Supplementary Methods

Quantification of PVS signal along major ACA vessels

Vascular inflow images and the corresponding Gd-enhanced PVS images were co-registered and analyzed in MATLAB. For each animal, a mid-sagittal slice covering the major ACA branches was selected (Fig. 1A). The analysis proceeded in four steps: ACA centerlines were segmented from the vascular image, perpendicular sampling lines were placed along these centerlines to extract paired V and PV intensity profiles, cross-sections with clearly resolved perivascular signal were retained, and the retained profiles were aligned to the vessel center and averaged across animals to produce the population radial profiles in Fig. 1C & D.

For ACA centerline segmentation, the vascular image of the selected slice was scaled to twice the slice maximum, smoothed with a Gaussian filter (σ = 2 pixels), and contrast-adjusted with imadjust. A binary vessel mask was obtained by Otsu thresholding with the threshold empirically scaled by a factor of 1.5, and the mask was then skeletonized to a one-pixel-wide centerline. Skeleton components smaller than 10 pixels were discarded. The cleaned skeleton was converted to a pixel graph, and at each graph edge a straight line of length 20 voxels (600 μm at 30 μm voxel size) was constructed perpendicular to the local vessel orientation and centered on the edge midpoint. Paired vascular and PVS intensity profiles were then sampled along this perpendicular line on the integer image lattice, yielding one cross-section per edge. Cross-sections were retained only if they showed a clearly resolved perivascular signal, defined as a single dominant vascular peak located near the profile center and flanked on both sides by distinct PVS peaks with a local PVS minimum at the vessel center. An example of the cross-section with its perpendicular sampling line is shown for the vascular and PVS images in Fig. 1B.

For population averaging, every accepted V and PV profile was linearly resampled to a common length of 20 points on a centered spatial axis so that the vessel center was aligned across cross-sections. Resampled profiles were averaged across all accepted cross-sections, and the standard

error at each position was computed as the across-cross-section standard deviation divided by the square root of the number of non-missing values at that position. Two versions of the population profile were generated, one in raw intensity units (Fig. 1C) and the other in which each individual profile was z-scored prior to resampling (Fig. 1D). Spatial position is reported in micrometers relative to the vessel center using a 30 μm voxel size.

Quantification of PVS signal in the parenchyma

Gd-enhanced PVS images were analyzed in MATLAB to detect perivascular enhancement around individual penetrating vessels in the cortical parenchyma, to test whether this enhancement exceeded the level expected by chance, to examine how it varied with cortical depth, and to track how it evolved from the infusion time point to 3 h post-infusion on the same vessels. The analysis was carried out on 2D patches cropped from axial and coronal slices in cortical regions, and all quantifications were performed in MATLAB.

To localize penetrating vessels and extract local image patches for analysis, the 3D PVS volume of each animal was first rotated around the vertical axis to align penetrating vessels with the image grid. The in-plane center of the cortical region of interest was selected interactively on an axial slice, and the cortical depth at that location was then selected on the corresponding coronal view. Another two adjacent axial slices spaced by one voxel above and below were also extracted at the chosen center point. From each slice a 41 by 41 voxel patch (roughly 1230 by 1230 μm at 30 μm voxel size) centered on that position was cropped. For each animal, the selection was repeated three times at each cortical depth to sample multiple spatial locations within each layer. This procedure was repeated at three cortical depths corresponding to 0 to 300, 300 to 600, and 600 to 900 μm from the pial surface, reported below as the surface, middle, and bottom layers.

To segment individual penetrating vessels and their surrounding parenchymal layers, intensities within each patch were rescaled to [0, 1] and a binary vessel mask was obtained by adaptive thresholding (adaptthresh, sensitivity 0.3, dark foreground), followed by morphological closing

with a disk structuring element of radius 1 voxel. The connected components smaller than 2 voxels were removed as noise. Each connected component in the resulting mask was treated as one penetrating vessel cross-section (Fig. 2B). For every vessel, three successive single-voxel dilations of the vessel mask defined three concentric ring-shaped layers at radial distances of roughly 30, 60, and 90 μm from the vessel edge, while the original component served as the vessel core. The maximum intensity within the core and within each of the three rings was extracted and labeled V, PV1, PV2, and TS, where TS was taken as the outer parenchymal reference. For each vessel, perivascular enhancement was quantified as the relative PV-to-tissue contrast, defined as (max (PV1, PV2) - TS) / TS. The distribution of this contrast across all segmented vessels was summarized as a histogram overlaid with a kernel density estimate (Fig. 2C, left).

To test whether the observed PVS enhancement exceeded the level expected under the null hypothesis of no perivascular signal, the experimental contrast distribution was compared against a control distribution obtained by randomizing vessel positions. The control distribution was generated by repeating the entire layer extraction 1000 times on the same image patches, with the vessel positions randomized on each repetition, and summarizing the pooled values as a kernel density estimate across the 1000 repetitions. The experimental and control distributions were then overlaid (Fig. 2C, right). A detection threshold was set at the 95th percentile of the control distribution, and vessels with a contrast above this threshold were considered to carry detectable perivascular enhancement. The red shaded area in the inset of Fig. 2C highlights the portion of the experimental distribution that lies above this threshold and exceeds the control distribution, representing the fraction of vessels carrying PVS signal above the chance level.

To compare perivascular enhancement across cortical depths, the same quantification was carried out separately on the surface, middle, and bottom layers. At each depth, only vessels with a contrast above the 95th-percentile detection threshold were retained. For every retained vessel,

the raw intensities at the vessel core and the three concentric rings were expressed as percentage signal change relative to the core, so that all vessels started from a common baseline at radial distance zero. The resulting radial profiles were averaged across vessels within each depth and are reported as mean plus or minus s.e.m. (Fig. 2D). Differences between the three depths at the 60 and 90 μm rings were evaluated by paired t-test.

To test whether the ability to detect PVS signal was confounded by vessel caliber and therefore reflected a partial-volume effect rather than true perivascular enhancement, the number of voxels in each segmented vessel mask was taken as a proxy for vessel size, and vessel sizes were compared between detectable and undetectable vessels (Fig. 2E). Segments exceeding 20 voxels (600 μm) were excluded, as they fall outside the characteristic spatial extent of individual penetrating vessels. Because vessel size distributions were heavily right-skewed and violated the normality assumption required by parametric tests (Shapiro-Wilk test, $p < 0.001$ for both groups), a two-sample Kolmogorov-Smirnov test was used to assess whether the two groups shared the same underlying distribution (MATLAB kstest2). The two-sample KS test showed no significant difference in vessel size distribution between PVS-detectable (n = 90, median = 4 voxels) and PVS-undetectable vessels (n = 1444, median = 3 voxels) ($p=0.3346$).

To test whether the detected PVS signal reflected true perivascular Gd accumulation rather than an imaging artifact or an iron-induced susceptibility effect, two additional analyses were performed on the pooled vessel population. First, to verify that the enhancement was specific to the infusion time point and not a persistent feature of the image, the raw intensities at the vessel core and the three concentric rings were averaged across detectable vessels at infusion and at 3 h post-infusion for comparison (Fig. 3B). Second, to track how PVS enhancement evolved over time on the same vessels, the infusion and 3 h post-infusion image volumes from each animal were co-registered, and the vessel masks generated at the infusion time point were reapplied to the post-infusion volume. This ensured that the vessel core and the three concentric rings were sampled

at identical spatial locations at both time points, so that each vessel contributed one paired measurement. For every vessel passing the 95th-percentile detection threshold at infusion, the infusion and post-infusion relative PV-to-tissue contrast values were compared by paired t-test (Fig. 3C, 200 vessels).

Quantification of PVS signal around hippocampal penetrating vessels across imaging contrasts

To confirm that the perivascular Gd enhancement observed around hippocampal penetrating vessels reflected true Gd accumulation in the perivascular space rather than a feature already present at baseline or introduced by the preceding iron infusion, signal intensities were compared across three sequential scans of the same animals: a baseline scan, a post-iron infusion scan, and a post-Gd infusion scan. The three scans were co-registered so that the same vessel and the same surrounding locations could be sampled at each time point. The analysis was carried out in MATLAB on coronal slices covering the dorsal hippocampus.

For every penetrating vessel, three ROIs were placed along a radial line centered on the vessel, one on the vessel lumen, one on the perivascular space, and one on the surrounding tissue, corresponding to radial distances of 0, 20, and 40 μm from the vessel center (Fig. 4D inset). The same three ROIs were then applied to the baseline, post-iron, and post-Gd scans of that vessel, yielding one intensity value per ROI per scan. A vessel was retained only if, on the post-Gd scan, the perivascular ROI was brighter than both the lumen ROI and the tissue ROI, which left 38 vessels from 8 animals. To place the three scans on a common scale despite their different absolute intensity ranges, the three ROI values of each vessel were z-scored within that vessel and within that scan, averaged across the 38 retained vessels separately for each scan, and plotted as mean plus or minus s.e.m. as a function of radial distance from the vessel center (Fig. 4D).

Phase-map validation of PVS signal

To test whether the bright perivascular signals detected on the MGE images reflected true Gd-induced T1 shortening in the perivascular space rather than phase-related artifacts such as local susceptibility offsets, chemical shift, or partial-volume phase cancellation, phase-difference and B0 field maps were reconstructed from the same raw multi-echo GRE k-space data used for the image-based PVS analysis and inspected at the same spatial locations as the bright perivascular voxels.

Phase-difference maps between the two echoes were computed as the angle of the Hermitian product $HP = S2 \cdot \mathrm{conj}(S1)$, where S1 and S2 are the complex images at the first and second echoes. This Hermitian formulation cancels any echo-independent background phase, such as coil phase offsets, and yields a wrapped phase-difference map whose value at each voxel depends only on the local frequency offset and the echo-time difference. The wrapped phase-difference map was then unwrapped in 3D using a region-growing phase-unwrapping algorithm (https://github.com/geggo/phase-unwrap), and the unwrapped phase difference was converted to a B0 field map in Hz by $B0 = -\Delta\phi / (2\pi \cdot \Delta TE)$, where ΔTE was the difference between the two echo times.