SUPPLEMENTAL MATERIAL

Supplemental Methods

Bromodeoxyuridine labeling

Bromodeoxyuridine (BrdU), a thymidine analog that is incorporated into cells during DNA synthesis, was used for S-phase labeling. Using a cumulative labeling protocol[1], rats (n= 6/group) were intraperitoneally injected once daily with BrdU (50 mg/kg, Sigma-Aldrich, St. Louis, MO) for 2 or 7 consecutive days, starting 24 hours after surgery. Rats receiving a daily BrdU injection in the 2-day and 7-day groups were sacrificed at 2 hours after the last BrdU injection. Rats in the 14-day group were injected daily with BrdU for 7 days starting 24 hours after surgery, and sacrificed 7 days after the last BrdU injection.

Immunohistochemistry

A series of coronal sections (6 µm thick) were obtained at the center of the lesion, corresponding to coronal coordinates for Bregma -1 to +1 mm^2. Three coronal sections were used for each immunohistochemistry experiment. Class IIa HDACs were examined using rabbit anti-HDAC4 (1:50, Santa Cruz Biotechnology) and rabbit anti-HDAC5 (1:50, Santa Cruz Biotechnology) antibodies. The following primary antibodies were used: mouse anti-microtubule-associated protein 2 (neuronal somatodendritic marker, MAP-2; 1:400, Millipore), mouse anti-phosphorylated neurofilament heavy chain (axonal marker, p-NFH; 1:500, Covance), rabbit anti-myelin basic protein (MBP; 1:400, Dako), rat anti-COUP-TF-interacting protein 2 (Ctip2; 1:250, Abcam), goat anti-parvalbumin (PV; 1:5000, Swant), mouse anti-adenomatous polyposis coli (APC; 1:20, GenWay), chicken anti-glial fibrillary acidic protein (GFAP; 1:500, Aves Labs) and mouse anti-BrdU (1:100, Dako).

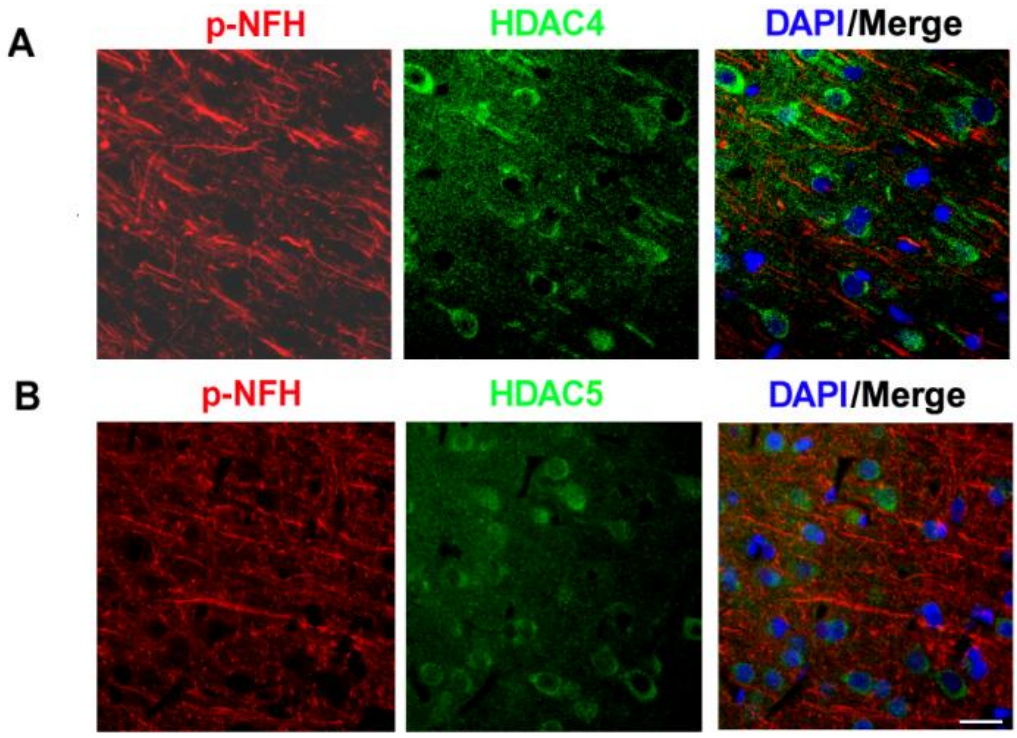
Brain sections were incubated with the primary antibodies listed above and with Cy3 or FITC (Jackson ImmunoResearch) conjugated secondary antibodies. Control experiments consisted of staining brain coronal tissue sections as outlined above, but omitting the primary antibodies. Counterstaining with DAPI (Vector Laboratories) allowed visualization of cells nuclei and determination of cortical layers.

TUNEL assay

TUNEL assay for the identification of apoptotic cells was performed using the ApopTag® Fluorescein in Situ Apoptosis Detection Kit (Millipore) following the manufacturer's manual.

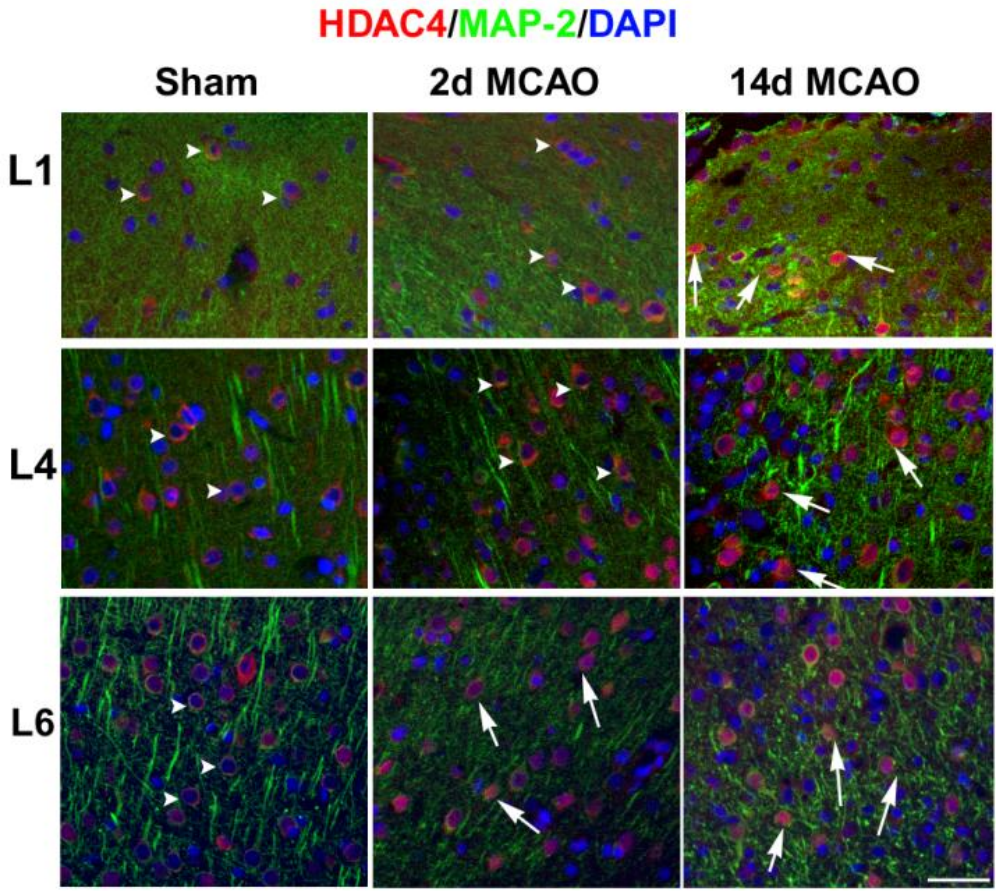
Supplemental Figures

Supplemental Figure I: HDACs 4 and 5 in p-NFH+ axons



Supplemental Figure I: Immunoreactivities of HDAC4 (**A**) and HDAC5 (**B**) were not detected in p-NFH+ processes of cortical neurons. Bar = 20µm.

Supplemental Figure II: Distribution of HDAC4 positive neurons across cortical layers 1, 4 and 6.



Supplemental Figure II: In superficial (L1,4) and deep (L6) cortical layers of sham-operated animals, HDAC4 immunoreactivity was predominantly detected in the cytoplasm of MAP-2+ neurons (arrowheads). Two days after stroke, nuclear HDAC4 (arrows) was increased in neurons of deep (L6), but not superficial (L1,4) cortical layers. Only 7 and 14 days after MCAO, increased nuclear HDAC4 immunoreactivity was also detected in superficial layers (L1,4). Bar = 20µm.

Supplemental References

1. **Zhang RL, Zhang ZG, Zhang L, Chopp M. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. *Neuroscience*. 2001;105:33-41**
2. **Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. Sydney ; Orlando: Academic Press; 1986.**