SUPPLEMENTAL MATERIAL

Supplemental Methods

The Induction of photothrombotic ischemia, BrdU injections, behavioural testing and transcardial perfusion of the animals were performed as described in the main text of the manuscript. Animals were randomly assigned to receive daily injections of cyclosporine A i.p. (10mg/kg body weight, n=7) or placebo i.p. (n=7) throughout the experiment.

For the detection of neurogenesis and microglial activation immunohistochemistry was performed as described in the main text of the manuscript. Since immunohistochemistry and Golgi-Cox stainings can not be performed within the same sections we performed βIII-tubulin immunohistochemistry as follows to visualize dendrite regeneration.\textsuperscript{1,2} First of all the endogenous peroxidase was blocked by a 0.3% \textsubscript{2}H\textsubscript{2}O\textsubscript{2} solution in Methanol/PBS. After washes in PBS and PBST the sections were preincubated with 5% normal horse serum (NHS; vector, Burlingame, CA, USA) in PBST for 30 minutes. Then the slices were incubated with a mouse monoclonal anti-βIII-tubulin antibody in 5% NHS/PBST solution over night at 4°C (1:1600, clone TU-20, Millipore, Billerica, MA, USA). The biotinylated universal secondary antibody (Vectastain kit, Vector, Burlingame, CA, USA) was diluted 1:85 in NHS/PBS and incubated for 30 minutes at room temperature. Immunoreactivity was visualized by the avidin-biotin complex method. The sections were developed in diaminobenzidine (Thermo-Fisher, Fremont, CA, USA). Finally they were mounted on glass slides, dried at room temperature and coverslipped with cytoseal XYL (Microm, Walldorf, Germany). Sagittal sections were scanned in equal light conditions with a digital camera (Roper Scientific, Ottobrunn/Munich, Germany) and digitized with the MCID image analysis system (Imaging Research Inc, St. Catharines, Ontario, Canada). Technical settings were not changed during image acquisition. For semi-quantitative analysis of neuroplasticity, optical density (OD) of βIII-tubulin immunoreactivity was determined in the immediate vicinity of the lesion and adjacent cortex both cranial and caudal to the infarct according to a modified protocol of Shyu and colleagues.\textsuperscript{2} In addition, the corresponding contralateral
cortex was also analyzed accordingly. Optical density of the stratum oriens was used as reference value for background staining.

All experiments were performed in a blinded fashion.

**Supplemental Results**

Rectal temperature, body weight and mortality were not different between the cyclosporine A and the placebo group. The adhesive removal test and the cylinder test showed no significant differences between the two groups (ANOVA P = 0.073 and P = 0.593, asymmetry scores (SD) for post-stroke days 1, 7, 14, 21 and 28 in the cyclosporine A and in the control group were -0.78 (0.15), -0.51 (0.22), -0.29 (0.37), -0.21 (0.13), 0.20 (0.20) and 0.71 (0.19), -0.61 (0.16), -0.49 (0.20), -0.45 (0.25), -0.40 (0.15) for the adhesive removal test and -0.17 (0.11), -0.24 (0.19), -0.09 (0.13), -0.14 (0.25), -0.11 (0.28) and -0.22 (0.09), -0.18 (0.08), -0.23 (0.06), -0.18 (0.08), -0.11 (0.06) for the cylinder test, respectively). There were no significant differences regarding BrdU-positive cells in the dentate gyrus (ipsilateral P = 0.85, contralateral P = 0.25), regarding BrdU/NeuN-double-positive cells in the dentate gyrus (ipsilateral P = 0.99, contralateral P = 0.09) and regarding the number of activated microglial cells in the dentate gyrus (ipsilateral P = 0.61, contralateral P = 0.21). There were no differences in the intensity of tubulin immunostaining between cyclosporine and placebo animals neither in the ipsilateral cortex (caudal to the infarct P = 0.85, cranial to the infarct P = 0.51) nor in the corresponding cortex of the contralateral hemisphere (caudal to the infarct P = 0.90, cranial to the infarct P = 0.71).
Online figure. Illustration of the area chosen for the dendrite analysis of Golgi-Cox stained neurons. A sagittal and a coronal view to the brain are shown. The darker orange area indicates the infarct. The red arrows indicate the areas chosen for neuron analysis.
Supplemental References
