SUPPLEMENTARY METHODS

MRI acquisitions and analysis. All MRI acquisitions were performed at the University of Virginia Molecular Imaging Core facilities in a 7T Clinscan system (Bruker, Ettlingen, Germany) equipped with a 30 mm diameter cylindrical RF Coil. For analysis of mouse intracranial vascular structure, mice were placed in the 7T Clinscan system under light anaesthesia with isoflurane (1–1.25% in oxygen). Structural imaging data of intracranial arteries (magnetic resonance angiography or MRA) was acquired with a high-resolution 3D isotropic Spiral Cine Phase Contrast (SCPC) technique: repetition time (TR) = 15 ms, echo time (TE) = 0.63 ms, field of view (FOV) = 25 x 25 mm, slice thickness = 0.01 mm, number of sagittal slices = 160, number of averages per phase-encode step (NEX) = 1 and flip angle (FA) = 20°. Total imaging time was of about 15 min per mouse. Structural imaging data of intracranial veins (magnetic resonance venography or MRV) was acquired with a high-resolution 3D isotropic SCPC technique with a saturation band positioned caudal to the slices, in order to saturate the arterial signal: TR = 17 ms, TE = 4.54 ms, FOV = 17 x 26 mm, slice thickness = 0.3 mm, number of sagittal slices = 160, NEX = 2 and FA = 90°. Total imaging time was of about 13 min per mouse. Vascular volume and diameters were quantified using semi-automatic segmentation tools provided in the OsiriX software. For analysis of mouse brain ventricular volume, mice were placed in the 7T Clinscan system under light anaesthesia with isoflurane and structural imaging data were acquired with a high-resolution 3D isotropic T2-weighted SPACE technique with the following parameters: TR = 3000 ms, TE = 139 ms, FOV = 26 x 20.5 mm, slice thickness = 0.13 mm, number of slices = 160 and NEX = 3, requiring a total acquisition time of about 16 min per mouse. Ventricular volumes were quantified using semi-automatic segmentation tools provided in the OsiriX software.

Measurement of blood-brain barrier integrity was based on previously published methodology with minor modifications[66]. Initially, a pre-contrast image was acquired after placing the mice in the 7T Clinscan system under light anaesthesia with isoflurane. Gadobenate dimeglumine (gadolinium or Gd, MultiHance, Bracco Diagnostics, Princeton, New Jersey) at 0.3 mmol/kg was then injected intravenously (i.v.), through a catheter inserted in the tail vein. To assess the rate of influx of a CSF tracer into the brain, mice were anaesthetized by i.p. injection of ketamine and xylazine in saline and an i.c.m. injection of 5 µL of Gd at a concentration of 25 mM in saline was performed. The Gd concentration of 25 mM was chosen based on previous published methodology[67] and on MRI acquisitions performed after i.c.m. injection of 1, 10 or 25 mM Gd (see Extended data Fig. 3). The mice were placed in the MRI apparatus and maintained under light anaesthesia with isoflurane. For MRI acquisition, maximum gradient strength of the system was 500 mT/m and the peak slew rate achievable was 6667 mT/m/ms. After i.v. injection of Gd or 10 min after injecting gadolinium into the CSF, a series of post-contrast T1-weighted images were taken through the head with the following parameters: TR = 500 ms, TE = 11 ms, FOV = 20 × 20 mm with a 192 × 192 matrix (104 µm × 104 µm resolution), slice thickness = 0.7 mm, number of slices = 22 and NEX = 2. For i.v. Gd injection 5 post-contrast images were acquired requiring about 16 min per mouse (each sequence taken every 194 s). For brain influx of Gd injected into the CSF, the total acquisition time was of about 52 min per mouse (194 s x 16 sequences per mouse), meaning that T1 images were acquired for approximately 1 hour post-injection.

MR-compatible physiological monitoring and gating system for mice (SA Instruments, Inc., Stony Brook, NY) was used for T1 and T2 acquisitions.

Measurement of gadolinium influx rate and modeling of tracer advection-diffusion (with sources/sinks model) within the brain were achieved using Lymph4D, a software developed in-

house and available online under GNU General Public license v3.0 at https://github.com/avaccari/Lymph4D. To allow for a direct comparison between the different conditions, the MRI stacks $S_i(x, y, z, t)$ of the imaged mice (4 vehicle and 4 Visudyne) were aligned to a Visudyne stack $V_1(x, y, z, t)$, chosen as reference. Before proceeding with the automated registration in the (x, y) plane, the stacks were manually aligned along the z (sagittal) direction, to ensure the correspondence between (x, y) (transversal plane) slices, and cropped both in the z and t (time) direction, to ensure that each set of coordinates (x, y, z, t) contained meaningful data. To optimize the slice alignment in the (x, y) plane, *scale pyramids*[68] (3 levels deep) were computed for both the image to be registered and the reference. Starting from the coarser scale, the optimal non-reflective transformation matrix (restricted to translation, rotation, and scaling) was evaluated by using an iterative process based on the *steep gradient descent* method with a *mean square error* (MSE) cost function. After reaching algorithm convergence at the largest scale, or the maximum number of allowed iterations, the alignment was refined using progressively finer scales. A linear interpolation mapping was then used to reconstruct the image registered using the optimized transformation matrix. The following alignment strategy was used. Given a stack S_i to be aligned to V_1, for every slice $z_j(t) = S_i(x, y, z_j, t)$ at a given location in the sagittal direction $(z = z_j)$, the optimal transformation matrix was evaluated at every available time (t) and the quality (MSE) of the resulting alignment measured. The transformation resulting in the lowest MSE (highest quality) was used to re-align all the $z_j(t)$ slices for each available time (t), ensuring consistent alignment along the temporal direction. This was key to the analysis of the transport model described next. After the alignment, rate of contrast agent influx in outlined regions of the brain (namely hippocampus and cortex) was obtained by measuring the gain in signal intensity after subtraction of the baseline values (sequence 1) to the images in sequence 2

and subsequent sequences. Also, after the alignment, contrast measurement, providing the difference between the maximum and the minimum values observed along the temporal direction during the experiment duration (variability in tracer signal intensity over time), was evaluated for a given (x, y, z) location in the S_i stack using:

$$\Delta L_i(x, y, z) = \max_t S_i(x, y, z, t) - \min_t S_i(x, y, z, t)$$

To evaluate the component of the transport mechanism within the brain a model combining the microscopic diffusive and the macroscopic advective (bulk motion) processes, with the presence of sources/sinks, was implemented. The model used is described by the following differential equation:

$$\frac{\partial \phi(\boldsymbol{x}, t)}{\partial t} = \nabla \cdot [D(\boldsymbol{x}, t) \nabla \phi(\boldsymbol{x}, t)] - \nabla \cdot [\phi(\boldsymbol{x}, t) \boldsymbol{u}(\boldsymbol{x}, t)] + \sigma(\boldsymbol{x}, t)$$

where:

$\phi(\boldsymbol{x}, t)$	is the *concentration* $\left[\frac{amount}{L^3}\right]$
$D(\boldsymbol{x},\ t)$	is the *diffusion coefficient* $\left[\frac{L^2}{T}\right]$
$\boldsymbol{u}(\boldsymbol{x}, t)$	is the *velocity field* $\left[\frac{L}{T}\right]$
$\sigma(\boldsymbol{x}, t)$	are the *sources/sinks* $\left[\frac{amount}{L^3 T}\right]$
$(\boldsymbol{x},\ t)$	are *location* $[L]$ and *time* $[T]$

The model was discretized using the forward-time central-space (FTCS) finite difference method[69] after the following assumptions. The concentration $\phi(\boldsymbol{x}, t)$ was considered proportional to the measured MRI signal intensity. The diffusion coefficient $D(\boldsymbol{x},\ t)$ was considered isotropic and constant over the duration of the experiment. Velocity field $\boldsymbol{u}(\boldsymbol{x}, t)$ and sources/sinks $\sigma(\boldsymbol{x}, t)$ were

considered constant over the duration of the experiment. Furthermore, due the difference in spatial sampling between the sagittal and transversal directions (0.7 mm vs. 104 μm), each transversal slice $z_j(t)$ was considered independently with the source/sink term summarizing the results of potential interactions with the neighboring slices along the sagittal direction. The final discretized model was used in an inverse problem framework to evaluate the parameters ($D(\boldsymbol{x})$, $\boldsymbol{u}(\boldsymbol{x})$, and $\sigma(\boldsymbol{x})$) generating the concentration spatiotemporal evolution $\phi(\boldsymbol{x}, t)$ that best matched the MRI data. For every location $\boldsymbol{x}$, the matching problem was posed as a constrained least-square optimization using data extracted from a spatiotemporal neighborhood of $\boldsymbol{x}$. The results of the optimization were presented as a 2D maps, one for each of the model parameters: isotropic diffusion coefficient (used in this study), velocity components and derived magnitude and direction, and sources and sinks. 2D maps of isotropic diffusion coefficient were further used to assess the area fraction of high, medium and low coefficient values, by means of the tools available in FIJI image processing software (NIH), and calculate the difference between Visudyne and vehicle in each of the stacks.

SUPPLEMENTARY REFERENCES

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