

SUPPLEMENTARY FIG. S6. Allocation table for each group and the timeline of the experiment. Rats submitted to sham operation (sham-op) or permanent occlusion of bilateral common carotid arteries (2VO). Mortality and blindness occurred only in rats with 2VO. Escape latency longer than mean plus three SD of sham-op rats was used as an arbitrary cutoff to determine blindness (total blinded rats, n=7). (A) Sham-op and 2VO rats were used for gene expression studies (RT-PCR, n=3, the left hemisphere of the brain) and protein expression studies (western blots, n=3, the right hemisphere of the brain) and immunohistochemical analysis (immunohistochemistry, n=3 or 4) at 1, 2, 4, and 10 weeks after operation. (B) Rats submitted to sham-op or 2VO that were treated with apocynin (Apo, IP injection of 10 mg/kg/day apocynin) or vehicle from days 0–56 post-2VO were used for behavioral analysis (n=6/group) and immunohistochemical analysis (n=6/group). Proinflammatory cytokine gene expression studies (real-time PCR, n=3) and immunohistochemical analysis in common carotid artery (immunohistochemistry, n = 6) were carried out at 1 and 4 weeks after 2VO operation treated with apocynin or vehicle. (C) In Nox1 knockdown study, AAV particles containing either Nox1 shRNA/AAV (Nox1shRNA) or Scb shRNA were stereotaxically injected into hippocampal CA1 subfield at 4 weeks before the 2VO or sham-op. Rats were allocated into four groups: sham-op rats injected with scramble shRNA AAV particles (ScbRNA control, n=10), sham-op rats injected with Nox1 shRNA AAV particles (Nox1shRNA control, n = 10), 2VO rats injected with scramble shRNA AAV particles (ScbRNA 2VO, n=10), and 2VO rats injected with Nox1 shRNA AAV particles (Nox1 shRNA 2VO, n=10). All groups were used for behavioral analysis (except Nox1shRNA control) from 10 weeks postoperation and for immunohistochemical analysis (n=5/group) at 15 weeks postoperation. Seven rats that failed behavioral training have been excluded from animals for odorant discrimination tasks (n = 8/group). All animals were sacrificed at 1 day after the behavioral test for histological study. 2VO, two-vessel occlusion; NADPH, nicotinamide adenine dinucleotide phosphate; Nox1, NADPH oxidase 1; RT-PCR, reverse transcription-polymerase chain reaction.