR and cloned into pEGFP-H2B-C1 (ref. 36) to replace EGFP in generating the DsRedex-H2B plasmids. In utero electroporation was performed as previously described27. In brief, a timed pregnant Swiss Webster mouse at E13.5 was anesthetized, the uterine horns were exposed, and ~1 µl of plasmid DNA (1–3 µg µl⁻¹) mixed with fast green (Sigma) was manually microinjected through the uterus into the lateral ventricle, using a beveled and calibrated glass micropipette (Drummond Scientific). For electroporation, five 50-ms pulses of 40–50 mV with a 950-ms interval were delivered across the uterus with two 9-mm electrode paddles positioned on either side of the head (BTX, ECM830). After the procedure, the uterus was placed back in the abdominal cavity and the wound was surgically sutured. The mouse was then placed in a 28 °C recovery incubator under close monitoring until she recovered and resumed normal activity. All procedures for animal handling and usage were approved by our institutional research animal resource center.

Retroviral and adenoviral in utero infection. Replication-incompetent GFP-expressing retrovirus was produced from a stably transfected packaging cell line (293gp NIT-GFP; a kind gift F.H. Gage). CMV-GFP adenovirus was acquired from Vector Biolabs. Mice were maintained according to protocols approved by the Institutional Animal Care and Use Committee at the University of California at San Francisco. Uterine horns of E11.5–E13.5 gestation stage pregnant Swiss Webster mice (Charles River Laboratories) were exposed in a clean environment. Retrovirus or adenovirus (~1.0 µl) was manually microinjected into intermediate zone of the cultured brain slice, using a beveled and calibrated glass micropipette (Drummond Scientific). All the time-lapse images were acquired using an inverted Leica TCS SP5 with an on-stage incubator (while streaming 5% CO₂, 95% O₂) and a ×40 air objective lens. Supplementary Movies 10 and 11 demonstrate that oRG mitotic behavior is not an artifact of brain sectioning.

Immunohistochemistry and confocal imaging. Mouse brains or cultured slices were fixed in 4% PFA in PBS at 4 °C overnight, incubated for 1 h at 24 °C in a blocking solution (10% (vol/vol) normal goat or donkey serum as appropriate, 0.1% (vol/vol) Triton X-100, and 0.2% gelatin in PBS), followed by incubation with the primary antibodies 3 d at 4 °C. Sections were then washed in 0.1% (vol/vol) Triton X-100) Triton X-100 in PBS and incubated with the appropriate secondary antibody for 1–2 h at 24 °C. The primary antibodies used were mouse monoclonal anti-β-III tubulin (clone TUJ1) (Covance, 1:500), rabbit polyclonal anti-Pax6 (Covance, 1:500), rabbit polyclonal anti-Tbx2 (Millipore/Chemicon, 1:500), goat anti-Sox2 (Santa Cruz sc-17320, 1:200) and mouse anti-phospho-ventin (MBL International D076-3s (Ser55) or D095-s (Ser82), 1:500). Secondary antibodies used were Alexa Fluor 488 (1:1,000), 546 (1:100), or 647 (1:1,000) conjugated to donkey anti-mouse, anti-rabbit or anti-goat (Invitrogen). DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI; Molecular Probes). Images were acquired with a Leica TCS SP5 broadband laser confocal microscope and analyzed with LCS confocal software (Leica), Imaris imaging software (Bitplane), Velocity (ImproVision) and Photoshop (Adobe). Data are presented as mean ± s.e.m., and Student’s t-test were used for statistical significance estimation.

Brain sectioning, cortical slice culture, viral infection in slices and time-lapse imaging. Brains were dissected out into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 5 KCl, 1.25 NaH₂PO₄, 1 MgSO₄, 2 CaCl₂, 25 NaHCO₃ and 20 glucose; pH 7.4, 310 mOsm l⁻¹. Brains were embedded in 4% low-melting-temperature agarose in ACSF and sectioned at 400 µm using a vibratome (Leica Microsystems). Brain slices then were transferred onto a slice culture insert (Millicell) in a glass-bottom Petri dish (MatTek) with culture medium containing (by volume) 66% Eagle’s basal medium, 25% Hanks balanced salt solution, 5% FBS, 1% N-2 medium, 1% penicillin/streptomycin/glutamine (all from Gibco) and 0.66% (wt/vol) d-(+)-glucose (Sigma). Cultures were maintained in a humidified incubator at 37 °C with constant 5% CO₂ supply. For experiments shown in Figure 2a,b, GFP-expressing retrovirus (1 × 10⁹ colony forming units) was manually microinjected into intermediate zone of the cultured brain slice, using a beveled and calibrated glass micropipette (Drummond Scientific). All the time-lapse images were acquired using an inverted Leica TCS SP5 with an on-stage incubator (while streaming 5% CO₂, 95% O₂) and a ×40 air objective lens. Supplementary Movies 10 and 11 demonstrate that oRG mitotic behavior is not an artifact of brain sectioning.

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