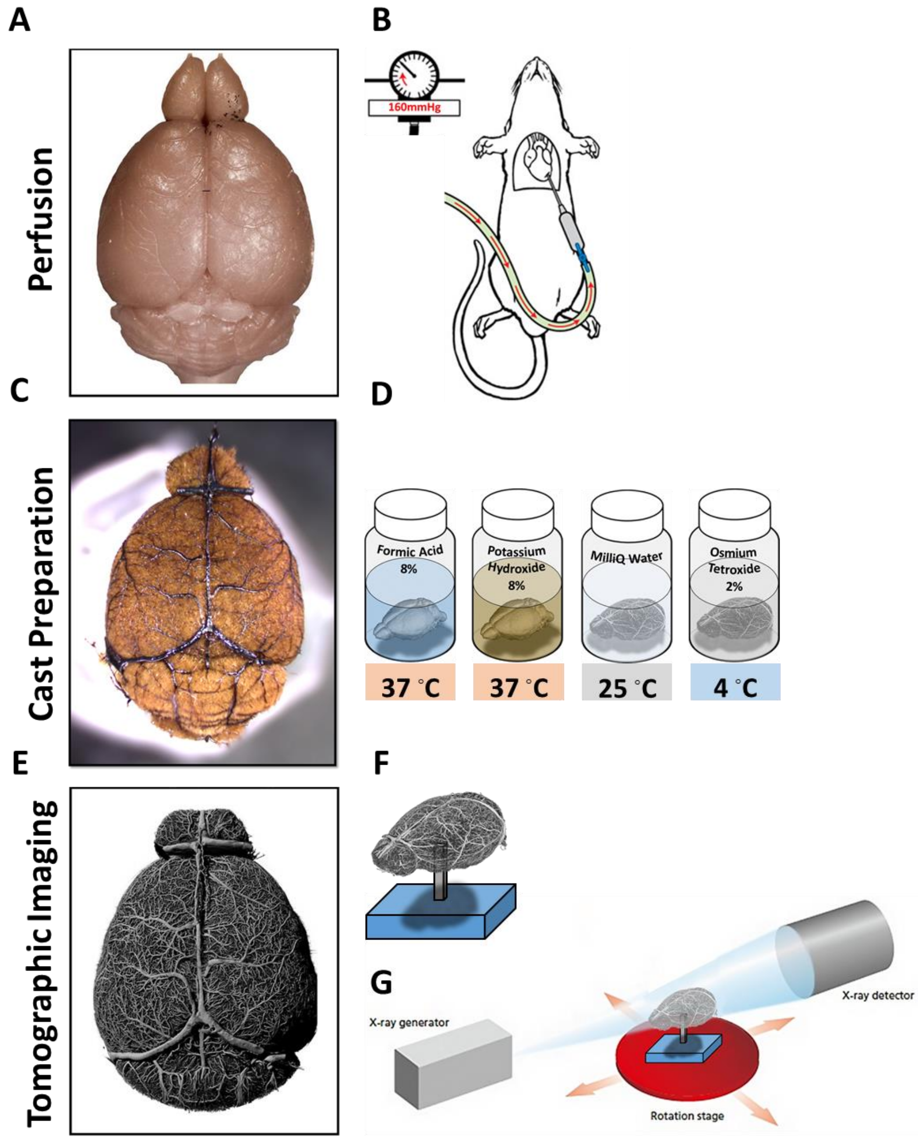
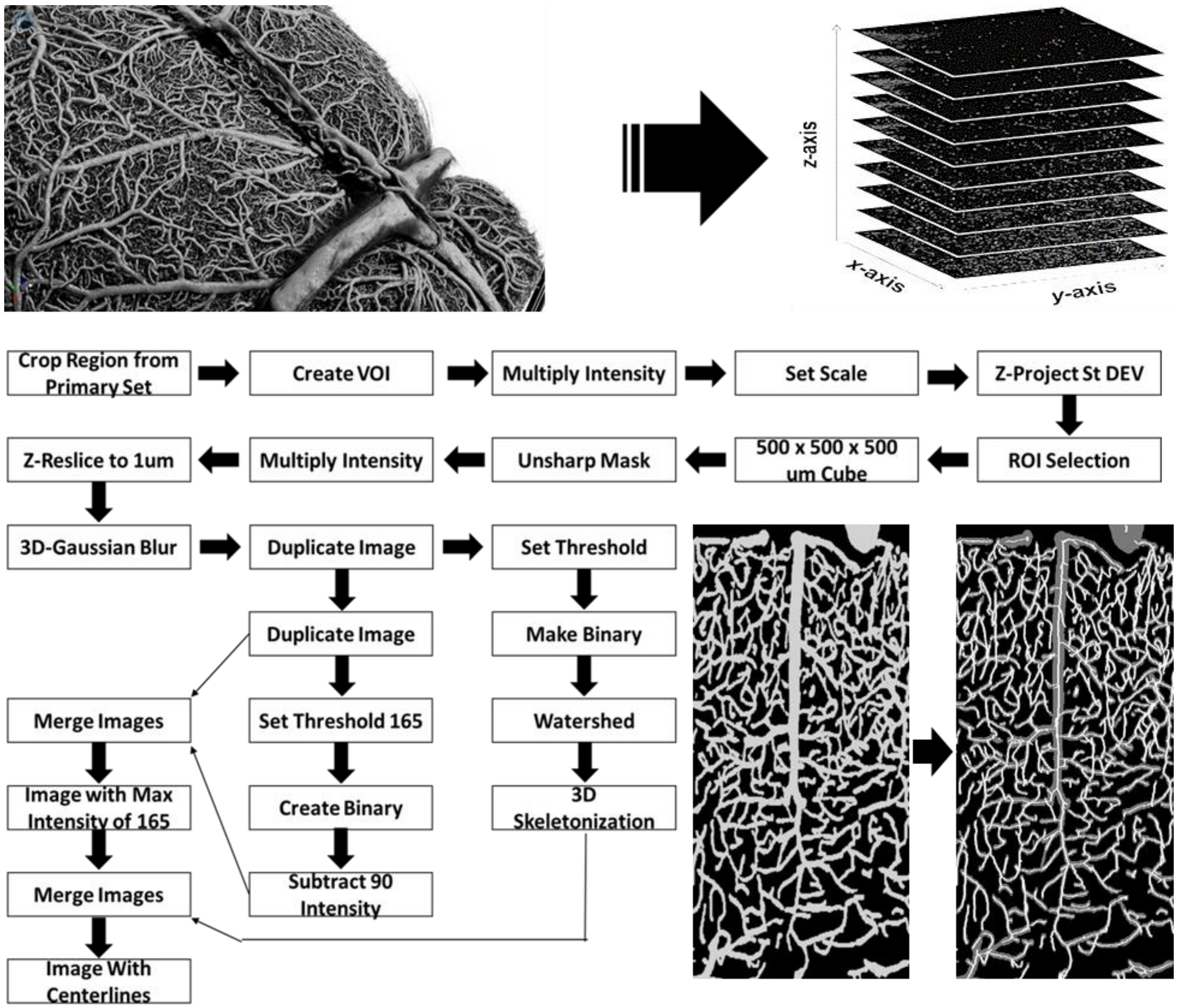
Supplemental Material

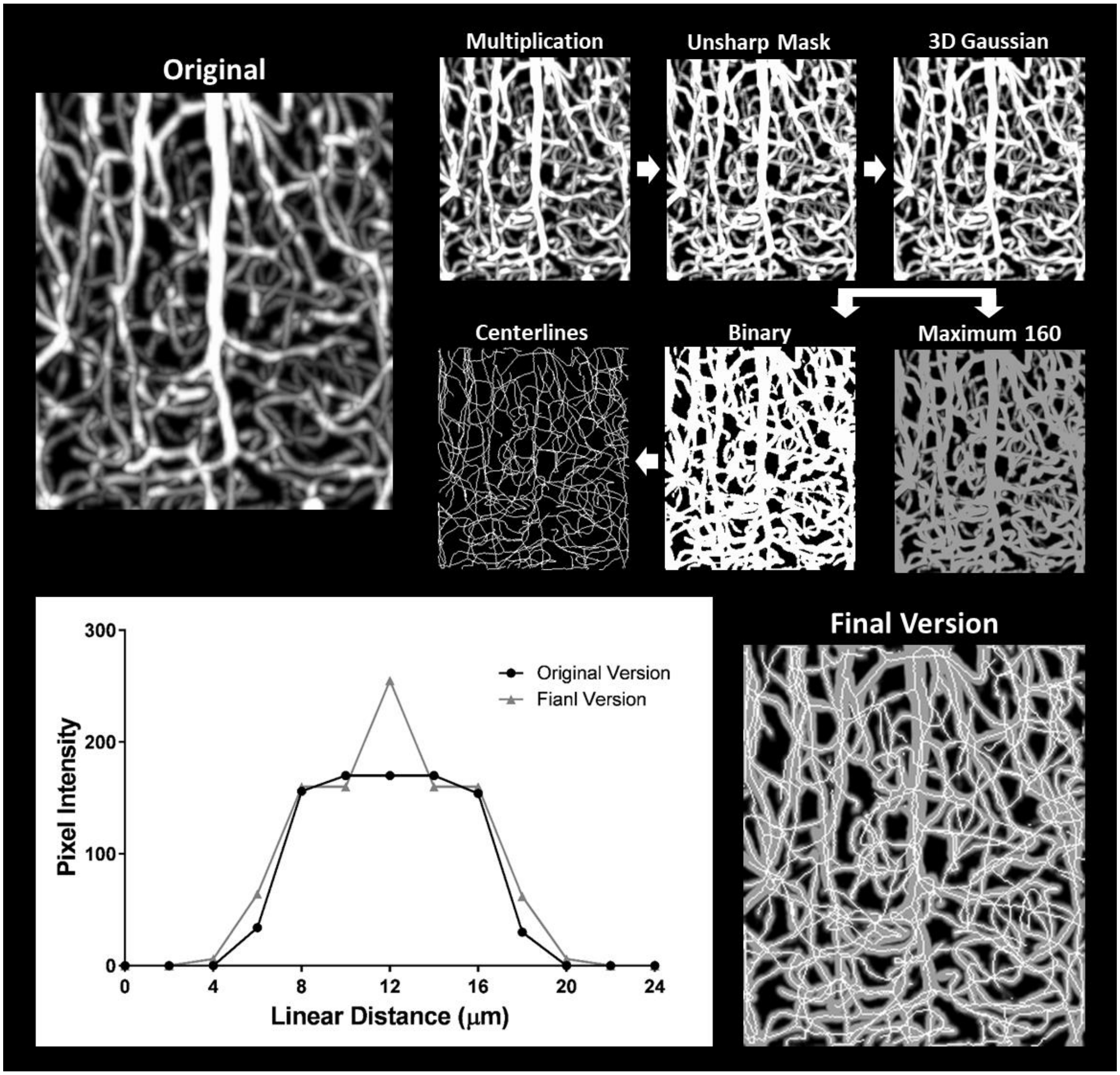


Supplemental Figure 1: Workflow of the procedure used to produce the cerebrovascular corrosion casts. (A) Perfusion of the adult mouse brain. (B) Transcardial perfusion via cannulated ascending aorta of the eluate, fixative, and casting resin at 160 mmHg. (C) Preprocessing of the vascular corrosion cast. (D) Decalcification, maceration, washing, and osmium embedding of the vascular corrosion casts. (E) Processing of the vascular corrosion cast for tomographic imaging.

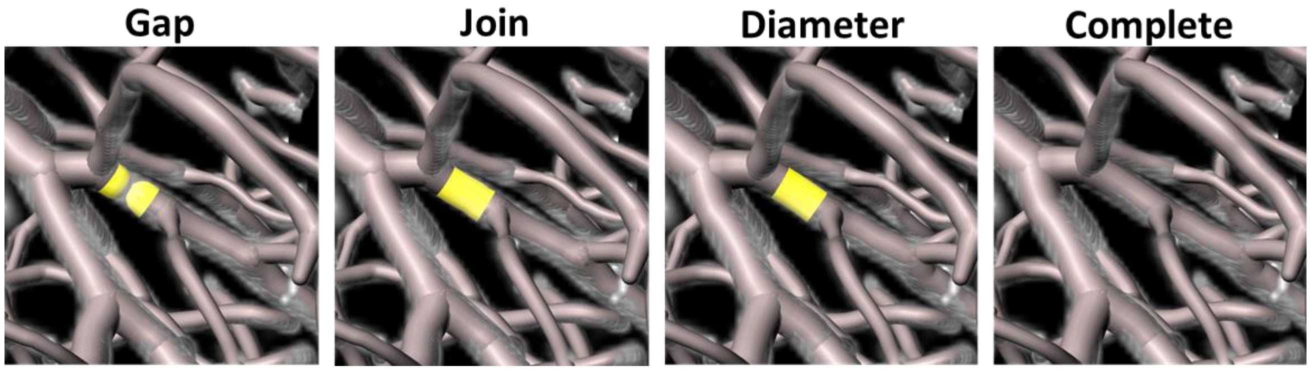
(F) Mounting and positioning of vascular cast on pedestals. (G) Setup of Image acquisition routine for μ computerized tomographic imaging of vascular corrosion cast.



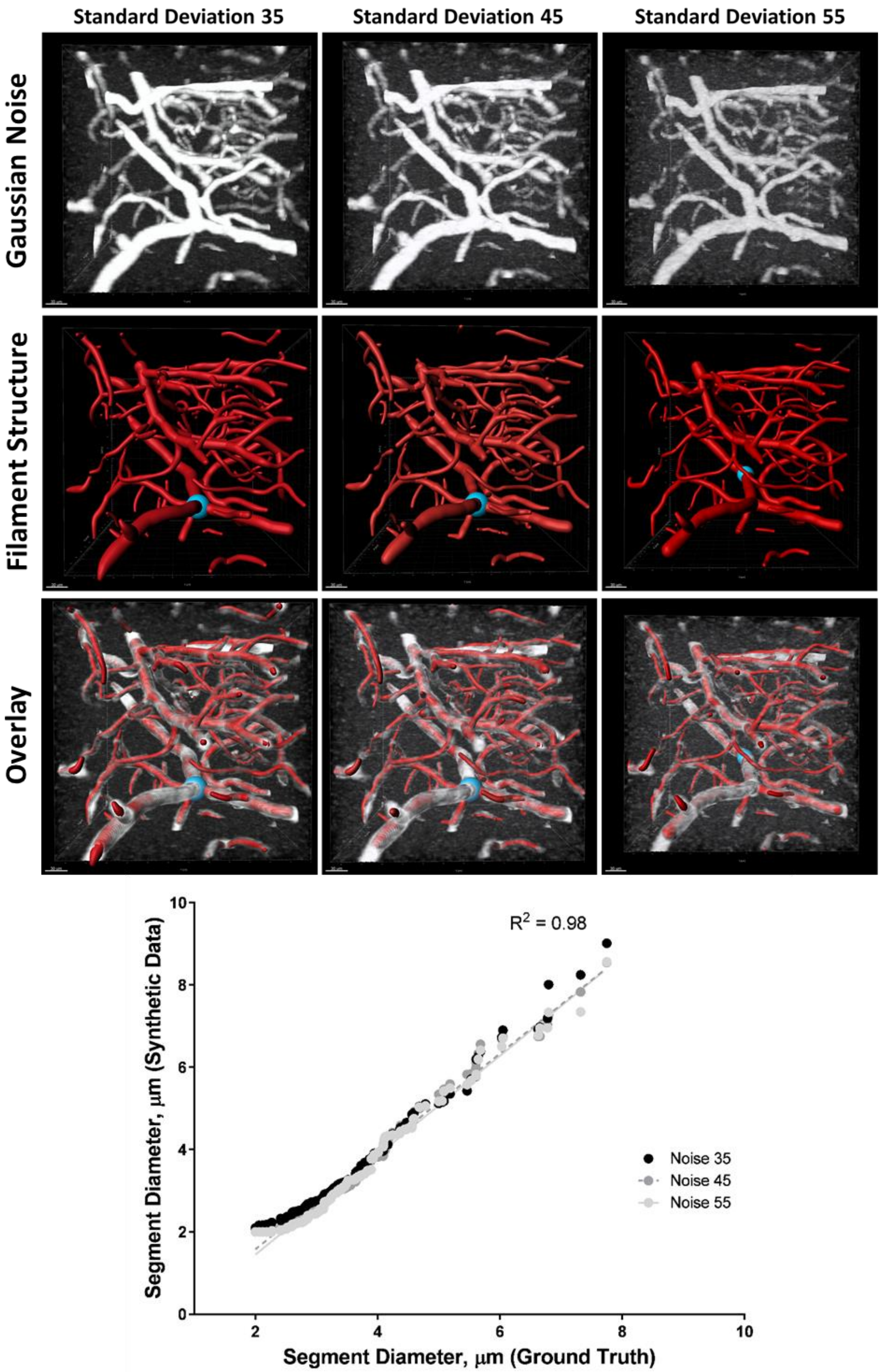
Supplemental Figure 2: Process flow diagram for brain vascular subregion optimization by segmentation and centerline insertion. Specific brain subregions were isolated into separate VOI image sets by 3-dimensional cropping of the primary data set. The intensity of each VOI data set was normalized to the average intensity of all VOIs by arithmetic adjustment. Standard deviation intensity projection of the image series was created to aid in the co-registration of the data set to a specified orientation. The final co-registered image series produced a 500 x 500 x 500 um VOI readjusted to a pixel size of 1um. The VOI data set was duplicated to produce three identical sub-blocks. The first sub-block was converted into a binary image series then used to extract the centerlines of vessels. The second sub-block served as a mask to subtract the intensity of all regions above 165 in the third sub-block. Finally, the centerlines of the first sub-block is merged with the third sub-block producing an image series with centerlines having a pixel intensity of 255 and vascular structures with a pixel intensity < 165.



Supplemental Figure 3: The post-processing of image data sets causes no artifactual alterations to vessel diameter or destruction of the native network architecture. The maximum intensity projection of a data set depicting cortical vessels after post-processing of an image by multiplication, unsharp mask, 3D Gaussian filter, maximum pixel intensity cutoff, binarization, centerline extraction and final image composition. Line graph demonstrating the linear pixel intensity profile (y-axis) across the entire diameter (x-axis) of a vessel before (black circles) and after the post-processing (gray triangle) of image data. The final version of the data set contains centerlines at a maximum pixel intensity of 255 while non-centerline pixel intensities are cut off at a maximum of 160.



Supplemental Figure 4: Manual error corrections following an automated filament trace function. Depiction of an erroneous gap (first panel, yellow) after automated filament tracing resolved by manually joining (second panel) the segment together followed by recalculating the diameter of the new portion of segment (third panel, yellow) from the image data set. Final version of a vessel filament that was manually corrected for a gap-error (fourth panel) that appeared following the automated filament trace function.


Standard Deviation 35
Standard Deviation 45
Standard Deviation 55
Gaussian Noise
Filament Structure
Overlay
R2 = 0.98
Noise 35
Noise 45
Noise 55
Segment Diameter, μm (Synthetic Data)
Segment Diameter, μm (Ground Truth)
0
2
4
6
8
10

Supplemental Figure 5: Effect of image noise on the three-dimensional vascular network reconstruction and analysis. Gaussian noise (top row) at a standard deviation of 35, 45, or 55, was artificially introduced into a 250 x 250 x 250 μm^3 data set. The quality of the generated filament structures (middle row) from each of the data sets was inspected. The specific positioning of the reconstructed filaments for each data set can be seen by overlay (bottom row). (A) Scatter plot of the measured filament diameters from each data set containing elevated Gaussian noise as a function to the filament diameters of the manually segmented and manually traced data set. Linear trend indicates agreement between the measured filament diameters. R^2 value indicates the strength of fit to the linear model.

Image Post-Processing

Function	Parameter(s)	Image Adjustment
Multiplication	Image = Image x 1.5	Used to shift up the average intensity of the original data set for thresholding.
Gaussian Filter	Sigma = 0.65 pixels	Convolution with Gaussian function. Smooths intensity of structures to aid in thresholding for binarization.
Unsharp Mask	Sigma = 1 pixel / Mask Weight = 0.06	Enhances local contrast to increase spatial resolvability.
Binarization	Moments Algorithm	To convert the 8-bit gray scale data set into a binary data set.
Centerline Extraction	3D Extraction from Binary Image	Used to obtain the center most pixel along the vessel structure's length in 3D space.
Maximum (Image Math)	Pixel I = Pixel I - (Pixel I - 160)	No pixel above 160. Primes image for centerline insertion at an intensity of 255. Only centerlines at 255.

Supplemental Table 1. Image post-processing functions used to optimize data sets prior to analysis. Multiplication: global pixel arithmatic; Gaussian Filter: convolving filter; Unsharp Mask: sharpen filter; Binarization: image format transformation; Centerline Extraction: morphological function; Maximum: global pixel arithmatic.

	Female		Male			
	Value	**SEM**	**Value**	**SEM**	**P-value**	**Units**
Age	3.0	0	3.0	0	n.r	Months
Weight	30	0.5	30	0.5	0.489	Grams
Temperature	36	0.1	36	0.1	0.125	C°
Systolic Blood Pressure	164	0.1	165	0.1	0.004	mmHg
Diastolic Blood Pressure	142	0.2	140	0.4	0.027	mmHg
Mean Arterial Pressure	149	0.2	148	0.3	0.057	mmHg
Whold Brain Analysis						
Vessel Diameter Range	2 - 220	-	2 - 220	-	n.r.	µm
Mean Diameter	36.6	2.4	54.3	10.6	0.095	µm
Number of Vessels	$1.4x10^{6}$	$2.6x10^{5}$	$1.8x10^{6}$	$3.0x10^{5}$	0.154	Segments
Intervessel Distance	20	5	32	13	0.037	µm
Total Vascular Volume	$2.2x10^{10}$	$4.0x10^{9}$	$1.3x10^{10}$	$1.7x10^{9}$	0.053	μm^{3}
Total Parencymal Volume	$1.5x10^{11}$	$5.1x10^{10}$	$4.7x10^{10}$	$1.7x10^{10}$	0.065	μm^{3}
Parencymal Volume (% Brain Volume)	76.7	6.6	62.3	8.7	0.111	%
Vascular Surface Area	$5.5x10^{9}$	$1.1x10^{9}$	$3.2x10^{9}$	$6.2x10^{8}$	0.075	μm^{2}
Vessel Surface Area / Vessel Volume	0.24	0.01	0.23	0.01	0.368	1 / µm
Vessel Population < 15 µm	50	6	40	3	0.084	% of Total
Vessel Population 20-80 µm	36.3	3	49	3	0.01	% of Total
BBB Interface (SA_c / V_p)	17.8	3.2	17.8	3.2	n.r.	mm^{2} / mm^{3}
Euler Number	$1.1x10^{6}$	$3.7x10^{5}$	$1.7x10^{6}$	$2.4x10^{5}$	0.11	Connections
Connectivity Value (Redundancy)	$5.2x10^{5}$	$2.2x10^{5}$	$1.2x10^{5}$	$6.2x10^{4}$	0.088	Cuts
Connective Density	$4.2x10^{-5}$	$8.0x10^{-6}$	$3.7x10^{-5}$	$4.7x10^{-6}$	0.333	1 / μm^{3}
Fractal Dimension	2.6	0.05	2.5	0.06	0.052	
Region Specific Analysis (Primary Somatosensory Cortex)						
Number of Vessels	660	54	468	107	0.071	Segments
Mean Diameter	14.76	0.22	14.37	0.31	0.175	µm
Vascular Surface Area	$5.3x10^{6}$	$5.5x10^{5}$	$4.5x10^{6}$	$3.3x10^{5}$	0.154	μm^{2}
Total Vascular Volume	$1.7x10^{7}$	$2.1x10^{6}$	$1.4x10^{7}$	$1.4x10^{6}$	0.164	μm^{3}
Total Parencymal Volume	$1.0x10^{8}$	$2.1x10^{6}$	$1.1x10^{8}$	$1.4x10^{6}$	0.164	μm^{3}
Intervessel Distance	38.6	2.4	43.1	1.5	0.1	µm
Volume of Vessels 15 µm	$6.5x10^{6}$	$1.2x10^{6}$	$3.8x10^{6}$	$7.7x10^{5}$	0.01	μm^{3}
Parencymal Zones < 15 µm	$3.2x10^{4}$	$6.5x10^{3}$	$2.4x10^{4}$	$2.7x10^{3}$	n.r.	Count
Parencymal Zones > 15 µm	$3.6x10^{5}$	$3.0x10^{4}$	$2.5x10^{5}$	$3.6x10^{4}$	0.04	Count
Network Composition, Vessels 6 µm	1835	490	1082	102	0.01	Segments
Euler Number	361	67	226	142	0.18	Connections
Connectivity Volue (Redundancy)	587	134	366	95	0.14	Cuts

Supplemental Table 2. Analysis of the whole brain cerebrovasculature and in the somatosensory cortex in female and male mice. Not reported = n.r..