SUPPLEMENTAL MATERIALS

MATERIALS AND METHODS

Evaluation of the cerebrovascular anatomy of ncx3+/+, ncx3+/- and ncx3-/- mice

Cerebral macrovascular morphology was assessed by examining the major cerebral blood vessels of the circle of Willis. To this purpose, transcardial perfusion of ncx3+/+, ncx3+/- and ncx3-/- mice was performed with a solution containing gelatine and waterproof Pelikan Ink (9:1), as described by Paxinos and Franklin (1995) and other authors (Paxinos, 1995; Huang et al, 1994; Lo et al., 2007). After this procedure, to analyze the cerebral microvascular density, the brains were cryopreserved and later serially sectioned on a cryostat at -20 °C. Microvascular density was assessed in the fronto-parietal cortex and in the striatum. Five sets of three sections (25 m thick) were cut 150 m apart, corresponding approximately to plates 12–16 in a mouse brain atlas (Paxinos and Franklin, 2000). Composite images spanning the full depth of the fronto-parietal cortex and the entire area of the striatum were obtained using an Axioskop 20 microscope (Zeiss, Germany) with motorized XYZ stage (Prior, USA) equipped with high resolution CCD camera. To determine the number of Ink-positive capillary profiles having a diameter less than 10 m, images were acquired and analyzed using the MCID Elite software. Cortical and striatal capillary densities were expressed as the mean ± S.E.M. of 15 composite images obtained from each brain. Data were expressed as the number of capillaries per mm^2 in 25 m thick slices.

LEGENDS FOR FIGURES

Figure 5: Cerebral vasculature analysis in ncx3+/+, ncx3+/- and ncx3-/- mice

A, Ventral view of large cerebral blood vessels of ncx3+/+, ncx3+/- and ncx3-/- mice perfused with Pelikan Ink. ***B*** and ***C***, Representative microscopic views of microvessel profiles in the cortex (***B***) and striatum (***C***) of ncx3+/+, ncx3+/- and ncx3-/- mice are shown. ***D***, Quantification of microvessel numbers per mm^2 in a 25 m thick slice in the cortex and in the striatum of ncx3+/+, ncx3+/- and ncx3-/- mice. n= 4 animals for each group. Cortical and striatal capillary densities were expressed as the mean ± S.E.M. of 15 composite images obtained from each brain.

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