Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain

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Pericytes are perivascular mural cells of brain capillaries. They are positioned centrally in the neurovascular unit between endothelial cells, astrocytes and neurons. This position allows them to regulate key neurovascular functions of the brain. The role of pericytes in the regulation of cerebral blood flow (CBF) and neurovascular coupling remains, however, under debate. Using loss-of-function pericyte-deficient mice, here we show that pericyte degeneration diminishes global and individual capillary CBF responses to neuronal stimuli, resulting in neurovascular uncoupling, reduced oxygen supply to the brain and metabolic stress. Neurovascular deficits lead over time to impaired neuronal excitability and neurodegenerative changes. Thus, pericyte degeneration as seen in neurological disorders such as Alzheimer’s disease may contribute to neurovascular dysfunction and neurodegeneration associated with human disease.

A properly functioning CNS requires regulation of CBF and oxygen supply to match neuronal functional activity, known as neurovascular coupling1–3. Neurovascular coupling is regulated by synchronous action of different cell types within the neurovascular unit, including neurons, vascular smooth muscle cells, endothelium and astrocytes4,5. Pericytes, mural cells of the capillary vessel wall6, are critical for stabilization of the capillary wall7–9 and maintenance of the blood-brain barrier10–12, and have been implicated in the regulation of capillary diameter and CBF13–15. Recent studies have demonstrated pericyte contractility in perfused brain slices, including contractile responses to different pharmacological vasoactive agents and electrophysiological stimulation13–15. Moreover, it has also been shown that active neurons increase their energy supply by dilating nearby arterioles and capillaries and that capillaries dilate ahead of arterioles in vivo in response to neuronal stimuli14,15. Neuronally evoked capillary dilation in vivo, but not arteriolar dilation, has recently been shown to depend on a rise in intracellular calcium in astrocytes15.

Some studies, however, dispute the proposed function of pericytes in neurovascular coupling. One study showed that pericytes in response to vasoconstrictors in vivo, but suggested that pericytes do not contribute to neurovascular coupling16. The other study, using genetically encoded microvascular mural cell labeling, functional calcium imaging and optogenetic light-driven cell activation, showed that spontaneous cardiac and respiratory-driven cerebral vasomotion and calcium currents are detectable only in arterioles but not in capillaries, that smooth muscle cells but not pericytes constrict vessels after light stimulation, and that capillary dilation in response to neuronal stimuli is not substantial, thus concluding that pericytes do not contribute to regulation of CBF responses17.

Pericyte degeneration is found in multiple neurodegenerative disorders involving early neurovascular dysfunction2,3,18, including mild dementia19, Alzheimer’s disease20–23 and amyotrophic lateral sclerosis24. Additionally, it has been shown that pericytes die after ischemic stroke and constrict capillary blood flow during the reperfusion phase of stroke14,25. Therefore, elucidating whether pericyte degeneration contributes to CBF dysregulation and neurovascular dysfunction, as seen in these neurological disorders, is extremely important. Moreover, blood oxygen level–dependent (BOLD) functional imaging signals, which depend on neurovascular coupling, are typically abnormal in early-stage Alzheimer’s disease, reflecting CBF dysregulation and aberrant hemodynamic responses, as shown by numerous studies26. Whether pericyte degeneration contributes to changes in BOLD signals in early Alzheimer’s remains, however, unknown.

To address whether pericyte degeneration affects neurovascular coupling, we studied hemodynamic responses in loss-of-function pericyte-deficient platelet-derived growth factor receptor-β (Pdgfrb+/−) mice, which at a young age develop a moderate, 25% loss of pericyte coverage of the brain capillary wall12. Given that most of the cortical vasculature in mice is composed of capillaries27, we hypothesized that if pericytes contribute to neurovascular coupling then Pdgfrb+/− mice12 will have reduced hemodynamic responses and diminished oxygen supply after neuronal stimulation, which over time will compromise neuronal function, leading to neurodegenerative changes.

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RESULTS
Impaired global and individual capillary responses
First, we found that the global CBF response to a hindlimb electrical stimulus as determined by laser Doppler flowmetry (LDF) in young Pdgfrb+/− mice, 1–2 months old was reduced by 30% compared to that in age-matched littermate Pdgfrb+/+ controls (Fig. 1a, 95% confidence interval (CI) of the mean difference [lower bound, upper bound] [4.9%, 54.9%]; Cohen’s d = 1.15). Next we used in vivo two-photon laser scanning microscopy (TPLSM) in Pdgfrb+/− mutants and controls to examine responses of individual capillaries (<6 µm in diameter) and arterioles (7–25 µm in diameter, including both precapillary and small penetrating arterioles) to a shorter electrical hindlimb stimulus in a well-defined region of primary somatosensory cortex (S1) (Fig. 1b) determined to be responsive by intrinsic optical signal (IOS) imaging. We first applied a low-pass and notch filter to all individual time courses of capillary responses to minimize cardiac and respiratory vasomotion artifacts (Fig. 1c) and then captured the time courses of diameter increase in each capillary by fitting a sigmoid parametric function to the individual capillary data traces in each animal (Supplementary Fig. 1). We computed time to 50% peak capillary dilation by averaging responses within individual animals and comparing data across animals (Fig. 1d and Supplementary Fig. 1). We found that capillaries in Pdgfrb+/− animals responded with a 6.5-s delay (95% CI [−8.6 s, −4.4 s], d = 2.69) in reaching 50% peak capillary dilation compared to Pdgfrb+/+ age-matched littermate controls (Fig. 1d; see Online Methods for details of diameter measurements), whereas the time to 50% peak arteriolar dilation was not different between the two groups (Fig. 1e, 95% CI [−4.2 s, 2.5 s], d = 0.25). Consistent with previous reports,14,15 we found that in control Pdgfrb+/+ mice arterioles reached 50% peak dilation with a delay of 2 s on average compared to capillaries, which dilated first (95% CI [−4.2 s, −0.1 s], d = 0.92). In contrast, vessel responses in Pdgfrb+/− mutants had the opposite order of dilation, with capillary diameter increases lagging behind arteriolar diameter increases by 3.5 s on average (95% CI [0.2 s, 6.8 s], d = 1.02).

Immunostaining analysis of CD13-positive pericytes in the stimulated cortical S1 area in a subset of control Pdgfrb+/+ mice studied for capillary dilation indicated a 77% pericyte coverage of lectin-positive endothelial profiles (≤6 µm in diameter) (Supplementary Fig. 2a–c), consistent with previous reports.8,11,12 Young pericyte-deficient Pdgfrb+/− mutants compared to age-matched littermate controls had an approximately 29% reduction in pericyte coverage, reflecting a 22% absolute loss of pericyte coverage (Supplementary Fig. 2a,b; 95% CI [17.7%, 26.6%], d = 7.16), as reported.12 Consistent with a delay in capillary diameter increases (Fig. 1c,d), analysis of TPLSM capillary line scans29 (Fig. 1g) indicated a 55% delay in stimulus-driven red blood cell (RBC) flow velocity increase in capillaries of Pdgfrb+/− mice compared to Pdgfrb+/+ controls (Fig. 1h,i; 95% CI [−104.9%, −6.0%], d = 1.08). Notably, there was no difference in the basal arteriolar or capillary vessel diameter between Pdgfrb−/− and Pdgfrb+/+ animals (Supplementary Fig. 3a; arterioles 95% CI [−3.8 µm, 2.9 µm], d = 0.12; capillaries 95% CI [−0.5 µm, 0.2 µm], d = 0.47), indicating that anatomical changes in the vessel diameter before stimulation did not influence vessel responses. Similarly, there were no baseline differences before stimulation between RBC flow velocity in arterioles or capillaries between Pdgfrb−/− and Pdgfrb+/+ animals (Supplementary Fig. 3b; arterioles 95% CI [−1.2 mm/s, 1.0 mm/s], d = 0.13; capillaries 95% CI [−0.2 mm/s, 0.3 mm/s], d = 0.14). The smooth muscle thickness in diving arterioles was also similar in Pdgfrb+/+ and Pdgfrb+/− mice.

Figure 1 Delayed capillary dilation and reduced capillary red blood cell flow in response to a hindlimb stimulus in 1- to 2-month-old pericyte-deficient Pdgfrb−/− mice. (a) CBF response to an electrical hindlimb stimulus (60 s, 7 Hz, 2 ms pulse duration) determined by LDF as a percentage of baseline change in Pdgfrb−/− mice (n = 9) and age-matched Pdgfrb+/+ (n = 10) littermate controls. Circles denote individual values derived from 3 independent LDF measurements per mouse; mean ± 95% CI, t-test, equal variances: t = 2.52, P = 0.02. (b) Representative z-stack projection of capillary (C) and arteriole (A) diameter measurement locations by TPLSM (inset; yellow boxes). Scale bar, 20 µm. (c) Time courses of diameter changes (mean ± 95% CI) in capillaries in Pdgfrb+/+ (red; n = 12 mice, 37 total capillaries) and Pdgfrb−/− (blue; n = 9 mice, 33 total capillaries) mice in the hindlimb S1 cortical area after an electrical hindlimb stimulus (10 s, 10 Hz, 2 ms pulse duration). Diameter changes are expressed relative to the respective basal capillary diameter before stimulation (value set to 0) and maximal diameter after stimulation (value set to 1). Vertical line denotes initiation of stimulus. Inset: sigmoid curve fits to the Pdgfrb+/+ (red) and Pdgfrb−/− (blue) time courses show average capillary diameter increase during the overall time course of dilation. (d) Mean time (± 95% CI) to 50% peak capillary diameter dilation increase in Pdgfrb+/+ (red; n = 12 mice, 37 total capillaries) and Pdgfrb−/− (blue; n = 9 mice, 33 total capillaries) mice from c (t-test, unequal variance: t = 6.48, P = 0.0002). Circles represent values per mouse derived from averaging individual capillary responses from sigmoid parametric fits to each capillary time course of dilation increase. (e) Time courses of diameter changes in arterioles in Pdgfrb+/+ (red; n = 8 mice, 18 total arterioles) and Pdgfrb−/− (blue; n = 11 mice, 20 total arterioles) mice in S1 cortex, in response to an electrical hindlimb stimulus (10 s, 10 Hz, 2 ms pulse duration). Arteriolar diameter changes are expressed relative to the respective basal and maximal diameters before and after stimulation, respectively, as for capillaries in c. Vertical line denotes initiation of stimulus. Inset: sigmoid curve fits to the Pdgfrb+/+ (red) and Pdgfrb−/− (blue) average arteriolar diameter increase during the overall time course of dilation increase. (f) Mean time (± 95% CI) to 50% peak arteriolar diameter dilation increase in Pdgfrb+/+ (blue; n = 8 mice, 18 total arterioles) and Pdgfrb−/− (red; n = 11 mice, 20 total arterioles) time (t-test, equal variance: t = 0.54, P = 0.60). Circles represent values per mouse derived from averaging the individual arteriole responses. Time to 50% peak diameter increase was determined from individual sigmoid parametric fits to each arteriolar time course of dilation increase. (g) Representative RBC TPLSM capillary line scans from Pdgfrb+/+ and Pdgfrb−/− capillaries illustrating RBC velocity changes after an electrical hindlimb stimulus (10 s, 10 Hz, 2 ms pulse duration). Dark lines closer to horizontal reflect faster RBCs. White lines: visual reference along RBC trajectories. Scale bar (left), 0.35 s. (h) Time courses of stimulus-driven RBC velocity changes in capillaries (mean ± 95% CI) from Pdgfrb+/+ (red; n = 12 mice, 38 total capillaries) and Pdgfrb−/− (blue; n = 9 mice, 39 total capillaries) mice. RBC velocity changes are expressed relative to the respective basal RBC velocity before stimulation (value set to 0) and maximal velocity upon stimulation (value set to 1). Vertical line denotes initiation of stimulus. Inset: sigmoid curve fits to the Pdgfrb+/+ (red) and Pdgfrb−/− (blue) average capillary RBC velocity increase during the overall time course of velocity increase. (i) Mean time (± 95% CI) to 50% peak RBC capillary velocity increase in Pdgfrb+/+ (red; n = 12 mice, 38 total capillaries) and Pdgfrb−/− (blue; n = 9 mice, 39 total capillaries) mice from h (t-test, equal variance: t = 2.35, P = 0.030). Circles represent values per mouse derived from averaging the individual RBC capillary velocity increase per mouse. Time to 50% peak RBC velocity increase was determined from sigmoid parametric fits of individual capillary time courses using the overall time course of RBC velocity increase. (j) Representative measurement locations on capillaries either covered by a pericyte (P, red, top) or in the absence of a pericyte (NP, bottom) in Pdgfrb+/+ mice crossed with NQ2-dsRed mice (Pdgfrb+/−/dsRed). Scale bar, 10 µm. (k) Relative dilation of pericyte-present and pericyte-absent capillary segments after an electrical hindlimb stimulus (10 s, 10 Hz, 2 ms pulse duration) in Pdgfrb+/−/dsRed mice determined 3 s after stimulus initiation. Responses (mean ± 95% CI) from 7 mice at 33 pericyte-present (red) and 34 pericyte-absent (blue) capillaries. Circles denote individual values per mouse derived from averaging all individual capillary dilation values per mouse per condition (t-test, equal variance: t = 2.59, P = 0.02). P values by two-tailed t-tests used as indicated in the legend for each data set for (d,f,i,k).
as shown by smooth muscle cell actin staining (Supplementary Fig. 3c,d; 95% CI [−1.1 μm, 0.4 μm], d = 0.64).

To assess whether loss of pericyte coverage was directly responsible for the loss of capillary dilation, we took advantage of the natural mosaic situation of the mouse model, whereby some segments of the capillary bed lack pericytes while others do not. We crossed PdgfrbR/− mice with NG2-dsRed (Cspg4-dsRed) mice (obtaining PdgfrbR/−;dsRed mice) to identify pericyte-covered capillaries. NG2-dsRed mice express dsRed in pericytes, smooth muscle cells and oligodendrocyte progenitor cells. These mice have been used previously to elucidate the role of capillary vasodilatation in vivo and to identify pericyte-covered microvessels. We determined capillary responses in PdgfrbR/−;dsRed mice and measured the diameter changes of capillaries where dsRed-labeled pericytes were present (either somata or processes; responses did not differ at these locations) and where no pericyte coverage was visible. Upon stimulation, active dilation of capillaries lacking pericyte coverage was barely detectable after 3 s, unlike those with pericyte coverage, which showed appreciable dilation in response to a stimulus (Fig. 1j,k and Supplementary Videos 1 and 2; 95% CI [0.2%, 2.1%], d = 1.38). These data suggest that capillaries lacking pericyte coverage in pericyte-deficient mice do not actively relax to generate neurovascular uncoupling.

To investigate further whether young, 1- to 2-month-old pericyte-deficient mice compared to controls develop a global deficit in neurovascular coupling, we studied changes in IOS acquired under 530-nm illumination. IOS at this wavelength has been demonstrated to reflect changes in hemodynamic responses. Pericyte-deficient mice compared to controls showed reductions in the peak signal amplitude and time to peak stimulus response (Fig. 2a,b), with an average 36% decrease in the peak amplitude (Fig. 2c; 95% CI [2.6%, 70.5%], d = 1.25) and a 2-s delay in 50% time to peak (Fig. 2d; 95% CI [−2.8 s, 0.2 s], d = 1.04), confirming abnormal neurovascular coupling. Notably, neuronal activity assessed by in vivo local field potential (LFP) recordings in somatosensory cortex of the same mice indicated no difference in the shape, amplitude or slope of the LFPs (Fig. 2e–h; amplitude 95% CI [−0.1 mV, 0.1 mV], d = 0.04; slope 95% CI [−0.2 mV/ms, 0.2 mV/ms], d = 1.16) after hindlimb stimulation, demonstrating that neuronal activity is unchanged in 1- to 2-month-old PdgfrbR/− mice. These data provide evidence for impaired hemodynamic responses in pericyte-deficient mice at an early stage, when neuronal excitability is still normal.

Arteriolar responses and endothelium-dependent vasodilation

We next studied whether changes in the arteriolar responses and endothelium-dependent vasodilation could contribute to neurovascular uncoupling in young PdgfrbR/− mice. Consistent with a comparable...
time to 50% peak arteriolar dilation (Fig. 1f), the stimulus-driven RBC velocity increase in arterioles was also similar in 1- to 2-month-old Pdgfrb+/- and Pdgfrb+/+ mice (Fig. 3a; 95% CI [-2.0 s, 4.1 s],  d = 0.38). We then imaged Alexa 633 hydrazide–positive arterioles (see Online Methods) in the S1 cortical region using acute brain slices from Pdgfrb+/- and Pdgfrb+/+ mice (Fig. 3b–e). Arteriolar constriction in response to phenylephrine was unchanged in Pdgfrb+/- arterioles compared to Pdgfrb+/+ arterioles (Fig. 3c,d; 95% CI [-5.7%, 14.7%],  d = 0.71). Similarly, there was no difference in smooth muscle relaxation induced by adenosine, an endothelium-independent vasodilator that acts as a direct vascular smooth muscle cell relaxant32, in Pdgfrb+/- and Pdgfrb+/+ arterioles preconstricted with phenylephrine (Fig. 3e; 95% CI [-12.1%, 17.2%],  d = 0.29). We then tested in vivo the effect of adenosine, finding no changes in CBF responses between Pdgfrb+/- mice and littermate controls, as determined by LDF using methods previously reported32 (Fig. 3f; 95% CI [-5.7%, 11.7%]).

Figure 2 Impaired hemodynamic response in 1- to 2-month-old pericyte-deficient Pdgfrb+/- mice determined by IOS imaging (530 nm) at a time point when neuronal activity is unaffected as determined by LFP recordings. (a) Example grayscale images of the visualized somatosensory cortex showing vasculature (far left) and IOS signals under 530 nm illumination (pseudocolored) in response to an electrical hindlimb stimulus (10 s, 10 Hz, 2 ms pulse duration) beginning at 0 s in Pdgfrb+/- mice and Pdgfrb+/- littermate controls. Peak signals and peak signal times are indicated. Scale bar, 0.5 mm. Boxes indicate regions of interests (ROIs) at least 30 µm away from large surface blood vessels for data shown in b. (b) IOS signal time courses in parenchymal regions for the examples shown in boxed ROIs in a. Arrows denote peak IOS signal times for Pdgfrb+/- (red) and littermate control Pdgfrb+/- (blue) mice. Curves are means of 10 trials for a representative individual mouse (± 95% CI) per group. (c,d) Mean (± 95% CI) change in IOS peak height (t-test, equal variance, single-tailed:  t = 2.35,  P = 0.02; e), and time to 50% peak response signal (t-test, equal variance, single-tailed:  t = 1.95,  P = 0.04; f) in 7 Pdgfrb+/- and 7 Pdgfrb+/- mice in ROIs away from large surface vessels. Each circle represents the value per mouse averaged from 10 trials as shown in b. (e,f) Representative traces from single trials illustrating a train of evoked LFPs. After a 10-s baseline, hindlimb stimulus was applied (10 s, 10 Hz, 0.1 ms pulse duration) followed by 10 s without stimulus in Pdgfrb+/- (top, red) and Pdgfrb+/- (bottom, blue) mice. (f) 100 averaged LFP traces from a single trial (mean ± 95% CI) for Pdgfrb+/- (red) and Pdgfrb+/- (blue) mice shown in e. Stimulus artifacts and LFPs are indicated in e,f. (g) Mean (± 95% CI) LFP peak amplitude (t-test, equal variance:  t = 0.07,  P = 0.95) and (h) slope (t-test, unequal variance:  t = 0.12,  P = 0.80) from Pdgfrb+/- (n = 5 mice; 2,500 total LFPs) and Pdgfrb+/- (n = 6 mice; 3,600 total LFPs) mice. Two-tailed t-tests unless otherwise indicated.
Acetylcholine (10 mM) to 50% peak arteriole RBC velocity increase per mouse. Circles denote individual values per mouse derived from averaging all times to 50% peak arteriolar RBC velocity increase per mouse. (Representative measurement of arteriole smooth muscle cell function in brain slices in the cortical S1 region in Pdgfrb+/+ and Pdgfrb−/− mice in resting conditions (aCSF, artificial cerebrospinal fluid) and after 100 µM phenylephrine (Phe; constriction) and 400 µM adenosine (relaxation). DIC: differential interference contrast image. Alexa 633: arteriole-specific vessel marker confirming vessel type. (c) Mean ± 95% CI arteriole diameter change relative to basal value in brain slices from 5 Pdgfrb+/+ mice and 4 Pdgfrb−/− mice. (d,e) Quantification (mean ± 95% CI) of arteriole constriction by phenylephrine (Phe; t-test, equal variance: t = 0.74, P = 0.47) after an electrical hindlimb stimulus (10 s, 10 Hz, 2 ms pulse duration) as in Figure 1g-i. (f-h) In vivo CBF response to adenosine (400 µM; Mann-Whitney U test, P = 1.00; f) acetylcholine (10 µM; Mann-Whitney U test, P = 1.00; g) and A23187 (3 µM; Mann-Whitney U test, P = 0.70; h) determined by DLP in 3 Pdgfrb+/+ and 3 Pdgfrb−/− mice. (g) Vasoactive agents were superfused over the exposed cortical surface. CBF measurements were performed in triplicate and an average individual value per animal was used for statistical analysis. Two-tailed t-tests unless otherwise indicated. Bootstrapped in f-h.

Changes in astrocytes in Pdgfrb−/− mice 6–8 months of age12. These data also suggest that the astrocyte-dependent component of CBF regulation is likely intact at early stages in Pdgfrb−/− mice. Similarly, there was no change in microglia numbers as shown by microglia-specific Iba1 immunostaining (Supplementary Fig. 4a,d; 95% CI [−5.1 cells per mm², 4.1 cells per mm²], d = 0.16). An increase in microglia is seen in Pdgfrb−/− mice only at a very late stage of 14–16 months of age, but not before12. Collectively, these studies suggest no detectable changes in endothelial, smooth muscle cell–dependent and astrocyte-dependent mechanisms of flow regulation in the present pericyte-deficient model.

Three-dimensional reconstructions of the vasculature revealed 10–16% lower capillary density across layers I–VI of the somatosensory cortex that, while not reaching significance, suggested a vascular capillary phenotype in Pdgfrb−/− mice compared to Pdgfrb+/+ controls (Supplementary Fig. 4e,f; 95% CI [−6.2%, 38.8%] across average of layers I–VI], d = 1.25 for capillary density), as reported12. In contrast, no changes were found in larger cortical vessels, including arterioles (Supplementary Fig. 4e,f; 95% CI [−140.3%, 152.5%], d = 0.07). To determine whether moderate microvascular reductions affect hemodynamic responses independently of pericyte coverage, we used another transgenic murine model with microvascular reductions but normal pericyte coverage, haploinsufficient mesenchyme homeobox 2 (Meox2−/−) mice33. Meox2−/− mice display approximately 30% reduction in the cortical capillary density due to primary endothelial...
hypoplasia, but have normal global CBF responses to neuronal stim-
uli\textsuperscript{12}. We confirmed reduced capillary density and normal pericyte
coverage in Meox\textsuperscript{2+/−} mice (Supplementary Fig. 5a–c; capillary
length 95% CI [30.4%, 39.8%], \(d = 16.94\); pericyte coverage 95% CI
\([-11.6\%, 15.3\%], \(d = 0.20\)) and showed that these mice have normal
stimulus-driven capillary and arteriolar responses as determined
by TPLSM (Supplementary Fig. 5d,e; capillary 95% CI \([-1.8\%, 0.66\%],
\(d = 0.91\); arteriole 95% CI \([-2.8\%, 1.2\%], \(d = 0.70\), consistent with

![Figure 4](image-url)

**Figure 4** Diminished brain tissue oxygen levels and oxygen delivery in young pericyte-deficient \(Pdgfrb^{+/−}\) mice. (a) Representative maximal intensity plots of rhodamine-labeled vasculature (left) and \(pO_2\) measurements for the same cortical regions at two different depths in 5- to 6-month-old \(Pdgfrb^{+/+}\) and \(Pdgfrb^{+/−}\) mice (center and right). Far right: maximal intensity projection examples of the vasculature in the first 100 \(\mu\)m depth and between 100 and 200 \(\mu\)m depth. Scale bars, 100 \(\mu\)m. (b) Histograms of distribution of \(pO_2\) measurements at 100 \(\mu\)m (left) and 200 \(\mu\)m (right) depth between 0 and 60 mm Hg. A total of 2,076 measurements at 100 \(\mu\)m and 2,344 measurements at 200 \(\mu\)m for \(Pdgfrb^{+/+}\) mice and a total of 2,173 measurements at 100 \(\mu\)m and 2,867 measurements at 200 \(\mu\)m for \(Pdgfrb^{+/−}\) mice were pooled from 3 mice in each group. (c) Percentage of image fields with \(pO_2\) values <15 mm Hg at 100 \(\mu\)m (left) and 200 \(\mu\)m (right) depth (mean ± 95% CI) from 3 mice per group. Individual values were obtained from 381–1,016 measurements per mouse per depth (Mann-Whitney \(U\) test, single-tailed: \(P = 0.03\) at 100 \(\mu\)m and \(P = 0.05\) at 200 \(\mu\)m; bootstrapped at both depths). (d) Pseudocolored IOS image sequence illustrating the activity map in the S1 region in response to hindlimb stimulation (300 ms mechanical vibration stimulus), showing baseline and peak responses of different phases including the initial dip (negative), followed by overshoot (positive peak) and undershoot (second negative deflection). Data were obtained using 627-nm illumination in 1- to 2-month-old \(Pdgfrb^{+/+}\) and \(Pdgfrb^{+/−}\) mice. Blues are negative signals and reds are positive signals. Scale bar, 0.5 mm. (e) Example IOS signal traces of intensity change in response to stimulus (at \(t = 0\) s) from the ROIs (box locations, at least 30 \(\mu\)m away from large surface blood vessels) of images in d. Curves are means of 10 trials for a representative individual mouse (± 95% CI) per group. Arrows indicate the peaks of overshoot. (f) Quantification (mean ± 95% CI) of peak intensity of the overshoot response (arrows in e) in 4 \(Pdgfrb^{+/+}\) and 5 \(Pdgfrb^{+/−}\) mice (\(t\)-test, two-tailed, equal variance: \(t = 4.39, P = 0.003\)).
previously reported normal global CBF responses\textsuperscript{12}. Whether additional compensatory mechanisms can contribute to normal global hemodynamic responses in $\text{Meox2}^{+/−}$ mice despite reductions in capillary length\textsuperscript{12} remains unknown. However, the analysis of vessel responses in $\text{Pdgfrb}^{+/−}$ and $\text{Meox2}^{+/−}$ mice suggests that a moderate reduction in capillary density alone does not lead to aberrant neurovascular coupling unless mice develop pericyte deficiency, as seen in $\text{Pdgfrb}^{+/−}$ mutants.

**Reduced tissue oxygenation and oxygen supply under stimulus**

Tight regulation of blood flow is critical for oxygen availability and normal brain function\textsuperscript{13,18}. Therefore, changes in CBF responses due to pericyte degeneration could potentially create a state akin to chronic hypoperfusion or hypoxia\textsuperscript{12} around the capillary-fed tissue, which may then lead to metabolic stress and accelerated neuronal damage and loss. To assess tissue oxygenation, we used in vivo TPLSM pO\textsubscript{2} (oxygen partial pressure) imaging in the cortex at two depths\textsuperscript{34} (Fig. 4a). $\text{Pdgfrb}^{+/−}$ mice had an overall shift in tissue oxygenation to pO\textsubscript{2} values lower than the typical 20–40 mm Hg range observed in normal mice (Fig. 4b). At 100 and 200 μm from the brain surface (Fig. 4a), a substantial portion of the tissue in $\text{Pdgfrb}^{+/−}$ mice had low pO\textsubscript{2} values (<15 mm Hg) compared to $\text{Pdgfrb}^{+/+}$ controls (Fig. 4c). 100 μm 95% CI [−7.7%, −1.1%], $d = 3.01$; 200 μm 95% CI [−27.5%, −10.0%], $d = 4.86$. In particular, in tissue far from arterioles at 200 μm depth, $\text{Pdgfrb}^{+/−}$ mice exhibited even lower pO\textsubscript{2} (<5 mm Hg), indicating potential existence of chronically hypoxic tissue pockets (Fig. 4a).

To study how the tissue oxygen supply changes in response to hindlimb stimulus, we next measured IOS at 627 nm in young $\text{Pdgfrb}^{+/−}$ and $\text{Pdgfrb}^{+/+}$ mice. At this wavelength, light is dominantly absorbed by deoxyhemoglobin and changes in IOS mainly reflect changes in oxygen consumption and/or delivery during hemodynamic response\textsuperscript{30,31}. The initial dip (Fig. 4d) is thought to be primarily the result of neuronal activity—that is, increase in oxygen consumption increases deoxyhemoglobin before a significant increase of CBF washes out deoxyhemoglobin—but it is possible that it could also result from an initial increase in cerebral blood volume and total hemoglobin due to stimulus\textsuperscript{30,31}. The positive peak (overshoot) (Fig. 4e) is predominantly due to the deoxyhemoglobin washout\textsuperscript{31}. $\text{Pdgfrb}^{+/−}$ mutants compared to $\text{Pdgfrb}^{+/+}$ controls showed a 34% reduction in the IOS signal peak (overshoot) and a delay in time to peak (Fig. 4d–f; 95% CI [15.8%, 52.6%], $d = 2.92$), indicating a reduction in oxygen delivery to the hindlimb S1 cortex that supports the observed deficit in neurovascular coupling.

To corroborate these data, we studied reduced nicotinamide adenine dinucleotide (NADH) changes in response to hindlimb stimulus. NADH is a molecule that is ubiquitously expressed and also intrinsically fluorescent in its reduced state (NAD\textsuperscript{+}), allowing it to function as a cellular metabolic indicator\textsuperscript{35}. Astrocytes labeled with sulforhodamine 101 (SR101) were used to control for the possible effect of hemodynamic changes due to stimulation on NADH fluorescence intensity\textsuperscript{36} (Fig. 5a). TPLSM in vivo imaging of NADH revealed an appreciable transient NADH signal increase in $\text{Pdgfrb}^{+/−}$ mice (compared to negligible change in $\text{Pdgfrb}^{+/+}$ controls) during functional activation (Fig. 5b; 95% CI [−0.1 fraction change, 0.0 fraction change], $d = 1.13$), indicating a reduction in oxygen delivery compared to consumption. Together these data show that the capillaries in pericyte-deficient mice are incapable of supplying sufficient oxygen to the brain either at rest or under stimulus.

We then sought to determine whether the changes observed in CBF regulation and oxygen delivery compared to consumption paralleled changes in lactate metabolism. Lactate, produced by astrocytes, is an alternative fuel source for neurons, requiring less oxygen for energy production than glucose\textsuperscript{37,38}. Using in vivo microdialysis of the somatosensory cortex, we found that 1- to 2-month old $\text{Pdgfrb}^{+/−}$ mice exhibited 47% higher interstitial fluid (ISF) lactate in the cortex than $\text{Pdgfrb}^{+/+}$ littermate controls (Fig. 5c). 47%; 95% CI [−8.1%, 102.7%], $d = 1.91$, $P = 0.04$; and serum metabolite levels (Supplementary Fig. 6a,b). Lactate 95% CI [−1.42 mM, 2.22 mM], $d = 0.32$; glucose 95% CI [−1.10 mM, 1.18 mM], $d = 0.05$ remained unchanged. Moreover, upon neuronal stimulation via high [K\textsuperscript{+}] retrodialysis, we found a 28% lower increase in stimulus-driven lactate levels in the ISF in $\text{Pdgfrb}^{+/−}$ mice compared to controls (Fig. 5e). 95% CI [5.2%, 50.5%], $d = 1.98$, possibly reflecting increased utilization of lactate released from astrocytes by activated neurons in a setting of low oxygen, when lactate becomes a preferred energy metabolite for neurons\textsuperscript{38}.

![Figure 5](https://example.com/figure5.png)
Impaired neuronal function and secondary neurodegeneration

Finally, we studied whether pericyte reductions causing early hemodynamic changes can lead to impaired neuronal function over time. As shown above, 1- to 2-month-old *Pdgfrb*+/− mice develop hemodynamic changes due to pericyte dysfunction, but do not show changes in neuronal excitability (Fig. 2e–h). Using voltage-sensitive dye (VSD) imaging of evoked membrane potential responses in the hindlimb S1 cortical area, we confirmed a normal depolarization pattern with no abnormalities in peak amplitude of response or response latency in 1- to 2-month-old *Pdgfrb*+/− mice (Supplementary Fig. 7a–d; time to peak 95% CI [−0.01 s, 0.05 s], d = 0.96; peak fluorescence change 95% CI [−0.2%, 0.2%], d = 0.07). Structural analysis by dual labeling of NeuN/TUNEL NeuN/SMI-312

Figure 6 Cortical neuronal activity, neuronal number, neuritic density and behavior in pericyte-deficient *Pdgfrb*+/− mice 1–2 and 6–8 months old, along with age-matched littermate controls. (a) Double immunostaining for NeuN (neuronal marker) and SMI-312 neurofilament (top) and for NeuN and TUNEL (bottom) in 1- to 2-month-old *Pdgfrb*+/+ and *Pdgfrb*+/− mice. (b,c) Quantification (mean ± 95% CI) of NeuN-positive cells (t-test, equal variance: t = 0.37, P = 0.72; b) and SMI-312-positive neuritic density (t-test, equal variance: t = 0.35, P = 0.73; c) in the S1 cortex in 5 *Pdgfrb*+/+ and 5 *Pdgfrb*+/− mice 1–2 months of age. In each animal 6 randomly selected fields from the cortex were analyzed in 5 nonadjacent sections (−100 µm apart) and data were averaged per mouse to obtain individual values (circles) as illustrated. (d–f) Novel object recognition (NOR) (d, n = 4 mice per group; t-test, equal variance: t = 1.17, P = 0.29), nest construction (e, n = 3 mice per group; Mann-Whitney U test, P = 1.00) and burrowing (f, food displacement; 7 *Pdgfrb*+/+ and 6 *Pdgfrb*+/− mice; t-test, equal variance: t = 0.31, P = 0.76) in 1- to 2-month-old mice; all values are mean ± 95% CI. (g) Representative pseudocolored VSD image sequence of cortical neuronal activity in response to a 300-ms hindlimb mechanical stimulus in 6- to 8-month-old *Pdgfrb*+/+ and *Pdgfrb*+/− mice. Dashed line indicates hindlimb (HL) region. Scale bar, 0.5 mm. (h–j) Representative VSD intensity traces (h), time to peak (i), time to peak, single-tailed, equal variance: t = 1.91, P = 0.05) and peak fluorescence change (j; mean ± 95% CI; t-test, single-tailed, equal variance: t = 2.09, P = 0.04) in 5 *Pdgfrb*+/+ and 4 *Pdgfrb*+/− mice 6–8 months of age. Arrows in h indicate peak VSD signal times for 6- to 8-month-old *Pdgfrb*+/− mice (blue) and *Pdgfrb*+/− (red) controls. Curves are means of 10 trials for a representative individual mouse (±95% CI) per group. VSD data showing no changes in cortical neuronal activity in 1- to 2-month-old *Pdgfrb*+/− mice are shown in Supplementary Figure 7. (k) Double immunostaining for NeuN and SMI-312 (top) and for NeuN and TUNEL (bottom) in S1 cortex in 6- to 8-month-old mice. (l,m) Quantification (mean ± 95% CI) of NeuN-positive cells (l, cortex: t-test, t-test, single-tailed, equal variance: t = 2.28, P = 0.03; hippocampus: t-test, equal variance: t = 2.05, P = 0.04) in 5 *Pdgfrb*+/+ and 5 *Pdgfrb*+/− mice, and SMI-312-positive neuritic density (m, cortex: t-test, single-tailed, equal variance: t = 2.48, P = 0.02; hippocampus: t-test, single-tailed, equal variance: t = 2.56, P = 0.01) in the S1 cortex and CA1 hippocampus in 6 *Pdgfrb*+/+ and 6 *Pdgfrb*+/− mice 6–8 months of age. In each animal 6 randomly selected fields from the cortex were analyzed in 5 nonadjacent sections (−100 µm apart) and data were averaged per mouse to obtain individual values (circles) as illustrated. (n–p) NOR (n, n = 4 mice per group; t-test, equal variance: t = 3.52, P = 0.01), nest construction (o, n = 5 mice per group; Mann-Whitney U test, single-tailed, P = 0.05) and burrowing (p, food displacement; 7 *Pdgfrb*+/+ and 6 *Pdgfrb*+/−; t-test, equal variance: t = 2.76, P = 0.02) in 6- to 8-month-old mice. All values are mean ± 95% CI. Two-tailed t-tests unless otherwise indicated. Bootstrapped in e,o.
neurons for NeuN (a neuronal marker) and SMI-312-positive neurofilaments revealed a normal number of neurons and normal neuritic density in the S1 cortical area (Fig. 6a–c; NeuN 95% CI [−222.0 cells per mm², 306.4 cells per mm²], d = 0.23; neuritic density 95% CI [−4.0% area, 5.4% area], d = 0.22) and hippocampus (data not shown). Dual labeling for NeuN and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for DNA fragmentation was negative (Fig. 6a), demonstrating no detectable neuronal cell death at this early stage. Novel object recognition (NOR), nesting and burrowing tests showed no behavioral deficits (Fig. 6d–f; NOR 95% CI [−8.0%, 22.7%], d = 0.83; nest score 95% CI [−0.4, 2.1], d = 1.55; food displaced as a result of burrowing 95% CI [−80.7 g, 107.4 g], d = 0.18). However, 6- to 8-month-old Pdgfrb+/− mice displayed abnormalities in the evoked cortical depolarization, peak amplitude of response and response latency (Fig. 6g–j; time to peak 95% CI [−0.2 s, 0.0 s], d = 1.21; peak fluorescence 95% CI [−0.0%, 0.3%], d = 1.38), had 15% lower neuron numbers in the S1 cortex and CA1 hippocampal subfield and 22% lower neuritic density (Fig. 6k–m; NeuN cortex 95% CI [−0.2%, 30.4%], d = 1.93; NeuN hippocampus 95% CI [−1.8%, 31.9%], d = 1.24; SMI-312 cortex 95% CI [2.2%, 43.6%], d = 1.43; SMI-312 hippocampus 95% CI [2.9%, 41.6%], d = 1.48), and exhibited occasional double-positive TUNEL+/NeuN+ cells (Fig. 6k) and changes in behavior (Fig. 6n–p; NOR 95% CI [5.5%, 30.8%], d = 2.48; nest score 95% CI [0.6, 1.4], d = 3.62; food displaced 95% CI [15.4 g, 137.0 g], d = 2.76). Collectively, these data suggest that pericyte-deficient mice develop age-related neurodegenerative changes as a continuum of the pathophysiological process initially triggered by pericyte degeneration.

As expected, global CBF responses to a stimulus progressively worsened in mice 6–8 months old, reduced by 58% relative to age-matched controls (Supplementary Fig. 8a; 95% CI [49.7%, 66.1%], d = 12.22), compared to a 30% reduction in Pdgfrb−/− mice 1–2 months old (Fig. 1a), which could be attributed to both a progressive pericyte reduction at 6–8 months—38% relative loss of coverage (Supplementary Fig. 8b,c; 95% CI [18.9%, 56.8%], d = 3.46) compared to 29% at 1–2 months (Supplementary Fig. 2a,b)—and added secondary neuronal changes, as suggested by our data (Fig. 6g–m).

**DISCUSSION**

The present study shows that pericyte-deficient Pdgfrb−/− mice develop early reduced global and individual capillary CBF responses to neuronal stimulus resulting in neurovascular uncoupling, limited oxygen supply to brain and metabolic stress. At this early stage characterized by impaired hemodynamic responses, we did not find detectable changes in neuronal, endothelial, smooth muscle cell–dependent and astrocyte-dependent mechanisms of flow regulation, suggesting that pericyte dysfunction is the primary factor driving CBF dysregulation in this loss-of-function model. We cannot, however, rule out the possibility that moderate reductions in capillary cortical density (10–16%), although found not to be meaningful in the present study, can still contribute indirectly to diminished global CBF responses in young Pdgfrb−/− mutants. By contrast, our finding of delayed stimulus–driven individual capillary responses would support direct causality between pericyte deficiency and diminished capillary dilation in this model. We also show that impaired hemodynamic responses lead over time to impaired neuronal excitability and secondary neurodegenerative changes. Thus, at a later stage, dysfunction in other neurovascular unit cell types including neurons may contribute indirectly to worsening abnormal hemodynamic responses initiated by pericyte degeneration.

It is conceivable that other vascular changes, such as blood-brain barrier breakdown and accumulation of blood-derived toxic products in the brain10–12, may contribute to neuronal dysfunction and neurodegenerative changes seen with age in pericyte-deficient mice. The exact contributions of impaired hemodynamic responses and blood-brain barrier breakdown to the pathophysiological process of neurodegeneration remain unknown.

Although extremely useful for understanding pericyte biology and providing important insights into their role in regulating neurovascular functions7,9–12, the current models of PDGF-BB and PDGFβR deficiency have their own limitations. For example, they cannot isolate the developmental impact of embryonic loss of PDGFβR signaling on vascular phenotype in the adult and aging brain. Additionally, smooth muscle cells also express PDGFβR, which contributes to vascular phenotype in the embryonic CNS7,9. Conversely, the role of pericytes and smooth muscle cells in adult brain could be different from their role in the embryonic CNS, as discussed9,12. The present study indicates, however, that smooth muscle cell function and responses to vasodilators or constrictors are not altered in arterioles of young Pdgfrb−/− mice. This is consistent with findings of intact stimulus-driven arteriolar diameter increases and normal RBC velocity arteriolar increase, and lack of basal differences in the arteriolar and capillary vessel diameter or basal RBC flow between young mutants and controls, as we report. Some studies have suggested that PDGFβR is expressed even by neuronal progenitors in the subventricular zone38, which has not been confirmed by others in the embryonic CNS40 or adult brain12,41. Nevertheless, the present study examines CBF responses in the cortex but not subventricular zone containing the neuronal progenitor pool.

Recent studies in NG2-cre transgenic mice expressing channelrhodopsin 2 (ChR2) found that light-driven optogenetic stimulation leads to constriction of smooth muscle cell–covered arterioles, but does not have an appreciable effect on pericyte-covered capillaries17. Because this study17 was focused on constriction responses of mural cells, it is difficult to make a direct comparison with the present findings focused on dilation of capillaries and arterioles in a loss-of-function model. Using optogenetic stimulation causing a hyperpolarization of the mural cells followed by relaxation and dilation of the vessels would allow more direct comparison with the present study. It has been suggested, however, that a controversy between the previous optogenetic study in transgenic mice17 and recent findings by others demonstrating pericyte contractility and capillary dilation responses in normal mice in vivo14,15 might be attributed to a drift in pericyte definition, particularly renaming pericytes on precapillary arterioles to smooth muscle cells42.

Additionally, one wonders whether a threshold for optogenetic stimulation of ChR2 in smooth muscle cells and pericytes in vivo is comparable between these two cell types in NG2-cre ChR2 mice17. Smooth muscle cells and pericytes are known to express different types and amounts of contractile proteins and Ca2+ channels43,44 and therefore might have different thresholds for optogenetic stimulation. Notably, another recent optogenetic study focused in Pdgfrb−/− mice expressing ChR2 has shown that strong two-photon optogenetic stimulation of pericytes leads to their contraction, constricting capillary lumens and reducing RBC flow in vivo (D.A. Hartmann, R.I. Grant and A.Y. Shih, Medical University of South Carolina, personal communication), opposite from the previous report in NG2-cre ChR2 mice17. Whether the differences in optogenetic ChR2 stimulation of pericytes and contractility are model-dependent or optogenetic light stimulus source and duration-dependent, as well as whether different Cre drivers can lead to differential ChR2 expression in different pericyte subpopulations, remains to be determined. However, our present data in control, non-transgenic mice corroborate
In contrast to all previous studies, the present study uses a loss-of-function approach to examine the effect of a loss of pericyte function on cerebrovascular regulation. We demonstrate that pericyte reductions lead to early hemodynamic cerebrovascular changes, before changes in neuronal excitability and structure and/or changes in function of other neurovascular unit cell types, which links pericyte reductions to an age-dependent continuous pathophysiological process of neurodegeneration. Therefore, pericyte degeneration as seen in neurological disorders\textsuperscript{19–24} associated with neurovascular dysfunction, such as Alzheimer’s disease\textsuperscript{2,3,18}, can contribute to impaired neurovascular coupling and diminished oxygen supply to brain, as shown by BOLD signal changes early in the disease\textsuperscript{26}. These hemodynamic changes, in turn, may contribute to neurodegeneration accompanying human disease. Thus, pericytes could be an important new therapeutic target for treating neurovascular dysfunction and neurodegeneration.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

K.K., A.R.N. and A.B. contributed to manuscript preparation and to experimental design and analysis, and conducted experiments. S.V.R. and S.S. contributed to experimental design and to data analysis and interpretation, and conducted experiments. A.A., D.L. and Y.W. conducted and analyzed experiments. B.V.Z. supervised and designed all experiments and analysis, and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Pdgfrb+/− mice and their littermate controls on mixed 129S1/SvImJ background were generated as we previously described28. All experiments were performed on 1- to 2-month-old or 6- to 8-month-old Pdgfrb+/− mice and littermate Pdgfrb+/+ controls unless otherwise specified. Pdgfrb+/− mice with pericytes expressing dsRed (Pdgfrb+/−;dsRed) were generated by crossing Pdgfrb+/− mice with N22–dsRed mice on a C57Bl6 background21. Mox2−/− mice and their controls on a C57Bl6 background were generated as we previously described28 and were used at 1–2 months of age. Both male and female animals were used throughout the experiments. Mice were housed in plastic cages on a 12-h light cycle with ad libitum access to water and a standard laboratory diet. During in vivo surgery and experiments, body temperature was maintained with electric heating pads, with thermal feedback and respiration monitoring. Intraperitoneal injections of 5% glucose in isotonic saline (0.2 mL/25 g) were administered every 2 h. Experiments were performed under isoflurane anesthesia (SomnoSuite, Kent Scientific) unless otherwise specified. For details, see specific experiments below. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California with National Institutes of Health guidelines and the Massachusetts General Hospital Subcommittee on Research Animal Care.

Cranial window. Animals were initially anesthetized with 100 mg/kg of ketamine and 50 mg/kg of xylazine, then fixed in a stereotaxic frame (Kopf Instruments) and transitioned to isoflurane anesthesia. A circular cranial window was drilled over the hindlimb region of the somatosensory cortex (center at AP = −0.94 mm, L = 1.5 mm). The window was filled with 2% low-melt agarose (Sigma, A9539) in artificial cerebrospinal fluid (aCSF) and covered with a 3-mm round coverslip. Animals were maintained on isoflurane anesthesia for subsequent experiments.

Laser Doppler flowmetry (LDF). CBF responses to hindlimb stimulation in anesthetized mice (−2% isoflurane) were determined using laser Doppler flowmetry measured through a cranial window, as previously described28,49. The tip of the laser Doppler probe (Transonic Systems Inc., Ithaca, NY) was stereotactically placed 0.5 mm above the cranial window over the area determined to be responsive from IOS imaging (see below). CBF was recorded from the somatosensory cortex hindlimb region following electrical stimulation of the hindlimb using a 60-s stimulus. The percentage increase in CBF due to stimulation was obtained after identification of dsRed pericyte-covered and uncovered capillary regions. Animals were maintained on isoflurane anesthesia for subsequent experiments.

In vivo two-photon microscopy. Diameter and velocity measurements. To identify the brain region responsive to hindlimb stimulation for two-photon microscopy, diameter and velocity experiments, red (630 nm) intrinsic optical signaling (IOS, see below) images were acquired in response to a 0.3-s electrical stimulus delivered to the contralateral hindlimb at 5 V at a rate of 7 Hz, 5 ms pulse duration (6002 Stimulator, Harvard Apparatus) using custom electrical probes. At the frequency used, this is equivalent to a single 5-ms pulse stimulus. These images were not used for data analysis.

Areas determined from IOS imaging to be responsive to hindlimb stimulation were located in the TPLSM and used for subsequent imaging. Mean arterial blood oxygen saturation (SpO2) and heart rate pulse were continuously monitored with a small-animal wrap transducer (TSD270B, Biopac Systems, Inc.) on the hind paw using an MP-150 Oxy200 (Biopac Systems, Inc.). The following physiological parameters were maintained during the experiment: mean arterial blood pressure was maintained at 90–120 mmHg; heart rate was maintained at 300–340 beats per minute; body temperature was maintained at 37 ± 1 °C. The surgical setup was described in vivo imaging and line scans were acquired at depths up to 400 μm below the pial surface using a custom-built Zeiss LSM 5MP multiphoton microscope coupled to a mode-locked Ti:sapphire laser (Mai Tai Deep See; Spectra Physics) set to 800 nm and a Nikon AR IR multiphoton microscope coupled to a mode-locked Ti:sapphire laser (Insight DS+; Spectra Physics) set to 820 nm for FITC excitation. FITC emission was collected using a 500–550 nm bandpass filter. Arteries were identified at the brain surface by morphology and direction of blood flow, and then followed into the brain for imaging.

An electrical stimulus (10 s, 10 Hz, 2 ms pulse duration) was delivered to the contralateral hind paw using custom electrical probes. Stimulus delivery and coordination with imaging were accomplished using a Digidata 1550 and accompanying pClamp software (Molecular Devices).

For diameter measurements, line scans were taken perpendicular to the vessels at 8.14–15 Hz, generating an image of vessel diameter over time. Diameter line scans were analyzed as thresholded diameter versus time images of each vessel with custom protocols written in Igor Pro 6 (WaveMetrics). First, images were smoothed with a 3 × 3 pixel Gaussian filter to remove noise and thresholded using the Igor Pro built-in “fuzzy entropy” threshold routine to generate a black-and-white (binary) image of the vessel diameter versus time. The Igor Pro custom analysis protocol identified the transitions between black/white and white/black in each line of the image, indicating the edges of the vessel, and then calculated the width of the vessel for each line in the image. The resulting diameter data were low-pass filtered (1 Hz cut off) and notch filtered (0.5 Hz), then smoothed with a 1-s window box filter. Subsequently, sigmoid parametric functions were fitted to the individual filtered vessel data traces to capture the overall time course of diameter increase and obtain the time to 50% peak vessel dilation data. Vessels with basal diameters <6 μm were considered capillaries and were confirmed by lectin-specific Lycopersicon esculentum lectin fluorescent staining to visualize brain capillary profiles and immunostaining (see below) for pericyte-specific marker CD13 and vascular smooth muscle cell actin (SMα) to be CD13-positive and SMα-negative. Vessels of basal diameter 7–25 μm were considered arterioles and were confirmed by immunostaining (see below) to be SMα-positive and CD13-negative.

Vessels outside the 627-nm IOS response area were excluded from analysis. Vessels imaged in the 627-nm IOS response region (5–7 vessels (capillaries and arterioles)) per animal on average) were analyzed. On the basis of the diameter analysis, any vessel that showed a focal drift occurring during the experiment or was affected by excessive breathing or movement artifacts was excluded. Only vessels with a diameter change larger than 1% after stimulus start were considered responding vessels and taken for further analysis. Using these criteria, a total of 50–70% of vessels measured responded. Under these conditions, the average maximal diameter measured upon stimulus was approximately 2.5% above basal diameter.

For in vivo two-photon vessel diameter and velocity experiments, data from analyzed vessels were averaged together by mouse for statistical analysis. For RBC velocity, line scans along the vessels were taken at a rate of 2.6 kHz (ref. 24). Velocity data were analyzed using a MATLAB algorithm as described28 and then low-pass filtered (1 Hz cut off) and notch filtered (0.5 Hz), then box filtered with a 1-s window to remove heartbeat and breathing artifacts using custom written protocols in Igor Pro to automate the filtering process. As in the diameter analysis, sigmoid parametric functions were then fit to the individual filtered vessel data traces to capture the overall time course of velocity increase and obtain the time to 50% peak RBC velocity data. Igor Pro code can be made available upon request.

For presentation in Figure 1c–h, the data were normalized such that the average basal diameter or velocity was set to zero and the maximum set to 1. Basal diameters or velocities were taken as the average diameter of the vessel during the 10 s before stimulus. For Supplementary Videos 1 and 2, high-magnification 512 × 512 pixel images were taken at 1.57 s/frame in the FITC channel only, after identification of dsRed pericyte-covered and uncovered capillary regions. Resulting image stacks were 3D Gaussian filtered and registered (StackReg, rigid body algorithm) in FIJI (ImageJ). The final movies were rendered in Photoshop (Adobe) and Fiji.

For experiments with Pdgfrb+/−;dsRed mice, the laser was set to 900 nm. FITC signal was collected as above, and the dsRed signal was collected using a 570–640 nm bandpass filter (Chroma). An electrical hindlimb stimulus was delivered as above (10 Hz, 10 s, 2 ms pulse width). Since the capillaries of Pdgfrb+/− mice dilate slower than capillaries of control mice (Fig. 1d), we chose the time point of 3 s, before 50% diameter change occurrence in Pdgfrb+/− mice, to specifically examine the active dilation of pericyte-covered capillaries versus pericyte-uncovered capillaries (Fig. 1k).

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Tissue oxygen partial pressure (pO2) measurements. Experiments were conducted as described previously^{25}. Briefly, 5- to 6-month-old Pdgfrb^{-/-} or Pdgfrb^{+/+} mice were anesthetized with isoflurane and then the femoral artery was cannulated to administer the dyes, to continuously monitor the blood pressure and heart rate, and to sample systemic blood gases (pCO2 and pO2) and pH. A tracheotomy was performed and a cranial window with dura removed was made. Blood pressure, heart rate, temperature and end-tidal pCO2 were monitored continuously during both surgery and experiment. Oxygen-sensitive dye (Pip-2-C343) was pressure-injected into cortical tissue using a glass micropipette inserted ~400 µm below the cortical surface. The cranial window was then sealed with a glass coverslip and isoflurane was discontinued and replaced with α-chloralose. Mice were ventilated with a mixture of air and oxygen and the following physiological parameters were maintained: arterial pO2 = 115 ± 15 mm Hg, arterial pCO2 = 37 ± 2 mm Hg, mean arterial blood pressure = 100 ± 10 mm Hg, and pH = 7.37 ± 0.02. Blood plasma was labeled by a small amount of FITC dye to obtain vascular images in the planes of pO2 measurement to coregister later with the three-dimensional vascular stacks. Tissue pO2 was measured at two depths (100 µm and 200 µm from surface) at 800-1,000 locations at each depth. At each pO2 imaging location, we excited phosphorescence by trains of femtosecond pulses from a Ti:sapphire oscillator at 840 nm, gated by an electro-optic modulator, and acquired decays by averaging multiple excitation cycles. Each cycle consisted of a 10-µs excitation gate followed by a 290-µs collection period. We averaged ~2,000 phosphorescence decays at each selected location for accurate pO2 determination, resulting in 0.6 s per single-point pO2 measurement. Blood plasma was subsequently labeled by rhodamine dye to obtain vascular stack images.

NADH measurements. NADH is intrinsically florescent with two-photon excitation and provides an indication of local tissue metabolism^{30,31,50}. The tissue was also stained with sulforhodamine 101 (SR101) dye (Invitrogen), which preferentially loads into astrocytes and oligodendrocytes and acts as a control marker to correct for fluorescence changes due to hemodynamic changes in response to stimulation^{31}. For these experiments, the window area was topically loaded with SR101 for 15 min before NADH imaging. Imaging was performed under isoflurane anesthesia (1.8–2%) using the two-photon microscope described above through a cranial window at a depth of 50 µm under the same stimulus used for vessel diameter and velocity measurements. The TPLSM excitation was set to 740 nm. NADH fluorescence emission was collected at 435–485 nm and SR101 fluorescence emission was collected at 650–710 nm. Interleaved NADH and SR101 images were taken at a rate of 6.12 s per pair of images at 50 µm depth. Images were loaded into ImageJ and ROIs were chosen so as to exclude the shadows of any large vessels. The SR101 signals were used to correct the average NADH ROI intensities for hemodynamic optical effects using a method reported previously^{51}.

Two-photon three-dimensional angiography. Mice were anesthetized with isoflurane and then injected with 100 µL of 1 mg/ml Dylight 594-labeled Lycopersicon esculentum (tomato) lectin (Vector; DL-1177) into the femoral vein. After a 15-min incubation, animals were transcardially perfused with 30 mL of PBS containing EDTA followed by 25 mL of 4% PFA in PBS at room temperature. The brain was removed and bisected down the midline, and cortices were separated from the rest of the brain. Cortices were placed between two microslides spaced 1.6 mm apart using metal washers and postfixed in 4% PFA overnight. Cortices were then washed with 0.1 M PBS and the somatosensory region extracted and embedded in a 4.5% agarose gel^{51}. Brains were imaged in 1-µm steps using a 20× objective at 810 nm using a serial two-photon tomography microscopy as described^{51}.

Using custom scripts and plug-ins, all obtained from TissueVision, images were processed in MatLab and stitched using Image^{52}. Projection images were made using Imaris x64 v8 (Bitplane Scientific Software). Blood vessels were traced in 50-µm-deep increments through the depth of the cortex using the Vida Suite software package written for MatLab^{52}.

Voltage-sensitive dye (VSD) imaging. A cranial window was created over the hindlimb region in the somatosensory cortex. RH-1692 VSD (Optical Imaging) dissolved in aCSF was applied to the exposed cortex for 90 min^{53}. The brain was then washed with aCSF and sealed with low-melt agarose and a coverslip as above. Under isoflurane anesthesia (~1.5%), images were captured at 5 ms/frame (184 × 124 resolution) using a half-inch CCD MicAM02-HR camera (SciMedia) coupled with MicAM BV_ANA acquisition software. RH-1692 was excited using a MHAB-150W (Moritex Corp.) light source with a 632/22 nm filter, and fluorescence collected with a 665 nm long-pass filter. The contralateral hindlimb was stimulated by a brief mechanical vibration lasting 300 ms. Alternating image setsDireccion were taken with and without stimulus to generate "stimulus trial" and "baseline" responses. Next the baseline image set was subtracted from the stimulus trials to eliminate any background signal. Ten baseline-subtracted trials were averaged to create the final profile for each mouse. Time courses were evaluated using a circular ROI centered over the hindlimb region as described previously^{54}. Time courses were plotted using Igor Pro 6 and analyzed with Igor Pro 6 and Excel. Pseudocolor images for presentation were generated in ImageJ.

Intrinsic optical signal imaging (IOS). IOS relies on changes in diffuse reflectance of the tissue due to changes in the tissue optical properties, notably changes in the blood volume and hemoglobin oxygen saturation^{55}. Intrinsic optical signals were imaged through a cranial window. Under isoflurane anesthesia set at 1%, images were captured at 30 ms per frame using a half-inch CCD MicAM02-HR camera (SciMedia; 2x binned to 184 × 124 pixel resolution; 1 pixel = 16.5 µm) with accompanying BV_ANA acquisition software.

IOS imaging at 530 nm illumination. At 530 nm the absorption extinction coefficients of oxy- and deoxyhemoglobin are equal^{25,26}. Therefore, at this wavelength, optical absorption of blood is dominated by the total hemoglobin concentration, independent of oxygen content, reflecting cerebral blood volume (CBV)^{26}. These changes also reflect CBF changes, as CBF and CBV changes are strongly coupled^{26}. Images were captured under a 530-nm green LED light source and collected through a 522/36 nm band-pass filter (Chroma). To match two-photon diameter and velocity stimulus conditions, images were acquired in response to a 10-s electrical stimulus delivered to the contralateral hindlimb at 5 V at a rate of 10 Hz, 2 ms pulse duration (6002 Stimulator, Harvard Apparatus) using custom electrical probes. The resulting image sets were low-pass filtered at 0.69 Hz and the baselines corrected for any drift using the BV_ANA software. Signal time courses were evaluated using 13 × 13 pixel ROIs chosen in the region of peak signal change such that the regions did not include any large visible vessels and were at least 30 µm from any large vessels. Time courses were plotted using Igor Pro 6 and analyzed with Igor Pro 6 and Excel. Pseudocolor images for presentation were generated in ImageJ.

IOS imaging at 627 nm illumination. At 627 nm, optical absorption of blood is dominated by the deoxyhemoglobin concentration^{25,26,28}. Images were captured under a 627-nm red LED light source, and collected through a 640/40 nm band-pass filter (Chroma). The contralateral hindlimb was stimulated by a brief mechanical vibration lasting 300 ms. A total of 10 trials were performed and image data were averaged and stored for further quantification. To quantify the image results, customized Matlab scripts were used. A baseline frame was first obtained as the average of 10 frames (300 ms) before stimulation. For each 300-ms average frame (averaged from 10 frames) collected after stimulus onset, the intensity change was calculated relative to the baseline frame on a point-by-point basis. An adaptive 2D Wiener filter was applied to remove ‘salt & pepper’ noise. Then regions of interest (ROIs; 13 × 13 pixels) were chosen such that the regions did not include any large visible vessels and were at least 30 µm from any large vessels, followed by quantification of the ROI intensity change over time.

In vivo electrophysiology. Following 530 nm IOS, electrophysiologic recordings were performed using custom-pulled borosilicate glass pipettes with filament (Sutter Instruments, outer diameter: 1.2 mm, inner diameter: 0.69 mm) filled with sterile aCSF, attached to a CV-7B headstage (Axon Instruments) and mounted on a micromanipulator. The electrode was stereotaxically placed in layer II (~200–300 µm deep) of hindlimb S1 at the peak responding location identified by 530 nm IOS. Data were acquired using a Multiclamp 700B amplifier and were taken with and without stimulus to generate "stimulus trial" and "baseline" responses. Next the baseline image set was subtracted from the stimulus trials to eliminate any background signal. Ten baseline-subtracted trials were averaged to create the final profile for each mouse. Time courses were evaluated using a circular ROI centered over the hindlimb region as described previously^{54}. Time courses were plotted using Igor Pro 6 and analyzed with Igor Pro 6 and Excel. Pseudocolor images for presentation were generated in ImageJ.

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peak LFP amplitude were quantified per trace. We performed 5 or 6 trials (repli-
cates) per animal and data were averaged per each animal from 5 *Pdgfb*+/+ mice (25 experiments total, 2,500 LFPs) and from 6 *Pdgfb*−/− mice (36 experiments total, 3,600 LFPs).

**Immunohistochemistry.** Mice were anesthetized with an i.p. injection of 100 mg/kg of ketamine and 50 mg/kg of xylazine, and transcardially perfused with 30 ml of phosphate buffer saline (PBS) containing EDTA followed by 30 ml of 4% PFA in PBS. Brains were removed and embedded into O.C.T. compound (Tissue-Tek) on dry ice. They were cryosectioned at a thickness of 18–20 μm. Sections were cut horizontally and those from depths of 50–150 μm, to coincide with TPLSM imaging depths, were used for analysis. Sections were subsequently blocked with 5% normal donkey serum (Vector Laboratories) with Triton (0.05%) for 1 h and incubated in goat anti-CD13 (1:100) primary antibody10,54, diluted in blocking solution overnight at 4 °C. To visualize CD13-positive pericytes, Cy3-conjugated bovine anti-goat (1:100; Jackson ImmunoResearch, 805-165-180) and scanned using a Zeiss 510 Meta confocal microscope with the following: 488-nm argon laser to excite Alexa Fluor 488, with emission collected through a 500–550 nm band pass (bp) filter; a 543-nm HeNe laser to excite Cy3, with emission collected through a 560–615 nm bp filter; and a 633-nm HeNe laser to excite Dylight 649, with emission collected through a 650–700 nm bp filter. z-stack projections and pseudocoloring were performed using ZEN software (Carl Zeiss Microimaging).

**Pericyte coverage analysis.** Pericyte coverage analysis was performed as previously described29,41. Briefly, CD13 and lectin staining was performed in PFA-fixed tissue sections as described above and signals from microvessels were separately subjected to automated threshold processing using ImageJ. The areas occupied by their respective signals were analyzed using the ImageJ area measurement tool. Pericyte coverage was quantified as a percentage of CD13-positive pericyte surface area covering lectin-positive capillary surface area per field (420 × 420 μm).

In each animal 5 randomly selected fields from the cortex were analyzed in 6 nonadjacent sections (−100 μm apart). Smooth muscle thickness analysis. SM-α staining was performed in PFA-fixed tissue sections sections as described above and signals from axial diving arterioles were subjected to threshold processing. The thickness of the smooth muscle layer was determined by subtracting the diameter of the lumen from the diameter of the lumen and SM-α signal combined. This was done at four different angles for each vessel and the average of the four was used as the thickness for a given vessel.

**Microvascular length measurements.** In a few experiments the length of lectin-positive capillary profiles was determined as we have previously reported29. Tissue sections were prepared and blocked as described above followed by incubation with *Lycopersicon esculentum* lectin (DL-1174, DyLight 488, Vector Laboratories). The capillary profile length (vessels < 6 μm in diameter) was measured using the ImageJ “Neuro J” plug-in length analysis tool from 5 randomly selected fields in the hindlimb S1 cortical area (420 × 420 μm) per section from 6 nonadjacent (−100 μm apart) sections per animal, as we described29. The length was expressed in millimeters of lectin-positive vascular profiles per mm² of brain tissue.

**NeuN-positive neuronal nuclei counting.** NeuN-positive neurons were quantified by using the ImageJ Cell Counter analysis tool. In each animal 6 randomly selected fields from the cortex were analyzed in 5 nonadjacent sections (−100 μm apart), as we reported29,54.

**AQP4-positive astrocyte endfoot coverage.** Aquaporin-4 and lectin signals from microvessels < 6 μm in diameter were separately subjected to threshold processing. The areas occupied by their respective signals were analyzed using the ImageJ Area measurement tool. Total aquaporin-4-positive area was expressed as a percentage of total lectin-positive area in each field. In each animal 6 randomly selected fields from the cortex were analyzed in 5 nonadjacent sections (−100 μm apart), as we reported29.

**GFAP-positive astrocyte counting.** GFAP-positive astrocytes were quantified by using the ImageJ Cell Counter analysis tool. In each animal 6 randomly selected fields (420 × 420 μm) from the cortex were analyzed in 5 nonadjacent sections (−100 μm apart), as we reported29.

**Microglia quantification.** The numbers of Iba1-positive microglia were counted using the ImageJ Cell Counter analysis tool. In each animal 6 randomly selected fields (420 × 420 μm) from the cortex were analyzed in 5 nonadjacent sections (−100 μm apart), as we reported29.

**Ex vivo brain slice imaging.** Brain slice preparation. Mice were intraorbitally injected with 50 μL of 1 mg/mL Alexa 633 hydrazide (Life Technologies) to identify arterioles29 and 50 μL of 1 mg/mL L. esculentum lectin (Vector Laboratories)9 to identify blood vessels at least 90 μm and 30 μm, respectively, before anesthesia (4% isoflurane), rapid decapitation and slice preparation on a Leica VT1000S vibratome. Coronal cortical slices (350 μm) containing the hindlimb area of S1 were cut in ice-cold high-sucrose, low-NaCl aCSF (85 mM NaCl, 2.5 mM KC1, 4 mM MgSO₄, 0.5 mM CaCl₂, 1.25 mM NaH₂PO₄, 25 mM NaHCO₃, 25 mM glucose, 74 mM sucrose, 0.5 mM ascorbate and 2 mM kynurenic acid) and stored in a submerged chamber containing normal aCSF (119 mM NaCl, 2.5 mM KC1, 1.3 mM MgSO₄, 2.5 mM CaCl₂, 1 mM NaH₂PO₄, 26 mM NaHCO₃, 11 mM glucose) with 2 mM kynurenic acid, saturated with 95% O₂:5% CO₂ at room temperature. Experiments were performed in a submerged chamber perfused with aCSF and saturated with 95% O₂:5% CO₂.

**Imageing of arterioles in live brain slices.** Acute brain slices were transferred to a Zeiss Axio Examiner upright DIC fluorescence microscope equipped with a Hamamatsu Orca-Flash 4.0 camera, and images acquired using Zen Pro 2012 software (Carl Zeiss Microimaging). Slices were continually perfused with 95% O₂:5% CO₂-saturated aCSF heated to 37 °C. After at least 10 min of acclimation in the recording chamber in aCSF, time-lapse images of arterioles were acquired every 5 s for 22 min (2 min of aCSF, 10 min of 100 μM phenylephrine, 10 min of 400 μM adenosine). Vessel internal diameters were measured at several locations along the vessel using the line capillary tool and peak-to-peak measurement calculation function in ImagePro Premiere software (MediaCybernetics). Experiments and images where focal changes occurred were excluded from analysis. Values for constriction by phenylephrine were calculated by comparing the vessel diameter of the last 3 min of phenylephrine application to aCSF baseline measurements. Relaxation by adenosine was calculated by comparing the diameter of the last 3 min of adenosine to the last 3 min of phenylephrine application.

**In vivo microdialysis.** In vivo microdialysis was performed to measure lactate and glucose in the ISF of isoflurane (−1%)-anesthetized mice. An intracerebral guide cannula (Bionanalytical Systems, MBR-5) was stereotaxically implanted over the somatosensory cortex (coordinates: AP:−0.5 mm, L:−1.5 mm, DV:0 mm) of the mouse. A 1-mm-long microdialysis probe (Bionanalytical Systems, MBR-1:5) with 38 kDa molecular weight cutoff extended from the tip of the cannula into the somatosensory cortex. Artificial cerebrospinal fluid (aCSF; Harvard Apparatus) was continuously perfused through the probe at a flow rate of 0.5 μL/min. After obtaining baseline values for 3 h, high ['K'] (100 mM) aCSF was perfused into the cortex for 60 min via retrodialysis to induce local neuronal stimulation. The microdialysates, collected every 30 min, were then analyzed for lactate levels (Sigma).

**Behavior testing.** Novel object recognition (NOR), nesting and burrowing tests were performed as previously described29.

**Blood pressure measurements.** Blood pressure measurements were performed using a non-invasive tail cuff method (CODA Monitor, Kent Scientific) on isoflurane-anesthetized mice under the same conditions and stimulus used for the two-photon diameter and velocity measurements (see above) to confirm that blood pressure does not change under those experimental conditions. Blood pressure measurements were taken before the stimulus, at the immediate
conclusion of the stimulus, and for several subsequent post-stimulus time points. Mean blood pressure was calculated using the formula \((2/3)\text{Diastolic} + (1/3)\text{Systolic}\) pressures. Mean blood pressure was \(83 \pm 7\) (mean \(\pm\) s.e.m.) before stimulus for both 1- to 2-month-old \(Pdgfrb^{+/−}\) and littermate control mice, and \(83 \pm 6\) and \(84 \pm 7\) for 1- to 2-month-old \(Pdgfrb^{+/−}\) and littermate control mice, respectively, at the conclusion of stimulus.

**Statistical analysis.** All animals were randomized for their genotype information. Immunostaining experiments were blinded; the operators responsible for the experimental procedures and data analysis were blinded to group allocation throughout the experiments. Other experiments were analyzed, but not performed, in a blinded fashion. Sample sizes were calculated using nQUERY assuming a two-sided \(α\)-level of 0.05, 80% power, and homogenous variances for the two samples to be compared, with the means and common s.d. for different parameters predicted from published data and our previous studies.

All data are expressed as mean \(\pm\) 95% confidence interval, unless otherwise noted. Grubb’s outlier test was used to test normality of the data (XLSTAT). For parametric analysis, the \(F\) test was used to determine the equality of variances between the groups compared; statistical significance across two groups was tested by Student’s \(t\)-test (Microsoft Excel 2010). Mann–Whitney \(U\) test was used for non-parametric analysis (Prism, GraphPad). Bootstrapping (1,000 sampling size) was performed on data that could not be statistically distinguished from a non-normal distribution to calculate 95% confidence intervals (XLSTAT)\(^{59,60}\). Effect size (for example, Cohen’s \(d\)) was calculated for all comparisons made (http://www.socscistatistics.com/effectsize/).

The accepted level of statistical significance was \(P \leq 0.05\).

A **Supplementary Methods Checklist** is available.

**Data availability.** All data analyzed during this study are included in this published article and its supplementary information files.

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