Supplemental Material.

Additional Methods

Vessels-neuroblasts analysis: 3 days after CTG injection P24 mice were anesthetized and transcardially perfused with 0.9% saline followed by 5ml of 1% then 5ml of 5% porcine type-A gelatin containing 1mg/5ml bovine serum albumin tetramethyl-rhodamine isothiocyanate (BSA-rhodamine, Sigma). The brains were immersion post-fixed in 4% paraformaldehyde in 0.1 M PB (pH 7.4) for 16 hours, cryoprotected in 30% sucrose in 0.1 M PB for 48 h, embedded in OCT cutting compound, frozen, and free-floating serial coronal sections (40 μm) cut on a cryostat. Sections were sequentially incubated in 1% bovine serum albumin and 1% normal donkey serum in 0.01M phosphate buffered saline, pH 7.4, 0.3% Triton X-100 (BSA-PBST) for 60min, primary antibody diluted in BSA-PBST at 4°C for 24 hours, washed in PBST (3 washes 5min duration), the appropriated Cy5 conjugated donkey secondary antibody (1:500, Jackson, West Grove, PA) at 22°C for 60min, counterstained with 50nM DAPI (4′,6-diamidino-2-phenylindole; Invitrogen) for 30 min at 22°C, and coverslipped with a DABCO (1,4-diazobicyclo-(2,2,2) octane)-based antifade mounting media. The following primary antibodies were used: anti-glial acidic fibrillary protein (GFAP, 1:5000, Dako) and anti-NeuN (1:1000, Chemicon) while FG can be visualized using a wide-band ultraviolet excitation filter. All fluorescent images for anatomical analysis were captured on an Olympus FluoView 500 confocal microscope. Confocal images z stacks were captured through the thickness of the slice every 1μm. To measure the

distance between cells and blood vessels 15 random cells were selected per field, at least 4 fields per animal from random points in the GCL, and 3 animals (148 cells in total). The distance from each cell to the nearest blood vessel was calculated using Neurolucida software (MicroBrightField) from the X, Y, and Z co-ordinates of the cell and closest vessel. As a control the distances from 15 random points in each field to the nearer blood vessel were measured (148 random points in total). To evaluate the orientation of blood vessels we again selected random migrating cells and measured the orientation of the vessel segment in contact with the cell. This orientation was represented as an angle with tangential represented by 0 degrees and a perfect radial orientation as 90 degrees. A random selection of points was used to determine the average vessel orientation in the bulb. Significance determined using the Kolmogorov-Smirnov Test.

Electron microscopy: 3 days after CTb injection P24 mice were anesthetized and transcardially perfused with 0.9% saline, followed by 4% EM-grade paraformaldehyde and 1% EM-grade gluteraldehyde in 0.1M PB (pH 7.4). The brains were immersion post-fixed for 16 hours and sectioned (25μm) as above. To assist antibody penetration sections were incubated in 30% sucrose and subjected to 3 freeze-thaw cycles from 22°C to -90°C. Sections were sequentially incubated in BSA-PBS for 60min, goat anti-CTb (1:5000, List Biologicals) diluted in BSA-PBS at 22°C for 24 hours, washed in PBST (3 washes 5 min duration), biotin conjugated donkey anti-goat secondary antibody (1:500, Jackson) at 22°C for 60min, washed in PBST (3 washes 5 min duration), incubated in an avidin-biotin-peroxidase reaction mix (ABC, Vector Labs), and reacted with diaminobenzidine (DAB, 0.5mg/ml), H_2O_2 (1.2% v/v), 0.2% NiCl, and 0.25% CoCl (Adams, 1981) following standard procedures for visualization of horseradish peroxidase.

The DAB stained sections were further processed for electron microscopy by infiltration with resin following our previously published protocols (Toida et al., 1998) and individual CTb/DAB labeled sections photographed at 100x (oil immersion lens, NA=1.4) with a transmitted light microscope. The identified cell was sectioned at 50nm on an ultramicrotome. Electron microscopy images were captured on an electron microscope (JEOL 1200EXII) with film (5.9cm x 8.2cm) at original magnifications at x1000 to x25000. Only cells in which identification was made at both the light and EM level were evaluated. After development EM negatives were digitally scanned at high resolution and inverted into a positive image.