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Supplemental Information

Transvascular Delivery of Hydrophobically Modified siRNAs: Gene Silencing in the Rat Brain upon Disruption of the Blood-Brain Barrier

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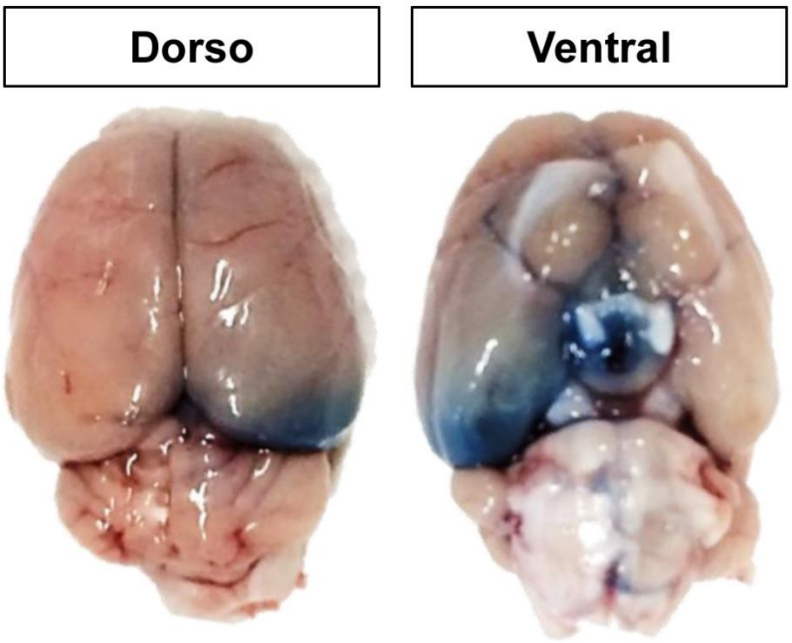

Figure S1. Single intracarotid administration of 25% Mannitol improves brain permeability to Evans blue dye.

Sprague-Dawley male rats ~9 weeks old were treated through the right carotid artery with a single dose of 25% mannitol (2.25 mL) followed by injection of 2% Evans blue dye (600 µL). Animals were euthanized after 48 hours. n = 2 animals.

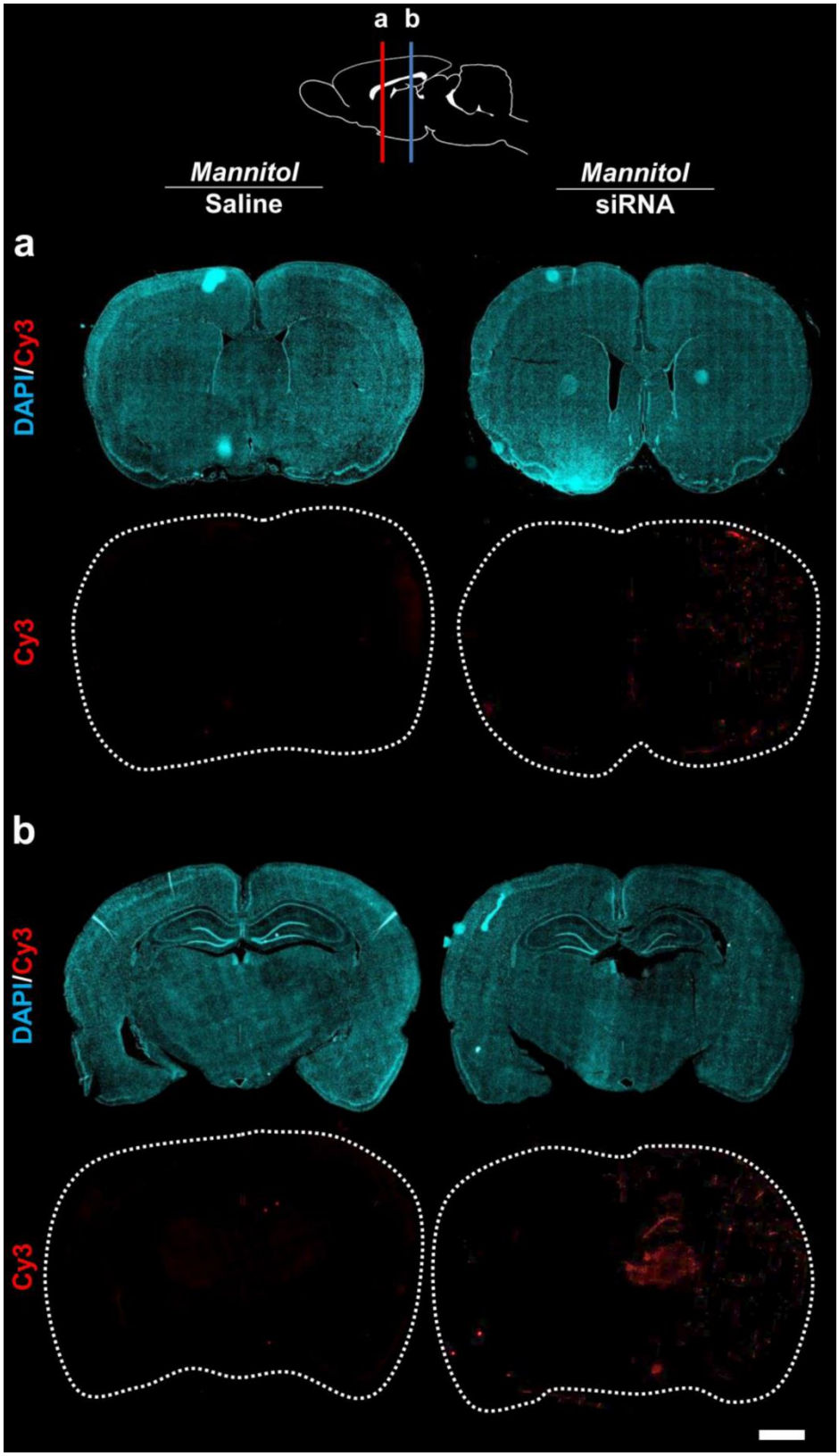

Figure S2. Unconjugated siRNAs present limited retention in the rat brain after transvascular delivery.

Cy3-labelled siRNAs 37 mg/kg were injected through the right carotid artery preceded by mannitol. Animals were euthanized 48 hours after injections and fluorescent tiled arrays obtained from coronal sections (20x objective). The schematic at the top shows the approximate positions of the (a) anterior (red line) and (b) posterior (blue line) sections. Nuclei (DAPI, cyan), Cy3-labelled siRNA (red). Scale bar = 2 mm. n = 2-3 animals/group.

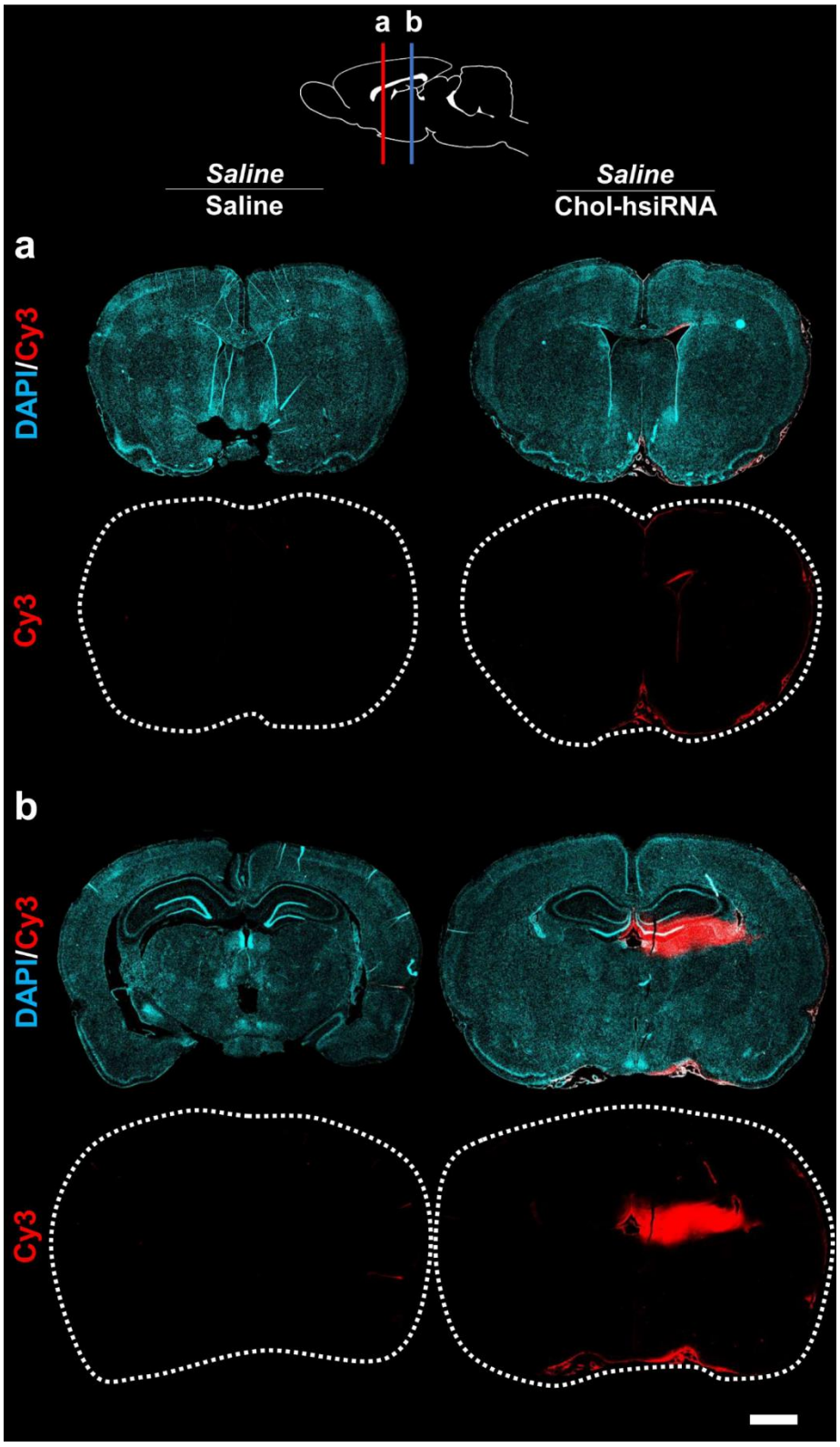

Figure S3. Cholesterol-hsiRNAs present limited transvascular delivery to the rat brain after intracarotid administrations.

Cy3-labelled Cholesterol-conjugated hsiRNAs (Chol-hsiRNAs) 33 mg/kg were injected through the right carotid artery preceded by saline. Animals were euthanized 48 hours after injections and fluorescent tiled arrays obtained from coronal sections (20x objective). The schematic at the top shows the approximate positions of the (a) anterior (red line) and (b) posterior (blue line) sections. Nuclei (DAPI, cyan), Cy3-labelled PC-DHA-hsiRNA (red). Scale bar = 2 mm. n = 2-3 animals/group.

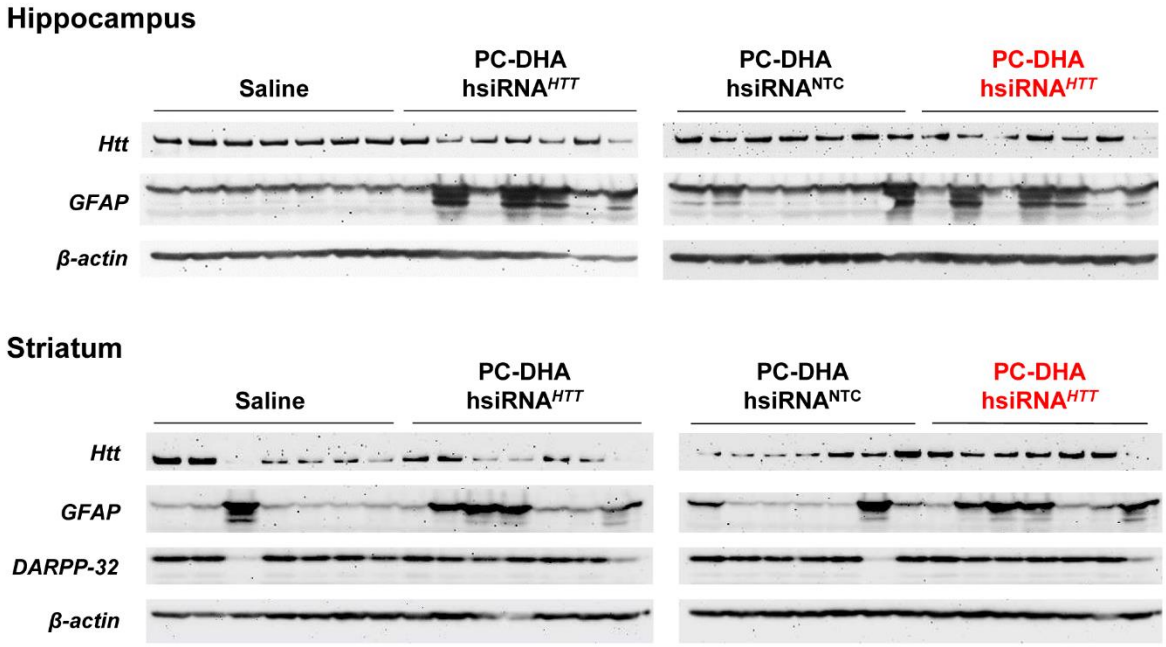

Figure S4. PC-DHA-hsiRNAs present differential reductions on target protein levels in the rat hippocampus and striatum after mannitol-induced blood-brain barrier disruption

PC-DHA-hsiRNAs (16 mg/kg) or saline were administered through the right carotid artery after pre-treatment with mannitol. Huntingtin (HTT)-targeting (PC-DHA-hsiRNA^{HTT}) and non-targeting control (PC-DHA-hsiRNA^{NTC}) sequences were used. HTT, Glial fibrillary acidic protein (GFAP) and Dopamine- and cAMP-regulated phosphoprotein (DARPP-32) expression levels were assessed by western blot 7 days post-injection. Blots depict protein levels from tissue biopsies collected from the dentate gyrus (hippocampus) and caudate putamen (striatum) of the injected side. PC-DHA-hsiRNA^{HTT} samples (red) were analyzed twice in two separate western blots: once with saline (left) and once with PC-DHA-hsiRNA^{NTC} controls. n=7-8 animals/group.

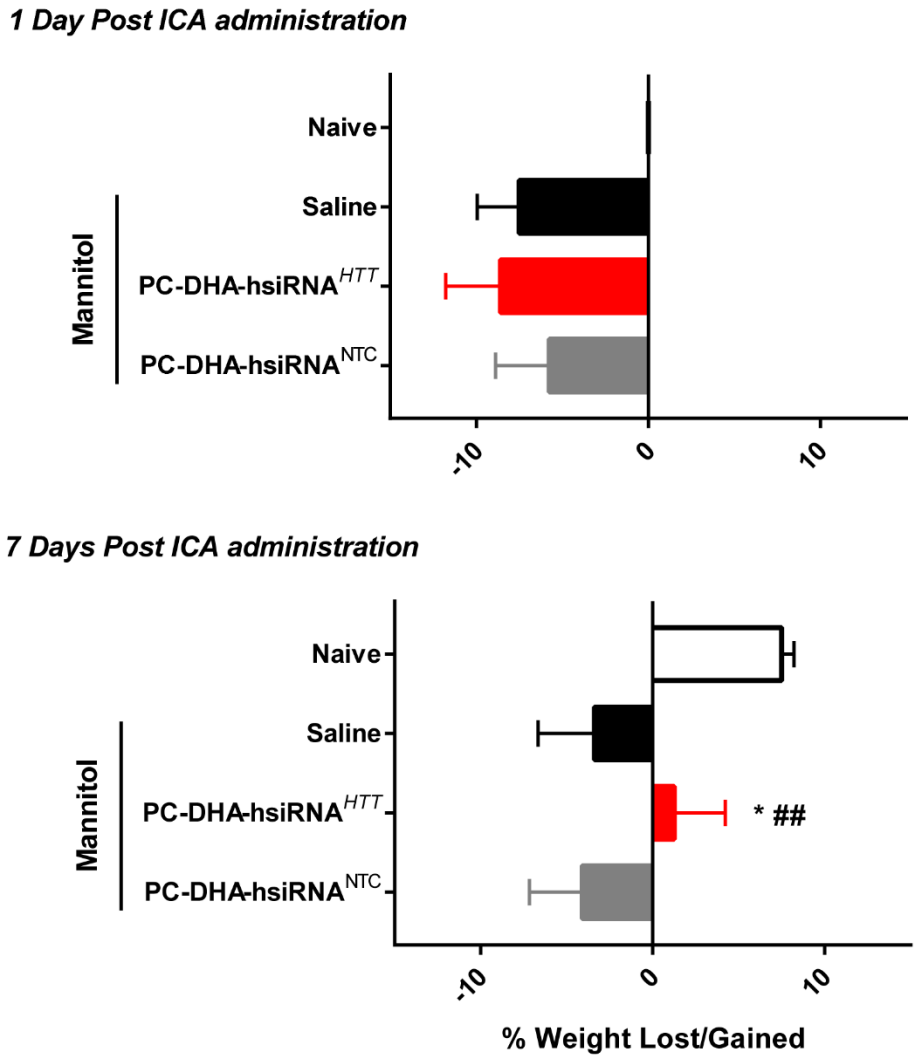

Figure S5. Percent change in body weight after transvascular delivery of PC-DHA-hsiRNAs by mannitol-induced blood-brain barrier disruption.

PC-DHA-hsiRNAs (16 mg/kg) or saline were administered through the right carotid artery after pre-treatment with mannitol. Animals were monitored for 7 days post-injection until they were euthanized for tissue collection. Percent loss or gain in body weight at 1 day (upper) and 7 days (lower). n = 3-7 animals/group, mean ± SD. One-way ANOVA with Tukey's post-hoc, *P<0.05 vs. Saline and ##P<0.01 vs. PC-DHA-hsiRNA^{NTC}.

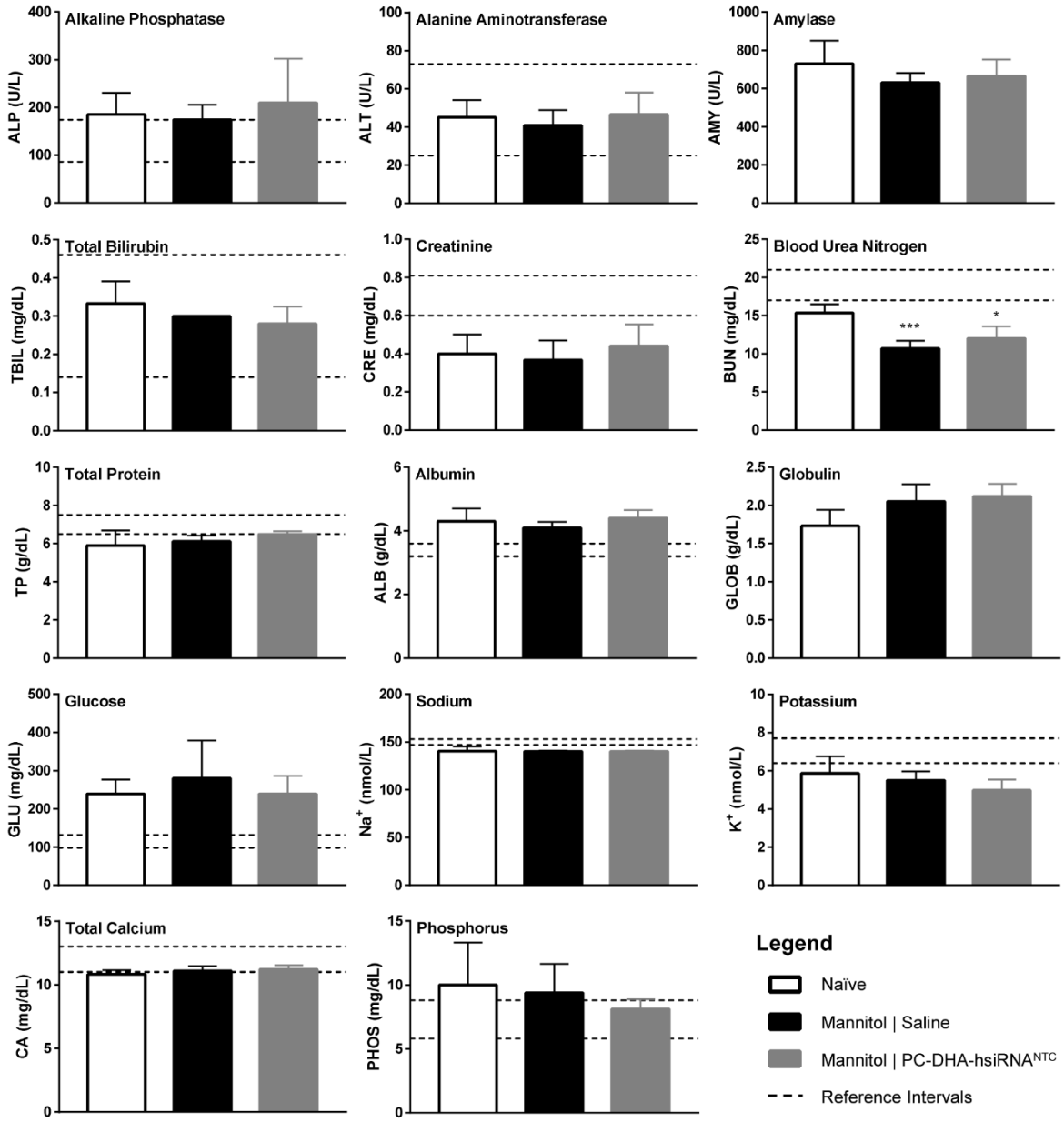

Figure S6. PC-DHA-hsiRNAs do not cause major acute systemic toxicities after mannitol-mediated transvascular delivery to the brain.

PC-DHA-hsiRNAs (16 mg/kg) or saline were administered through the right carotid artery after pre-treatment with mannitol. A comprehensive diagnostic blood chemistry profile of 14 biochemical markers was analyzed 7 days post-injection using a VetScan 2. Dashed lines indicate reference intervals reported in Fox JG et al. (2015) *Laboratory Animal Medicine*. n = 3-8 animals/group, mean ± SD. One-way ANOVA with Tukey's post-hoc, *P<0.05 and ***P<0.001.

Supplemental Methods

Optimization of intracarotid administrations

Male Sprague-Dawley rats (age 8-9 weeks, weight ~290 g) were cannulated using two different strategies for administration of 0.9% saline or 25% mannitol (Hospira, Cat. # NDC 0409-4031-01) solutions, followed by an infusion of Cy3-labelled asymmetric hydrophobic siRNAs (hsiRNA). hsiRNAs where conjugated to phosphocholine docosahexanoic acid (PC-DHA). The first approach consisted of direct administrations into the common carotid artery (CCA) performed by implanting a catheter in the right CCA to deliver 16 mg/kg Cy3-labelled PC-DHA-hsiRNA. The second approach consisted of indirect administrations conducted by implanting a catheter in the right external carotid artery (ECA) and positioning it to deliver 37 mg/kg Cy3-labelled PC-DHA-hsiRNA into the right CCA. After injections, catheters were removed and the respective arteries ligated. For other surgical details regarding anesthesia, catheters, volumes and rates of injection refer to materials and methods in the main text. Animals were euthanized 48 hours after injections and perfused with phosphate-buffered saline (PBS). Brains were removed and sliced using a rat brain matrix and fixed in 10% formalin overnight. Tissues were embedded in paraffin and sliced in 4 µm sections for assessment of biodistribution and toxicity.

TUNEL assay

For terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), 4 µm sections were obtained from paraffin-embedded tissues. Sections deparaffinized in xylene in 3 rounds of incubation 5 min/each. Samples were incubated in 100% ethanol for 5 min and this step repeated 2 times. Slides were treated in 95% ethanol for 5 min and this step also repeated 2 times. Sections were washed in deionized water for 5 min twice. Tissues were permeabilized using 500 µL of PBS Triton X-100 0.5% for 20 min and subsequently washed with PBS. 50 µL of TUNEL reaction mixture from the *in situ* cell death detection kit (Sigma Aldrich, Cat# 11684795910) was added to each slide and incubated for 1 hour at 37°C. Slides were washed with PBS twice for 5 min and stained with DAPI (1:10,000, Molecular probes, Cat. #D1306) for 2 min. Permafluor Mounting media (ThermoScientific, Cat. #TA-030-FM) was used to place glass coverslips, and slides left to dry at 4°C overnight protected from light. Imaging was carried out using a Leica DMi8 Fluorescent microscope fitted with a Hamamatsu C11440 ORCA-Flash 4.0 camera and a 40x objective. Images were acquired under the same light intensity settings and exposure times set individually for each channel.

Comprehensive Diagnostic Blood Chemistry Profiles

Naïve Sprague-Dawley rats or animals subjected to mannitol-mediated BBB disruption were euthanized 7 days post intracarotid administrations. Blood was collected from the right atrium into a sodium heparin tube and kept on ice or at 4 °C until analysis. Blood samples were loaded on a comprehensive diagnostic profile rotor (Abaxis, Cat. #500-0038) and analyzed through a VetScanVS2. Reference values for each of the blood chemistries were obtained from Fox, J.G. (2015) *Laboratory animal medicine*.