Supplementary Data

Supplementary methods

Animals. Six macaque monkey embryos were used for the immunohistochemistry (IHC) electron microscopy (EM) study at E37, E42, E45 (n=2), E61 and E65, collected through cesarean sections performed by veterinarians at Yale University. Brain was removed and immersed in the fixative containing 4% paraformaldehyde, 0.2% picric acid, and 0.2% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) during 4 days at 4C.

Immunohistochemistry. Coronal 80-μm-thick slices were cut by a vibratome, cryoprotected in 30% sucrose, freeze-thawed with liquid nitrogen, and processed for immunolabeling with rat anti-GFAP (Invitrogen, Eugene, OR, USA; dilution 1:5,000), or rabbit anti-GFAP (Dako Cytomation, Denmark; dilution 1:1,000) polyclonal antibodies overnight at room temperature. Then, the slices were immersed in solution of biotinylated goat anti-rabbit or goat anti-rat antibodies (both Jackson Immunoresearch Inc., West Grove, PA; 1:300) and developed by the Elite ABC kit (Vector Laboratories, Burlingame, CA) with Ni-intensified 3,3'-diaminobenzidine-4HCl (DAB) as a chromogen, post-fixed with 1% OsO_4 and embedded in Durcupan (ACM; Fluka, Buchs, Switzerland) on microscope slides. Controls in which the primary antibody was replaced with normal rat serum showed absence of staining (see Fig. S1 and suppl. Table 1, cells NC1, NC2).

Electron microscopy and 3D reconstruction were performed as we previously described in detail [41-43]. Selected neocortex segments from caudal, lateral or rostral neocortex were cut into 70-nm-thick sections. Series of 150-200 consecutive sections were collected and evaluated in a JEOL 1010 electron microscope equipped with Multiscan 792 digital camera (Gatan, Pleasanton, CA, USA). Serial images of mitotic or arbitrarily selected interphase cells were made at 12,000x or 15,000x magnification and three dimensional reconstruction and volume estimation of cell bodies, their processes and anti-GFAP DAB deposits were performed using Reconstruct software [119], publicly available at http://www.bu.edu/neural/Reconstruct.html. We reconstructed 46 dividing cells and 5 interphase cells from GFAP stained tissue, and 2 dividing cells from negative control tissue.

Methodological considerations

For estimation of the content of GFAP in the studied cells, we quantified the volume of the cells and the volume of the immunoreaction end-product. We performed systematic analysis of uninterrupted serial sections with complete 3D reconstruction of the cell body and proximal

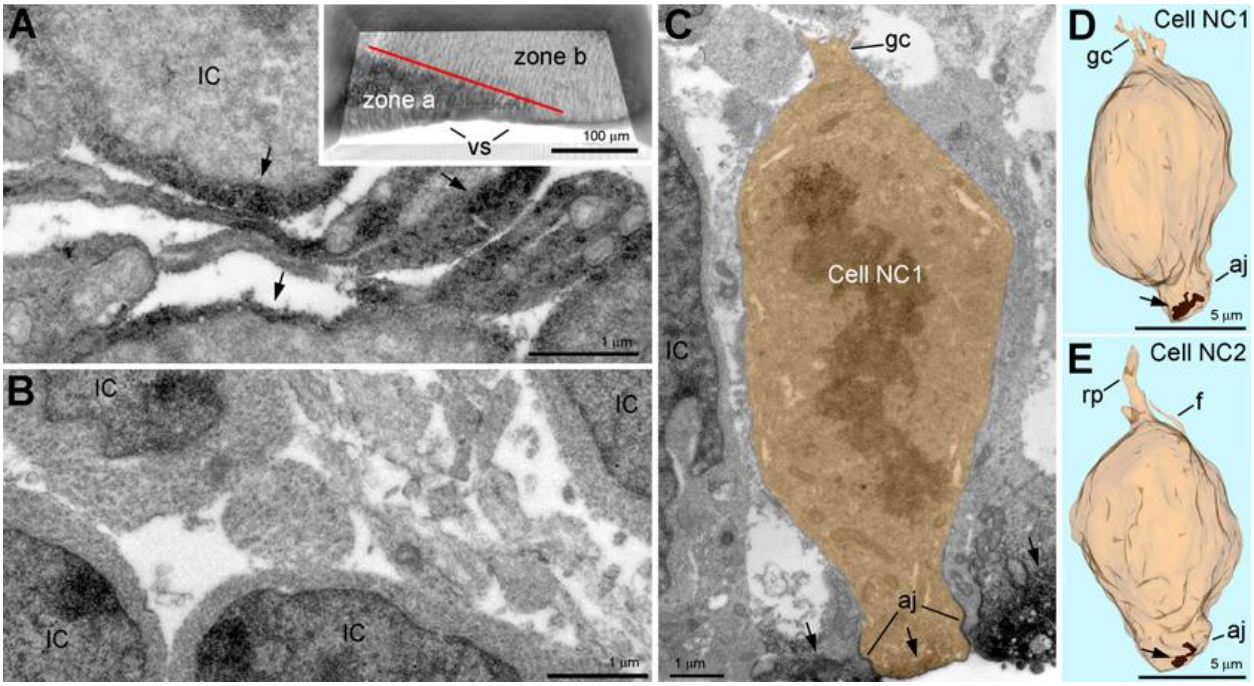
segments of their basal processes. However, immunohistochemistry has specific caveats related to antibody specificity, reagent penetration and possible batch effects that may bias the results. Therefore, we performed a series of analysis intended to asses this limitations.

First, given the low level of staining in some cells, we used negative controls, replacing the primary antibodies with normal serum (Fig. S1). The results showed non-specific DAB precipitate both at light and electron microscopic level in cells located on the very surface of the slice, but this widespread staining disappeared when we studied the tissue located slightly below the surface (Fig. S1A, B). The only staining found was located in the apical aspect of the cells, due to exposure to the ventricular space (Fig. S1C, E). This pattern of staining was easily disregarded as artifactual, as it was different to the one observed when using the antibody, in which most of the labeling was present in the basal aspect of the cell.

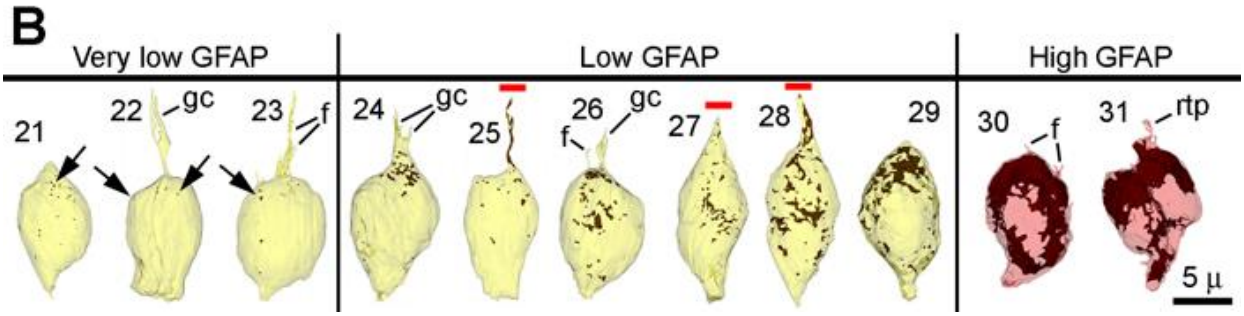
Second, to assess possible bias due to antibody penetration we compared the staining in cells located superficially or deep in the tissue. Some of the cells located deep in the tissue showed very low levels of GFAP labeling (Fig. S2 cells 21-23) that were below the levels found in cells located more superficially. However, the rest of cells showed similar levels and range of variation found in superficially located cells. Therefore, we performed all the comparisons and correlations excluding those eleven cells located deeply, to asses possible bias in the interpretation of the data. We did not find relevant changes in the differences by age or in the correlation of GFAP levels and mitotic phase (Fig. S2), apart from expected decrease in the effects due to reduced sample size. Therefore, we show and discuss the results considering all 46 cells analyzed.

Third, we further confirmed the specificity of the immunolabeling using two alternative GFAP antibodies (produced in rabbit and rat) that identified similar patterns of labeling and also heterogeneous degrees of GFAP accumulation in different cells (Suppl. Table 1).

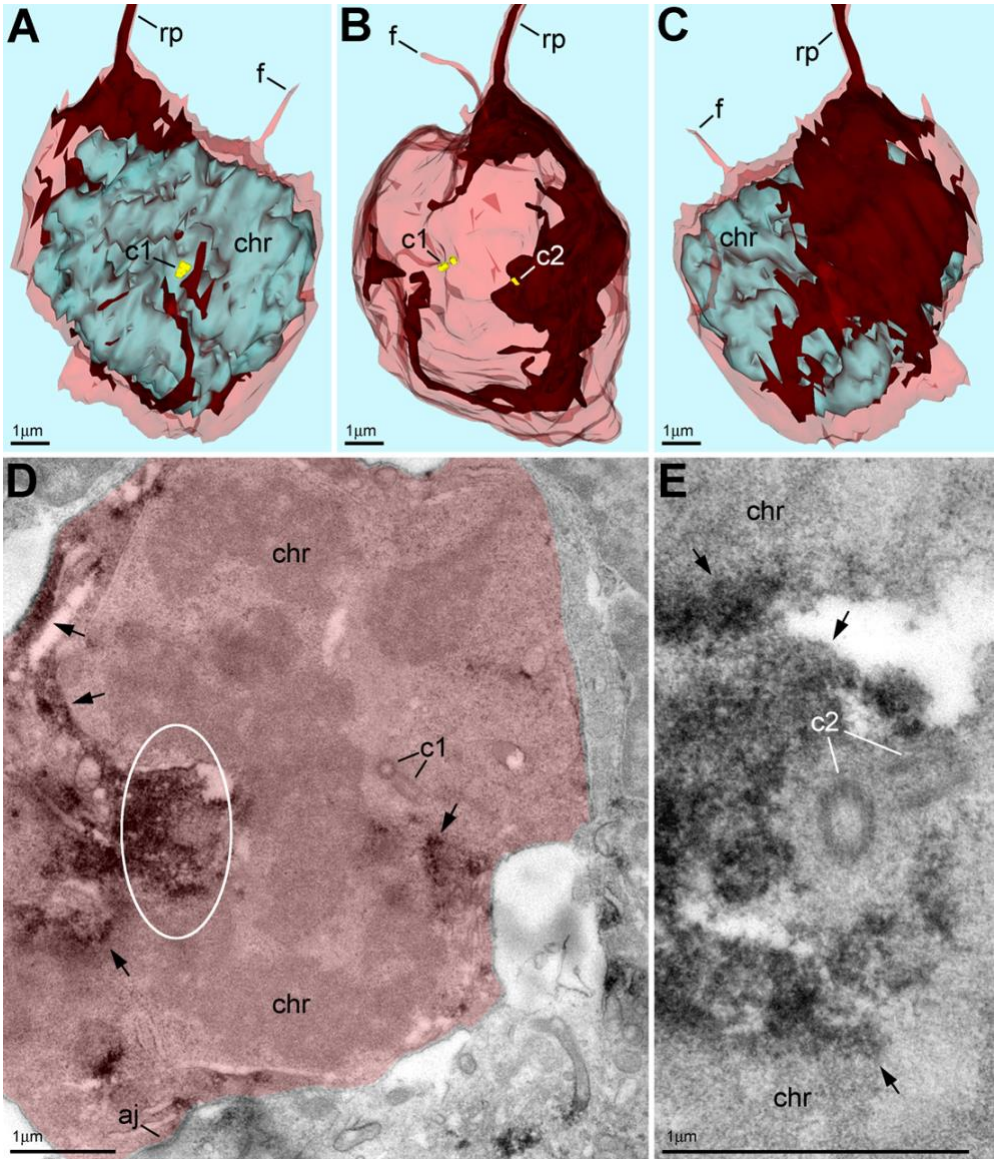
Thus, we could conclude that our study relied on specific GFAP immunostaining, with minor influence due to antibody penetration that did not interfere with our interpretation of the data.



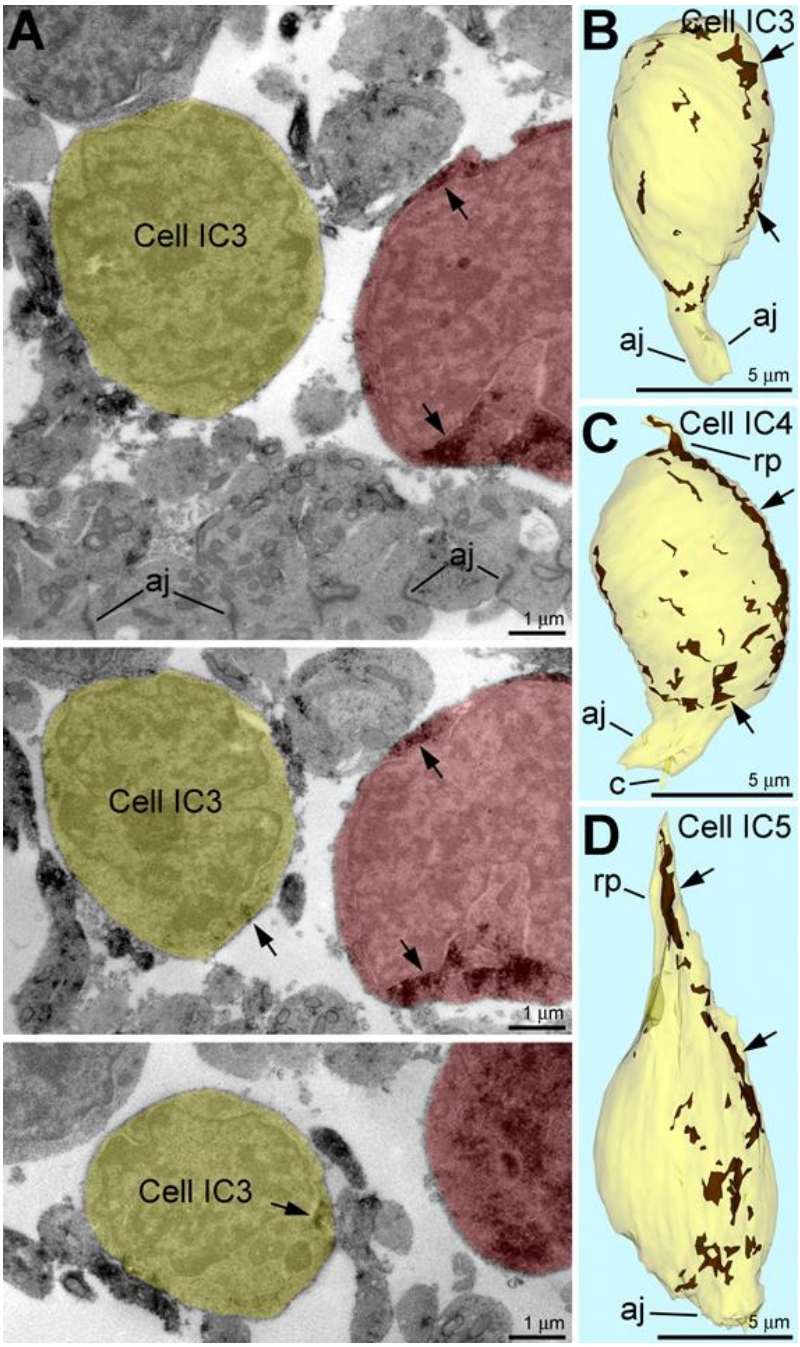
Suppl. Fig. 1 Negative control with GFAP antibodies replaced with normal rat serum in E45 rhesus monkey embryo. Insert in A shows a specimen trimmed as trapezoid for cutting of ultrathin sections. Zone “a” is the very surface of the slice with non-selective staining visible in all cell bodies and processes (arrows) in A. Zone “b” is located 2-3 micron deeper from the surface of the slice, and shows many cells lacking staining, as illustrated in (B). C-E. Electron microscopy analysis with 3D reconstruction of two mitotic cells (semitransparent orange; cell codes are indicated according to Table S1) situated ~3-10 µm deep in the slice demonstrates minor intra-cellular DAB precipitation (dark brown; arrows) near the ventricular surface whereas the remaining of the cell is negative. Abbreviations: aj, adherens junction; f, filopodium; IC, interphase cell nucleus; gc, growth cone-like process; rp, truncated radial process; vs, ventricular surface



Suppl. Fig. 2 Assessment of differences in penetration of the antibody. Montage of 3D reconstructed mitotic cells (basal is up) located deep in slices from VZ of E45 immuno-labeled for GFAP (brown). Some cells (# 21-23) showed very low levels of GFAP labeling (arrows) suggesting possible uneven penetration of the antibody, while the rest (#24-31) showed similar levels to cells located more superficially (see also Fig. 3). To assess the possible bias in our quantitative analysis, we repeated it after excluding these 11 cells located deep in the tissue. The remaining 35 cells showed similar differences in average GFAP content between E45 (3.5%) and E61/65 (6.9%) while the correlation between GFAP content and mitotic phase was reduced (rho= 0.38, p (two tailed)=0.023) as expected after decreasing the number of cases.



Suppl. Fig. 3 Asymmetric distribution of GFAP between putative daughter cells in anaphase stage from cerebral VZ of E65 monkey embryo. (A-C) Three images of the reconstructed cell body rotated 90 degrees. The conglomerate of chromosomes (chr; depicted blue) is omitted in B. GFAP depositions (brown) are lower around one of the centrosomes (c1) and higher around the other one (c2). (D, E) Electron micrographs of the reconstructed cell (highlighted red) exemplify different amounts of anti-GFAP reaction end-product depositions (arrows) around centrosomes 1 and 2. White ovoid indicates approximate position of centrosome 2 shown in another serial section in E. This cell and others showing asymmetric division are also illustrated in Fig. 2 as cells #41, 42 and 43. Abbreviations: aj, adherens junction; f, filopodium; rp, radial process



Suppl. Fig. 4 GFAP-positive interphase cells from E45 rhesus monkey (Cell IC3, IC4 and IC5 in Table S1). A. Serial micrographs show that Cell IC3 (semitransparent yellow) contains relatively low amount of GFAP (arrows) whereas an adjacent cell (semitransparent red) is immunopositive in all the sections. B-D. 3D reconstructed Cells IC3, IC4 and IC5 (yellow) show relatively low GFAP content (brown; arrows). Images B-D show basal side up. Cell IC3 has

smooth surface; cells IC4 and IC5 are emitting radial processes (rp) that are truncated in the series of sections. Abbreviations: aj, adherens junction; c, cilium