SUPPLEMENTAL MATERIAL

Supplemental Methods

AAV injection in mouse hippocampus

Stereotaxic AAV injections into the CA1 sector of the hippocampus were performed in 4-week-old C57Bl/6 mice. Under isoflurane anesthesia (1.5 % to 2.0 %), a midline skin incision was made between the bregma and interaural line. A 2 mm hole was drilled in the skull and AAV-PHB or AAV-control virus in 1 µl Ringer solution (titer: 10^{12}-10^{13} genomic copy/ml) were injected into CA1 using a glass micropipette. The stereotaxic coordinates were 1.5 mm posterior to bregma, 0.9 mm lateral to the midline and 2.1mm below the dura. The solution was slowly injected over 30 min and the needle was left in place for an additional 10 min. The needle was then slowly withdrawn and the incision closed. AAV-injected mice were used for experiments after three weeks, a period determined in pilot studies to be necessary for the full expression of viral mediated gene expression.

Transient forebrain ischemia by bilateral common carotid artery occlusion

Mice were anesthetized with a mixture of isoflurane (1.8-2%), oxygen (30%), and nitrogen (70%). Fiber-optic probes were glued to both parietal bones (3 mm lateral, 2 mm caudal to bregma) and connected to a laser-Doppler flowmeter (Periflux System 5000; Perimed, Järfälla, Sweden) for continuous monitoring of cerebral blood flow (CBF) in the neocortex. During surgery body temperature was monitored and maintained between 36.5° and 37.5°C using a thermostatically controlled heating pad. Through a midline incision of the neck, 4-0 surgical threads were loosely placed around both common carotid arteries and the arteries were tied for 22 min. Only animals that exhibited 90% reduction in CBF within the first minute of occlusion and CBF recovered by 80% after 10 min of reperfusion were included in this study [1]. After surgery, mice were kept at 37°C for 24 hours to prevent post-ischemic hypothermia. Thereafter, mice were returned to general housing (22-24°C). Sham-operated mice underwent the same procedures, except that their arteries were not occluded. Twenty-one % of all animals used in this study were excluded because of insufficient CBF reduction. About 11% of animals died after surgery and were also excluded from the study. The percentage of excluded animals was similar in AAV-vector and AAV-PHB treated groups. Animals were randomly assigned to the treatment groups.

Caspase-3 activity assay

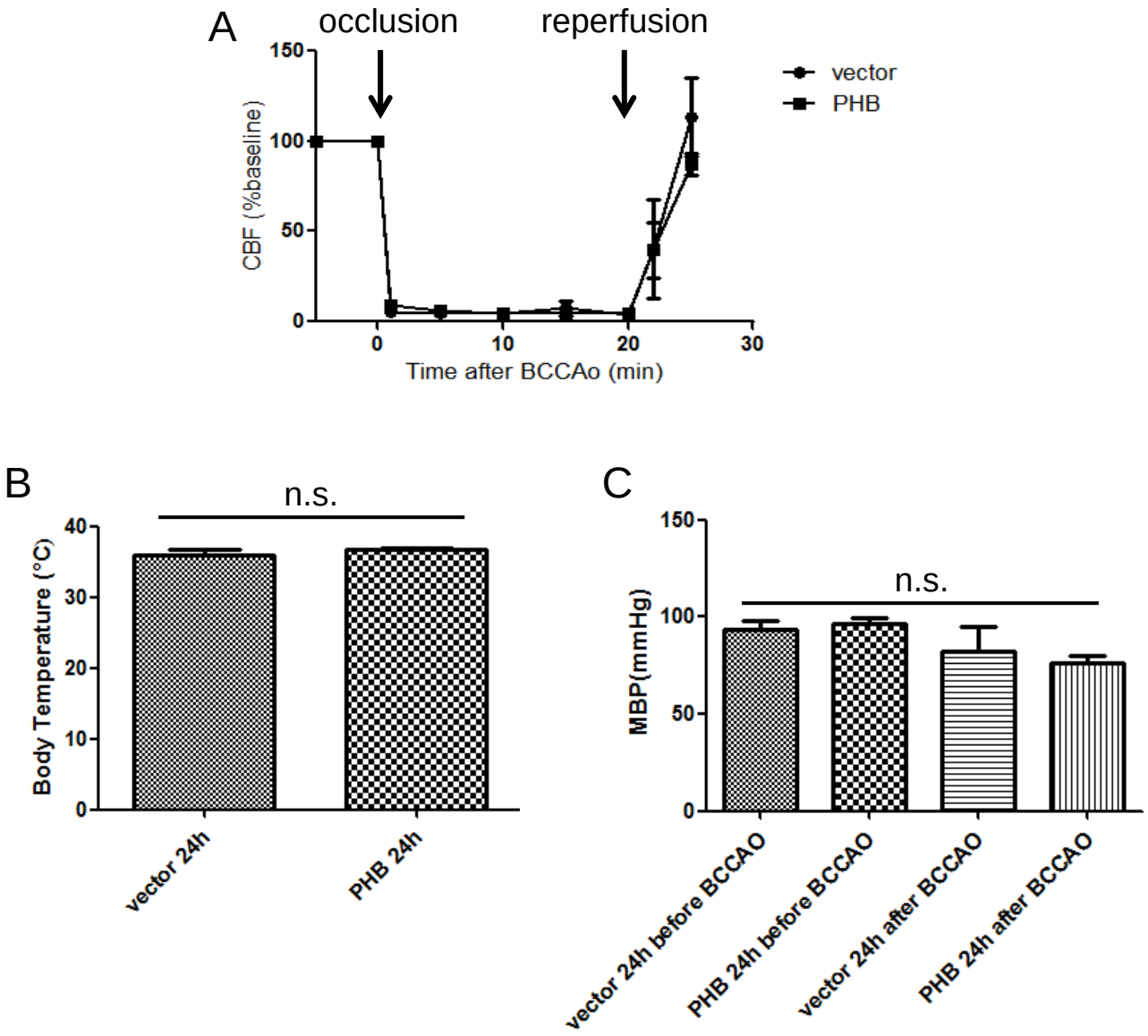
Caspase-3 activity in hippocampal tissues was assayed as described previously [2]. Briefly, at the designated time points the hippocampus was quickly dissected and frozen in liquid nitrogen. The tissue was homogenized in lysis buffer (25mMol/L Hepes, pH 7.4, 0.1% Triton X-100, 5mMol/L $MgCl_2$, 2mMol/L DTT, 1.5mMol/L EDTA, 1mMol/L EGTA, 1x protease inhibitor cocktail). After a 10 min spin in a table top centrifuge at 10,000xg, the supernatant was mixed with equal volume of 2x assay buffer containing DEVD-afc as caspase-3 substrate in a 96 well plate. After 30 min of incubation at 37^{o}C, the fluorescence intensity was measured in a fluorescence plate reader (405 nm excitation, 505 nm emission wavelength, respectively). The fluorescence intensity was normalized to the amount of protein in samples and expressed as relative fluorescence intensity per mg protein (RFI/mg protein).

Hippocampal slice preparation and electrophysiology.
400 µm hippocampal slices were prepared using a vibratome as described previously [3]. The slices were maintained at room temperature in a submersion chamber with artificial cerebrospinal fluid (ACSF) containing (in mMol/L) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 24 $NaHCO_3$, and 15 glucose, bubbled with 95% O_2/5% CO_2. Slices were incubated for at least 2 hours before the experiments and the operator was blinded to the treatment groups. For electrophysiology experiments, slices were transferred to recording chambers (preheated to 32 ºC) where they were superfused with oxygenated ACSF. Monophasic, constant-current stimuli (100 µsec) were delivered with a bipolar silver electrode placed in the stratum radiatum of area CA3, and the field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum radiatum of area CA1 with electrodes filled with ACSF (Re = 2–4 MΩ). Baseline fEPSPs were monitored by delivering stimuli at 0.033 Hz. fEPSPs were acquired, and amplitudes and maximum initial slopes measured, using pClamp 10 (Axon Instruments, Foster City, CA). LTP was induced with a high-frequency stimulation (HFS) protocol consisting of two 1-second long 100 Hz trains, separated by 60 seconds, delivered at 70-80% of the intensity that evoked spiked fEPSPs.

References for Supplemental Methods

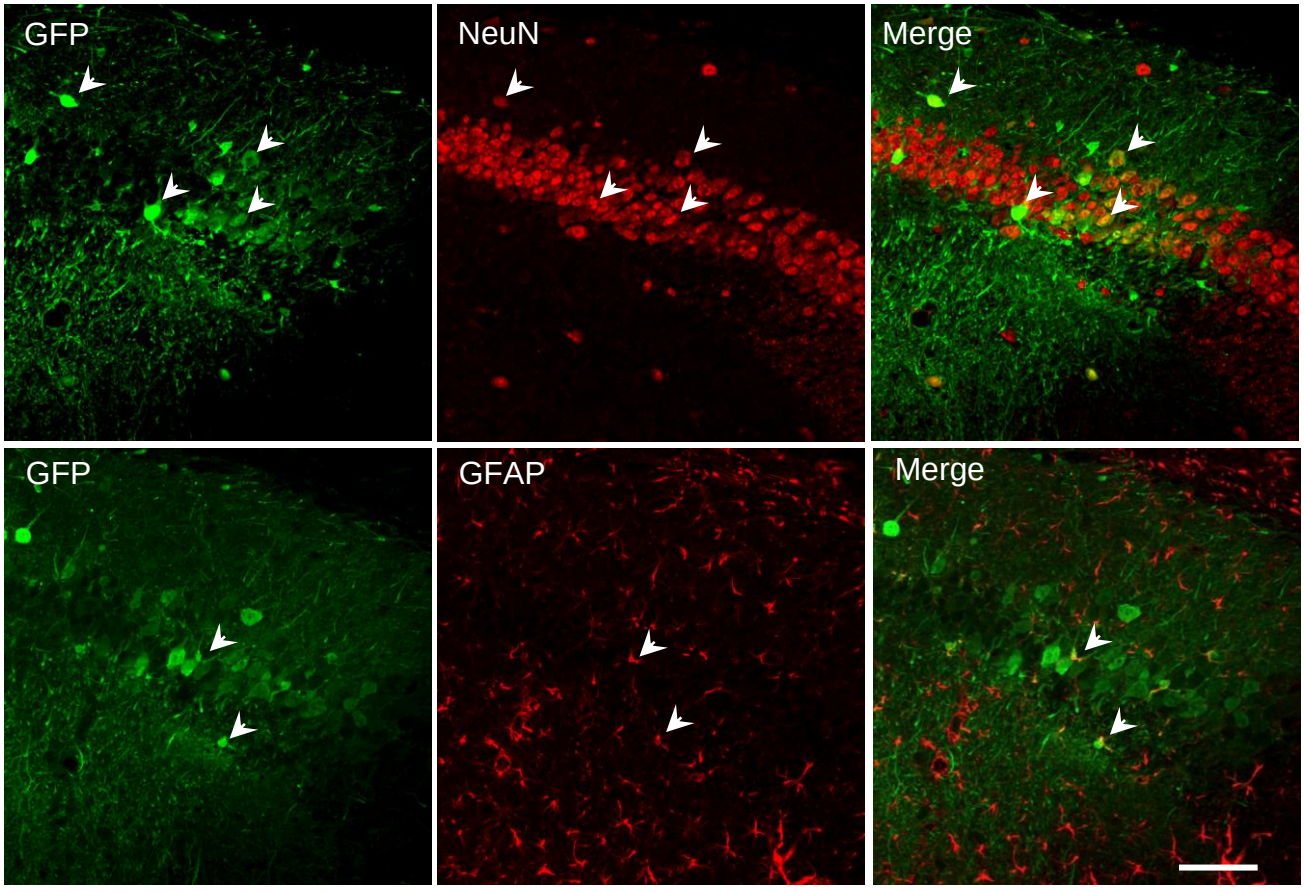
1. Shimamura M, Zhou P, Casolla B, Qian L, Capone C, Kurinami H, et al. Prostaglandin e type 1 receptors contribute to neuronal apoptosis after transient forebrain ischemia. *J Cereb Blood Flow Metab*. 2013;33:1207-1214
2. Zhou P, Qian L, Glickstein SB, Golanov EV, Pickel VM, Reis DJ. Electrical stimulation of cerebellar fastigial nucleus protects rat brain, in vitro, from staurosporine-induced apoptosis. *J Neurochem*. 2001;79:328-338
3. Ma T, Hoeffer CA, Wong H, Massaad CA, Zhou P, Iadecola C, et al. Amyloid β-induced impairments in hippocampal synaptic plasticity are rescued by decreasing mitochondrial superoxide. *J Neurosci*. 2011;31:5589-5595

Supplemental figure I



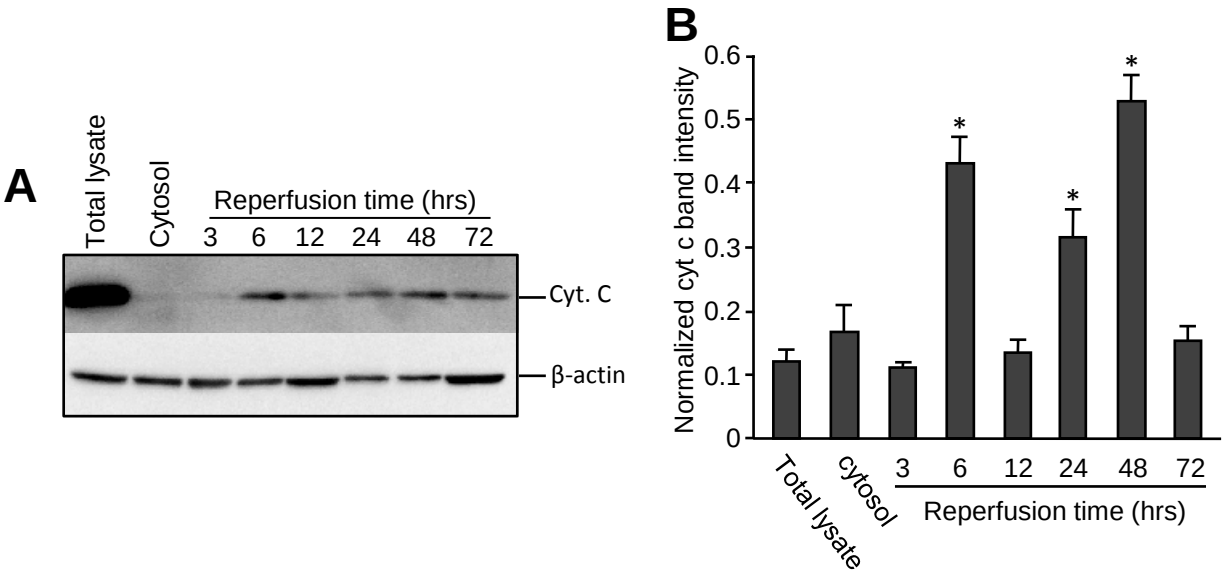
Supplemental figure I. Physiological parameters of mice before and after BCCAO. (A). Cerebral blood flow (CBF) measurement by a laser-Doppler flowmeter before and after BCCAO. (B). Body temperature measurement of mice 24 hrs after BCCAO/reperfusion. (C). Mean blood pressure (MBP) measurements 24hrs before and after BCCAO. All measures were obtained from 4 mice and statistically not significant (n.s.).

Supplemental figure II



Supplemental figure II. AAV-mediated PHB expression in the mouse hippocampus. Three weeks after AAV injection, brain sections were immunostained with the neuronal marker NeuN (red) and glial marker GFAP (red) to assess the identity of GFP positive cells. Most GFP-expressing cells co-localize with NeuN positive cells (top panels, arrows), while few GFP cells co-localize with GFAP positive processes (Lower panels, arrows). Scale bar is 75 μm.

Supplemental figure III



Supplemental figure III. Biphasic cyt c cytosol release from mitochondria following BCCAO in hippocampal CA1. (A) Temporal pattern of cyt c release in hippocampus after BCCAO detected by western blotting. Hippocampal CA1 tissues were dissected and the cytosolic fractions (10µg/lane) prepared at different reperfusion time points following BCCAO were loaded on gels. (B) Protein band intensity quantitation from data in (A). *$p<0.05$ compared to cytosolic fractions of non-stroked mice by one-way ANOVA analysis followed by Dunnett's test; n=4/group. The difference between the peaks at 6hrs and 48hrs after BCCAO is not statistically significant.