Cerebral oxygenation during locomotion is modulated by respiration

Qingguang Zhang, Morgane Roche, Kyle W. Gheres, Emmanuelle Chaigneau, Ravi T. Kedarasetti, William D. Haselden, Serge Charpak & Patrick J. Drew

In the brain, increased neural activity is correlated with increases of cerebral blood flow and tissue oxygenation. However, how cerebral oxygen dynamics are controlled in the behaving animal remains unclear. We investigated to what extent cerebral oxygenation varies during locomotion. We measured oxygen levels in the cortex of awake, head-fixed mice during locomotion using polarography, spectroscopy, and two-photon phosphorescence lifetime measurements of oxygen sensors. We find that locomotion significantly and globally increases cerebral oxygenation, specifically in areas involved in locomotion, as well as in the frontal cortex and the olfactory bulb. The oxygenation increase persists when neural activity and functional hyperemia are blocked, occurred both in the tissue and in arteries feeding the brain, and is tightly correlated with respiration rate and the phase of respiration cycle. Thus, breathing rate is a key modulator of cerebral oxygenation and should be monitored during hemodynamic imaging, such as in BOLD fMRI.
A n adequate oxygen supply is critical for proper brain function, and deficiencies in tissue oxygen is a noted comorbidity in human diseases and aging. For these reasons, there has been a great deal of interest in studying dynamics of cerebral oxygenation. However, there is a gap in our understanding of how behavior, such as natural exercises like locomotion, affects cerebral oxygenation. In natural environments, animals and humans have evolved to spend a substantial portion of their waking hours locomoting. As exercise is known to have a positive effect on brain health, a better understanding of the basic brain physiology accompanying these behaviors can give insight into how exercise can improve brain function. During movement, neuromodulator release and neural activity in many brain regions is elevated, and there is an increase in cardiac output and respiratory rate. How these changes in local and systemic factors interact to control cerebral oxygenation is a fundamental question in brain physiology but is not well understood. Most cerebral oxygenation studies are performed in anesthetized animals (but see refs. 4, 17, 18), or non-invasively in humans. Anesthesia causes large disruptions of brain metabolism and neural activity, and non-invasive human studies are impeded by technical issues, making accurate determination of any aspect of brain tissue oxygenation problematic.

In addition to the importance of understanding oxygen dynamics in the brain to basic physiology, a better grasp of natural oxygen dynamics in the brain will greatly aid in the interpretation of functional MRI signals, which allow non-invasive imaging of neural activity. Previous work in awake primates has shown that tissue oxygen signals correspond well with changes observed with blood-oxygen level dependent (BOLD) fMRI. Neuromodulated BOLD signals are generated by vessel dilation. However, in addition to the BOLD fMRI signals of a neural origin (i.e., those generated by neurovascular coupling), BOLD signals can arise from sources that are not directly linked to underlying neural activity, such as pure vascular effects, respiration, cardiac pulse rate, and autonomous hemodynamic regulation. Resolving the nature of non-neural sources of BOLD contrast has been an area of active research, and will be helpful in better spatially resolving the neurally-generated BOLD signals.

Here we investigated how and by what mechanisms voluntary exercise impacts brain tissue oxygenation. We used intrinsic optical signal (IOS) imaging, electrophysiology, and optogenetics to elucidate how vasodilation, neural activity, and systemic factors combine to generate changes in brain oxygenation. All experiments were performed in awake mice that were head-fixed on a spherical treadmill or rotating disk that allowed them to voluntarily locomote. We find that cerebral oxygenation rises during locomotion in cortical regions that do not experience vasodilation, as well as when vasodilation is blocked. Oxygen levels increase in the arteries that supply the cortex during exercise, consistent with an increase in systemic oxygenation. Finally, oxygen fluctuations are correlated with spontaneous and locomotion-evoked changes in respiration rate, as well as the phase of the respiration cycle, also consistent with a dynamic regulation in systemic oxygenation.

Results
Locomotion drives localized cortical vasodilation. We first assessed the spatial extent of cortical hemodynamic responses and their relationship to voluntary locomotion using IOS imaging (Fig. 1a). Imaging was done through a thin-skull window over the right-hemisphere (Fig. 1b). When the brain is illuminated with 530 nm light, reflectance decreases report dilations of arteries, capillaries and veins, which correspond with increases in cerebral blood volume (CBV). During locomotion, we observed region-specific changes in reflectance. There was a pronounced decrease in reflectance (corresponding to an increase in CBV) in forelimb/hindlimb representation of somatosensory cortex (FL/HL), while in frontal cortex (FC) there was no change, or a slight increase in reflectance (Fig. 1b). To better localize the area of decreased CBV, we used a smaller region of interest (ROI, 2–4 mm rostral and 0.5–2.5 mm lateral from bregma, ~4 mm²) more rostral in FC than in our previous study. We also assayed cerebral blood flow (CBF) using laser Doppler flowmetry, which will evaluate flow changes in a ~1 mm² area. The locomotion-evoked CBF showed similar spatial pattern of responses (Fig. 1c) as CBV. We quantified how locomotion affected both CBV and CBF in two complimentary ways. We calculated the locomotion-triggered average, generated by aligning the IOS or laser Doppler signals to the onset of locomotion (see “Methods”) using only locomotion events ≥ 5 s in duration (Fig. 1d). The locomotion-triggered average showed no significant change in CBF in FC (n = 5 mice, Wilcoxon signed-rank test, p = 0.22), and a large increase in FL/HL (n = 5 mice, Wilcoxon signed-rank test, p = 0.03). The locomotion-triggered average showed significant increase in optical intensity in FC (n = 11 mice, Wilcoxon signed-rank test, p < 0.001) while decrease in optical intensity in FL/HL (n = 11 mice, Wilcoxon signed-rank test, p = 0.0122). We also calculated the hemodynamic response function (HRF) using only locomotion events. When we quantified the net CBF using the HRFs, locomotion actually drove a significant decrease in flow in FC and increase in FL/HL (Supplementary Fig. 1), using all locomotion events. When we quantified the net CBV using the HRFs, locomotion actually drove a significant decrease in flow in FC and increase in FL/HL (Supplementary Fig. 1). Using the HRFs to quantify the net CBV, we obtained same conclusions as derived from locomotion-triggered average. This shows that locomotion and the accompanying cardiovascular changes do not drive global increases in CBF/CBV, rather CBF/CBV increases are under local control. This lack of non-specific flow increase in the cortex during locomotion is likely because of autoregulation of the feeding arteries at the level of the circle of Willis and larger resistance arteries, as well as increased blood flow to the muscles.

To assess neural activity during locomotion, we measured the local-field potential (LFP) and multi-unit activity (MUA) in a separate group of seven mice (six sites in FL/HL and four sites in FC) using multi-channel linear electrodes (Fig. 1e). We used electrophysiological measures of neural activity, as they are more sensitive than calcium indicators (which fail to detect about half of the spikes even under ideal conditions), and do not disrupt normal neural activity as genetically encoded calcium indicators can do. Since gamma-band (40–100 Hz) LFP power has been observed to be the strongest neural correlate of hemodynamic signals in rodents and primates, and humans, and increases in gamma-band activity are also closely associated with the increases in metabolic demand, we quantified how locomotion affects neural activity by generating locomotion-triggered averages of gamma-band LFP power and spiking (see “Methods”). We observed that both the gamma-band LFP power and spike rate increased during locomotion across all layers in both FL/HL (Fig. 1f, h, i) and FC (Fig. 1g, i). The slow rise in neural activity a few hundred milliseconds before the onset of locomotion is due to low-pass filtering of the MUA signal (5 Hz, see “Methods”) and windowing (1 s duration) required to estimate the LFP power, as well as the ramping up of neural activity due to arousal changes seen before voluntary locomotion. As optogenetic stimulation of fast spiking inhibitory neurons has been shown to induce large increases in blood oxygenation in somatosensory cortex, we
sorted recorded spikes into fast spiking (FS, putatively inhibitory) and regular spiking (RS, putatively excitatory) spikes (see “Methods”). We found that FS and RS neurons exhibited a similar degree of rate increases during locomotion in both FL/HL and FC areas (Supplementary Fig. 2).

Taken together, our results show that a short bout of locomotion increases neural activity, which is followed by an increase in CBV and CBF in FL/HL, and a small decrease or no change in CBV and CBF in FC. Together with our previous work\textsuperscript{11,34}, these results suggest that coupling between neural activity and hemodynamics are brain region-specific (Fig. 1j), as seen in many other neurovascular coupling studies in the cortex and other brain regions\textsuperscript{35}. The lack of observed vasodilation in the FC is not due to a lack of sensitivity of our IOS imaging paradigm, as if the vasodilation in FC had the same relationship to neural activity as in FL/HL, we would expect to see a 2%
decrease in the reflectance (Fig. 1j), which is easily detectable with our IOS setup. As tissue oxygenation reflects the balance between oxygen supply and utilization, we would expect that in FL/HL, increased activity of the neurons will be more than matched by an increased blood supply, leading to an increase in tissue oxygenation. However, increased neural activity in FC during locomotion will not be matched by an increase in the blood supply and should lead to a decrease in oxygenation in FC.

Locomotion drives cortex wide increases in brain oxygenation. To test if the brain region-dependent differences in neurovascular coupling drove regional differences in brain oxygen dynamics during locomotion, we measured partial pressure of tissue oxygen ($PtO_2$) in awake, behaving mice (n = 37 mice, 23 in FL/HL, and 14 in FC; 148.2 ± 28.3 min of recording for each mouse) using Clark-type polarographic electrodes (Fig. 2a). Signals from these electrodes are similar to those obtained with BOLD fMRI, but with sub-second response time (Supplementary Fig. 3a, b), long-term stability (Supplementary Fig. 3c, d) and higher spatial resolution. We measured oxygen dynamics at different cortical depths by sequentially advancing the probe from the cortical surface into deeper layers. We observed a laminar-dependence of resting $PtO_2$ in awake mice, with lower oxygenation in layer 1 than other layers in both FL/HL and FC (Supplementary Fig. 4a, b, see also refs. 4,17), though this is probably too thin a section of brain to be distinguishable with laminar fMRI. Resting $PtO_2$ levels were similar at each cortical depth in both FL/HL and FC (Supplementary Fig. 4a, b). No difference in onset time or peak time was observed in the HRFs of $PtO_2$ across layers, though the onset time was shorter in FC (Supplementary Fig. 4d, e), consistent with fMRI measurements using ultrashort stimuli. Note that because we are measuring tissue oxygenation in this case, any oxygen dynamics in the vasculature will be temporally blurred by the diffusion dynamics of oxygen in the tissue. These results, together with the observation that resting $PtO_2$ is similar in somatosensory cortex and olfactory bulb glomerular layer, indicate that the spatial distribution of oxygen in the brain under normal (non-anesthetized) physiological condition is relatively homogenous. This quantification of laminar tissue oxygen dynamics should aid interpreting the complicated dynamics of laminar fMRI signals.

Locomotion produces large, sustained dilation of arteries and increases in CBF and CBV in somatosensory cortex. These locomotion-induced dilations were not due to systemic effects, as they have been shown to be unaffected by drugs that do not cross the blood brain barrier (BBB) that increase or decrease the heart rate and are blocked by local neurovascular coupling. The locomotion-induced dilations are comparable in magnitude to those elicited by episodic whisker stimulation, which is known to elevate oxygenation, so one would expect increases in tissue oxygenation in FL/HL during locomotion. As anticipated, we observed increases in $PtO_2$ during locomotion in FL/HL in all layers (Fig. 2b–d). Because the supply of blood to FC does not increase, but neural activity does, one would expect a decline in tissue oxygenation during locomotion. Surprisingly, we also observed a very similar $PtO_2$ increase in FC (Fig. 2b–d) to that observed in FL/HL, despite small decreases in CBV or CBF, and an increase in neural activity. The elevation of $PtO_2$ in FC during locomotion suggests that other factors can increase oxygenation in the brain. Polarographic probes provide measures of oxygen tension over a small region of brain tissue, and the response may be affected by vasculature and density surrounding the probe. To distinguish compartment-specific oxygen tension in the tissue, arterial and venous blood spaces, we then mapped the spatial distribution of locomotion-evoked brain oxygenation response using optical imaging spectroscopy (Fig. 2e). Taking advantage of differences in the optical absorption spectra of oxyhemoglobin (HbO) and deoxyhemoglobin (HbR), we collected reflectance images during rapid alternating green (530 nm) and blue (470 nm) illumination. Note that spectroscopic measurements report oxygen concentrations in red blood cells, while polarography reports average oxygen concentration in tissue near the electrode. The oxygen levels in the tissue will differ from that in the blood somewhat due to the constraints of oxygen diffusion from the blood into the tissue and ongoing metabolic processes in neurons and glial cells. Using cerebral oxygenation index (HbO-HbR), the spectroscopic measures of hemoglobin oxygenation were similar to measurements from the tissue using polarographic probes: both methods yielded an increase in oxygenation during locomotion in both FC and FL/HL (Fig. 2f, g). These oxygenation changes persisted even when the heart rate increase associated with locomotion was pharmacologically blocked or occluded (Supplementary Fig. 5b, d, e), indicating they were not driven by increased cardiac output during locomotion. Moreover, locomotion-induced elevations in oxygenation were present in the parenchyma, arterial and venous blood (Fig. 2f). As oxygen levels in the brain strongly depends on the arterial oxygen content, we made direct measurements of oxygen partial pressure in the center of pial arteries ($PaO_2$) using two-photon phosphorescence lifetime microscopy (2PLM, Fig. 2h) with a new phosphorescent probe (Oxyphor 2P) which has a very high brightness, improving measurement speed and imaging depth. We asked if the oxygen levels increased in the center of large pial arteries that supply blood to the brain. As blood in these arteries will have minimal time to exchange oxygen in their transit through the heart and carotid artery to the brain, oxygen levels in these arteries will track systemic oxygenation levels. We
measured PaO2 in cortical and olfactory bulb arteries and found that PaO2 increased during locomotion (Fig. 2i). Taken together, these measurements are consistent with an increase in systemic blood oxygenation that leads to a brain-wide increase of oxygenation in the tissue and vascular compartments during locomotion. The increase in oxygenation accompanying the decrease in CBV and CBF in FC suggests that neurovascular coupling is not the only process controlling brain oxygenation during locomotion.

**Cortical oxygen increases even when vasodilation is blocked.**

Our observation that locomotion induced localized blood flow/volume increases, but cortical-wide increases in brain oxygenation, led us to hypothesize that activity-dependent vasodilation may not be necessary for an increase in oxygenation. To test this, we pharmacologically blocked glutamagentic and spiking activity by infusing/superfusing a cocktail of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 0.6 mM), (2R)-amino-5-phosphonopentaonic acid (AP5, 2.5 mM) and muscimol (10 mM) to suppress local neural activity. We first infused a cocktail of CNQX/AP5/muscimol via a cannula into FL/HL25, while concurrently monitoring neuronal activity, CBV and blood oxygenation (n = 4 mice, Fig. 3a).

The cocktail infusion suppressed resting gamma-band LFP power by 80 ± 12% and spiking activity by 82 ± 3% relative to vehicle infusions. Similarly, the standard deviation (SD) of these neural activity measurements during resting periods, an indicator of spontaneous neural activity levels, was decreased by 75 ± 18% in the MUA amplitude. To quantify CBV responses, we selected a semicircular ROI centered on the cannula and with a radius specified by the distance between the electrode and cannula (Fig. 3a), to ensure the ROI only included suppressed cortex25. Accompanying this neural activity blockade, baseline reflectance from the ROI increased (indicating decreased CBV), and the locomotion-evoked decrease in reflectance (vasodilation) was almost completely suppressed (Fig. 3b–d), consistent with our previous study25 showing that intracerebral infusion of a cocktail of CNQX/AP5/muscimol suppressed sensory-evoked CBV increase. However, the block of neural activity was less effective during locomotion (Fig. 3b), likely due to the large increases of neural and modulatory drive to the cortex that occur during locomotion15,14. Nevertheless, this is conducive for testing our hypothesis, as a complete block of vasodilation and an incomplete block of neural activity increases should lead to a decrease in oxygenation. However, if there is no oxygenation decrease, or the oxygenation increases, this would indicate that the oxygenation of the inflowing blood is elevated during locomotion. When locomotion-induced vasodilation was blocked, the locomotion-evoked increase in HbO-HbR persisted, though the increase was smaller (Fig. 3b–d, Supplementary Fig. 5b, c, e). This increase was surprising, as we were able to completely block the locomotion-
induced vasodilation, and there was still a small locomotion-induced increase in neural activity, which should result in a net decrease in oxygenation.

We further studied the effects of the suppressed vasodilation on oxygen responses in the tissue in a separate set of mice using polarographic electrodes \(n = 9\) mice, 5 in FC and 4 in FL/HL). We topically applied a cocktail of CNQX/AP5/muscimol to the cortex, while measuring spontaneous and locomotion-evoked neural activity and PtO2 in the superficial cortical layers (100–200 µm below the pia). The efficacy of the cocktail in suppressing neural activity was monitored with two electrodes spanning the oxygen measurement site\(^{25,30}\) (Fig. 3e).

Similar to intracortical infusions, superfusing the cocktail potently suppressed resting gamma-band LFP power by 89 ± 8% and the SD by 77 ± 21% (Fig. 3g). Resting PtO2 increased by ~70% following the suppression of neural activity and vasodilation (before: 23.09 ± 10.60 mmHg; after: 34.64 ± 11.11 mmHg; Fig. 3h), consistent with neural signaling being a major component of metabolic demand\(^{47}\).

To quantitatively assay locomotion-evoked oxygen and neural responses, we calculated the linear kernels (i.e., the HRF) relating PtO2 and the gamma-band LFP power to locomotion. To ensure that vasodilation was blocked, we only analyzed those animals \(n = 4\) mice, 3 in FC and 1 in FL/HL) that showed >50% suppression of locomotion-evoked neural activity. In these animals, application of CNQX/AP5/muscimol reduced peak amplitude of gamma-band LFP neural response function (NRF) by 81 ± 8% (Fig. 3i). If activity-dependent vasodilation is the only determinate of tissue oxygenation, we would expect the HRF of PtO2 shows profound reductions, since the vasodilation was blocked by the suppression of neural activity (Fig. 3b–d). However, the peak amplitude of PtO2 HRF was not changed (82 ± 51% of before cocktail application, Fig. 3i). Taken together, these results show that suppressing vasodilation does not block the locomotion-evoked oxygen increases.

Respiration drives changes in tissue and blood oxygenation.

One possible driver of the increases in cerebral oxygenation is the increase in respiration during locomotion. Changes in respiration affect blood oxygen levels in the carotid artery\(^{48,49}\) in anesthetized animals, and in humans, inhalation of 100% oxygen can elevate brain oxygen levels\(^{50}\). However, it is not known if normal fluctuations in respiration rate can impact cerebral oxygenation during normal behaviors. We tested whether respiration was correlated with oxygenation during locomotion by simultaneously measuring cortical tissue oxygenation and respiration (Fig. 4a). Locomotion was accompanied by a robust increase in respiratory rate (Fig. 4a, Supplementary Fig. 6), and fluctuations in respiratory rate on the time scale of seconds were linked to fluctuations in PtO2 (Fig. 4a). We quantified how well the fluctuations of respiratory rate and gamma-band LFP power (which has been shown to be the LFP band most correlated with...
vasodilation\textsuperscript{25,30} correlated with the fluctuations in PtO\textsubscript{2} by calculating the cross-correlation. During periods of rest, increases in gamma-band LFP power were correlated with decreased oxygenation (Fig. 4d, e), which was unexpected as gamma-band power increases during rest are correlated with vasodilation\textsuperscript{25,30,51}. Because the decrease takes place with near zero time lag (Fig. 4d, e), it seems as though the dilatation induced by spontaneous neural activity are insufficient relative to the metabolic demand. In contrast, respiratory rate increases were correlated with increased oxygenation with a slight delay, consistent with the transit time of the blood from the lungs to the brain (Fig. 4b, c). When periods of locomotion were included, the correlation between gamma-band LFP power and oxygenation was positive, suggesting that the coupling depends on animal's state\textsuperscript{31} (Fig. 4d, e). The coupling between other frequency bands of the LFP and oxygen increases was negative (Supplementary Fig. 7), consistent with previous reports showing decreases in the power of these bands during voluntary locomotion\textsuperscript{32} (see also Supplementary Fig. 8). Because cortical excitability and respiratory rate are correlated during locomotion (likely due to the reciprocal connections between respiratory and modulatory regions\textsuperscript{32}), we sought to disentangle their respective contributions to cerebral oxygenation using partial coherence analysis\textsuperscript{53}. We found that the coherence between respiratory rate and PtO\textsubscript{2} was not due to the co-varying neural component (Supplementary Fig. 9b), nor was the coherence between gamma-band power and PtO\textsubscript{2} affected by removing the respiratory rate contribution (Supplementary Fig. 9c). Thus, the partial coherence analysis indicates that respiration and neural activity (and likely vasodilation) affect tissue oxygenation independent of each other. The correlated fluctuations in respiratory rate and PtO\textsubscript{2} suggests that the oxygen tension of arterial blood should also track the respiratory rate. To test this, we simultaneously monitored respiration and PaO\textsubscript{2} in the pial arteries using 2PLM. In mice with irregular respiration, where respiratory rate transients of a few seconds occurred without locomotion, PaO\textsubscript{2} followed respiration rate fluctuations (Fig. 4f), showing that changes in respiration rate can alter the oxygenation of the arterial blood entering the cortex.

We then asked if PaO\textsubscript{2} tracked the phase of respiration, that is, whether the concentration of oxygen in the blood entering the brain fluctuated in phase with the inspiration-expiration cycle. This requires measuring PaO\textsubscript{2} at rates high enough (>5 Hz) to capture fluctuation in PaO\textsubscript{2} due to respiration (nominally 2.5 Hz).
As measurement of PaO₂ with the 2PLM method is based on the lifetime of the phosphorescence decay of the dye, accurate quantification of the oxygen concentration requires averaging of decays, which amounted to ~3000 decays at our laser power (corresponding to ~0.75 s of data), too slow to capture inspiration-expiration linked changes in PaO₂. Therefore, we took advantage of the respiration periodicity. When the respiratory rate is very regular, the phosphorescence decays can be aligned and binned according to their place in the phase of the respiratory cycle (Fig. 4g), analogous to how erythrocyte-related transients can be detected in capillaries, or analyzing the signal in the frequency domain. In animals (n = 5) with long bouts of highly regular respiration rate (average frequency 2.5 Hz, SD ≤ 0.6 Hz, average frequency to SD ratio > 4), we tested whether fluctuations of PaO₂ tracked the respiratory cycle with a phase randomization test (see “Methods”). Six arteries (three in cortex, and three in olfactory bulb) out of nine had significant PaO₂ fluctuations synchronized with the respiratory cycle (Fig. 4h–j). Note that even though three arteries did not pass the phase randomization test and were excluded, the plot in Fig. 4g remained significant when including all nine arteries (Wilcoxon signed-rank test, p = 0.0039). These arteries showed oscillations in PaO₂ at the frequency of respiration that were significantly larger than would be expected by chance (reshuffling test, see “Methods”). This shows that arterial blood flowing to the brain is not saturated at rest. It also shows that oxygen tension in blood tracks sub-second respiration dynamics, so increase in respiration can drive rapid increases in systemic blood oxygenation that will impact brain oxygenation.

These results above indicate that the blood was not saturated with oxygen at rest. This suggests that changing the availability of oxygen could change the saturation of arterial oxygen, even without locomotion or a change in respiration. In humans, arterial oxygenation can be elevated through an oxygen challenge, where the subject inhales a gas mixture with elevated oxygen levels. We addressed this using an oxygen challenge (see “Methods”). During periods of rest, in both FL/HL (n = 2 mice) and FC (n = 2 mice), inhaling 100% oxygen substantially increased oxygenation of cerebral blood (Supplementary Fig. 10), as seen in human fMRI. Importantly, during oxygen challenge, there were no significant changes in respiration rate or behavior of the mouse (Supplementary Fig. 10). Taken together, these results show that systemic blood oxygenation increases in mice, from either respiration increase or environmental oxygen availability, can drive increases in brain oxygenation.

**Modeling respiratory contribution to cortical oxygenation.** We then asked what the relative contributions of increased arterial oxygenation and vasodilation were to changes in PiO₂. Recent work has shown that substantial oxygenation exchange occurs not only at capillaries, but also around penetrating arterioles in the cortex. To better understand how increases in blood oxygenation impact tissue oxygenation around arterioles, where the simple geometry of the vasculature allows us to better capture the dynamics of oxygenation changes due to vasodilation and systemic oxygenation changes, we simulated oxygen transport and consumption in blood and the brain parenchyma (Fig. 5a). In this model, we dynamically varied arterial oxygenation, cerebral metabolic rate of oxygen (CMRO₂), vessel diameter and blood flow. For this model, we used experimentally-determined quantities for the values of arterial oxygenation, and vessel diameter dynamics. We used published values for CMRO₂ (Supplementary Table 1) for these simulations (Fig. 5c). The free parameters were chosen such that tissue oxygenation predicted by the model matched our oxygen measurements in FC and FL/HL (Fig. 5d). Consistent with our data (Fig. 3h), the model also showed an increase in tissue oxygenation when neural activity (and metabolism) were suppressed (Fig. 5d). Moreover, using this model, we were able to tease out the relative contributions of vasodilation and increased arterial oxygenation to tissue oxygenation changes in both FC and FL/HL. In FL/HL, the large increase in CMRO₂ during locomotion were counteracted by approximately equal increases in arterial oxygenation due to vasodilation and increase in arterial oxygenation. In FC, the small increase in CMRO₂ and vasoconstriction was totally offset by the increase in arterial oxygenation (Fig. 5e). These simulations show that both increases in respiration and vasodilation (with the accompanying increase in blood flow) contribute to modulating tissue oxygenation in the cortex during behavior. Next, we asked if the increase in FC oxygenation could be caused by a decrease in CMRO₂ during locomotion. In this case, the increase in oxygenation could be alternatively explained by a 15% reduction in CMRO₂ (Supplementary Fig. 11), which is only likely in deep sleep or under anesthesia. A recent study has shown that elevations of neural activity are highly correlated with increases of CMRO₂, which suggests that a decrease in CMRO₂ accompanying an increase in neural activity is highly unlikely. These simulations show that increased arterial oxygenation that accompanies increases in respiration can lead to increases in tissue oxygenation, even in brain regions that lack a marked hyperemic response. In addition, the increase in arterial oxygenation will also increase the oxygen tension in the tissue around the capillary bed, though the actual changes will depend on the details of the capillary geometry and the movement of individual red blood cells, which is hard to capture without detailed anatomical models, and will depends on the details of flow dynamics. Taken together, the experimental data and the simulation support the notion that increases in respiratory rate play an important role in regulating cerebral oxygenation.

**Discussion**

Respiration is not the only physiological change that accompanies exercise, and it bears considering other mechanisms that could account for the cerebral and arterial oxygenation changes seen here. Exercise causes large changes in cardiac output and blood pressure, and can be accompanied by changes in blood CO₂ and lactate levels, but we think they are unlikely to be the cause of the nonspecific increase in cerebral oxygenation that we saw here. First, for increases in cardiac output to raise global oxygenation in the cortex (independent of any changes in systemic oxygenation), it would need to drive an increase in CBF. Our laser Doppler experiments show that CBF does not rise in FC, as they are likely buffered by resistance arterioles and autonomic regulation of the circle of Willis (but see ref. 58). Additionally, when heart rate and blood pressure increases during locomotion were blocked (with the beta blocker atenolol, which does not cross the BBB) or occluded (with the muscarinic receptor antagonist glycopyrrolate which also does not cross the BBB), there was no change in the locomotion-evoked CBV change (Supplementary Fig. 5, see also ref. 40). Therefore, systemic cardiac output increase cannot explain the increases in cerebral oxygenation seen during locomotion. Second, while CO₂ is a strong vasodilator, and can drive increases in cerebral oxygenation under hypercapnia conditions by dilating blood vessels, rodents become hypocapnia during sustained exercise. Exercise-evoked changes in CO₂ would tend to cause cerebral vasoconstriction and would tend to drive a deoxygenation. Again, this mechanism could not drive the observed increase in blood and tissue oxygenation in FC without corresponding flow increases and vasodilation. Sustained, high intensity exercise can cause increases in blood lactate over tens of minutes, but there is no way that these lactate changes could
drive changes in cerebral oxygenation seen in our experiments on the time scale of seconds. So, while many systemic variables change during voluntary locomotion, with the exception of increases in respiration rate, none would be able to increase oxygen in the arteries or in tissue within a few seconds of locomotion onset, nor could they explain the breathing cycle-locked oscillations in the blood oxygenation or respiration-related fluctuations at rest. Thus, unless there is some heretofore unknown physiological process taking place during exercise, the most parsimonious explanation is that increases in respiration are the origin of the oxygenation increase in the brain observed here.

While our studies were performed in mice, there are respiration-driven fluctuations in the arterial blood of ungulates, suggesting it is a general property of mammals. Our finding may have useful implications for human fMRI work. Though the effects of respiration rate on CO₂ levels (which will cause changes in arterial diameter and blood flow on the scale of tens of seconds to minutes) have been appreciated in human neuroimaging, changes in systemic oxygenation due to respiration changes will be more rapid (of the order of a few seconds). While it is generally presumed that arterial blood is saturated in humans, arterial oxygen tension decreases substantially with age and acutely during sleep, and oxygen challenge in humans and monkeys raises blood oxygenation just as in our mice (Supplementary Fig. 10). This suggests that respiration may play a more important role in cerebral oxygenation in humans than is currently appreciated, particularly as respiration rate is actively modulated during cognitive tasks. Respiration in humans is known to be increased following auditory or visual stimulation, and patterns of respiration differ from individual to individual, which might play a role in cerebral oxygen dynamics. Recent work has shown that respiration is actively modulated during cognitive tasks in humans, and respiration dynamics predict task performance. There is an emerging consensus that global brain activity is coordinated with respiration phase and rate in both animals and humans. Because respiration will be modulated by tasks, there may be spurious, spatially distributed, non-neuronal BOLD signals locked to the stimulus driven by respiration changes. Fortunately, these artifacts can be removed by regressing out the effects of respiration, either using measures of systemic blood oxygenation, or measures of respiration itself.

The role of increased respiration in increasing brain oxygenation during behavior observed here is likely facilitated by the reciprocal connections between respiratory centers and the locus coeruleus and other brain regions involved in arousal. Consistent with a tight interplay between respiration and metabolic demand in the brain, activation of the locus coeruleus, which will cause increases in alertness, and also causes concomitant increases in neural activity and blood flow in the cortex. This tight interplay at the behavioral and anatomical levels between cortical arousal and respiration may help maintaining healthy oxygenation for optimal cortical function.

Methods

Experimental design. Cerebral oxygenation, laminar electrophysiology, CBF and CBV data were acquired from separate groups of awake, behaving mice during voluntary locomotion. All experimental procedures were approved by the Pennsylvania State University and INSERM Institutional Animal Care and Use Committee guidelines.

Animals. A total of 78 C57BL/6J mice (58 male and 20 female, 3–8 months old, 25–35 g, Jackson Laboratory) and 5 Thy1-GCaMP6f mice (4 male and 1 female, 3–12 months old, 25–35 g, Jackson Laboratory) were used. Recordings of laminar cortical tissue oxygenation were made from 37 mice (23 male and 10 female) in FL/HL and 14 (7 male and 7 female) in FC using Clark-type polarographic microelectrodes. Simultaneous measurements of cortical tissue oxygenation using polarographic electrodes, respiration and local field potential were conducted in 9 mice (5 male and 1 female) in FL/HL and 4 (2 male and 2 female) in FC. Six of these mice were also used for laminar cortical tissue oxygenation measurements. Local field potential and spiking activity of different cortical layers were measured using laminar electrodes in a separate set of 7 male mice (4 in FC and 3 in FL/HL, 3 mice were measured in both FL/HL and FC simultaneously). CBV measurements using IOS imaging (with 530 nm illumination) were conducted in 11 male mice. CBF measurements using laser Doppler flowmetry were performed in 5 male mice. Tissue oxygenation measurements using spectroscopy (using alternating 470 nm and 530 nm illumination) were conducted in 11 male mice (4 mice were implanted with cannula and electrode). Oxygen measurements with 2PLM were conducted in adult Thy1-GCaMP6f (GPS11) mice (n = 5). Tissue oxygenation measurements using spectroscopy during oxygen challenge (using alternating 470 nm, 530 nm and 660 nm illumination) were conducted in 4 mice (2 male and 2 female, 2 in FL/HL and 2 in FC). Mice were given food and water ad libitum and maintained on 12-h (7:00–19:00) light/dark cycles. All experiments were conducted during the light period of the cycle.
Surgery. With the exception of mice imaged with 2PLM, all surgeries were performed under isoflurane anesthesia (in oxygen, 5% for induction and 1.5–2% for maintenance). A custom-machined titanium head bolt was attached to the skull with cyanoacrylate glue (#32002, Vibra-tite). The head bolt was positioned along the midline and just posterior to the lambda cranial suture. Two self-tapping 3/2" #000 screws (J.I. Morris) were imbedded into the skull contralateral to the measurement sites over the frontal lobe and parietal lobe. A stainless-steel wire (#792800, A-M Systems) was wrapped around the screw imbedded in the frontal bone for use as an electrical ground for cortical tissue oxygenation and neural recordings. For CBF measurement using laser Doppler flowmetry (n = 5 mice), CBV (n = 11 mice) measurement using IOS imaging or brain oxygenation measurement using spectroscopy (n = 11 mice), a polished and reinforced thin-skull (PoRTS) window was made covering the right hemisphere (125.34.4071). For simultaneous measurement of tissue oxygenation and neural activity (n = 9 mice), we implanted two electrodes to measure LFP signals differentially. Electrodes were made from Teflon-coated tungsten wire (#795350, A-M Systems) with a –1 mm length of insulation stripped from the tip. The electrodes were inserted into the cortex to a depth of 800 µm at 45° angle along the rostral/caudal axis using a micromanipulator (MP-285, Sutter Instrument) through two small burr holes made in the skull. The two holes for the electrodes were made –1.15 mm apart to allow insertion of the oxygen probe between the two electrodes in following experiments. The holes were then sealed with cyanoacrylate glue. For spectroscopy imaging experiments with intracortical infusion (n = 4 mice), two small craniotomies were made at the edge of the thinned area of skull, and a cannula (dunny cannula: C315DCS; guide cannula: C315GS-4, Plastic One) was inserted into the upper layers of cortex at a 45° angle via one craniotomy. The stereotrode was placed 1.75 ± 0.5 mm away from the cannula through the other craniotomy. The screws were tightened, and cannula was connected to the micromanipulator via the midline suture using cyanoacrylate glue and black dental acrylic resin (#1530, Lang Dental Manufacturing Co.) to minimize skull movements. For tissue oxygenation (n = 37 mice) and laminar electrophysiology (n = 7 mice) experiments, the measurement sites were marked with ink and covered with a thin layer of cyanoacrylate glue. For oxygen measurements using 2PLM, we used mice chronically implanted with a cranial window over FL/HL (1.0 mm lateral from bregma) over the contralateral hemisphere. In experiments investigating effects of suppressing vasodilation on cortical tissue oxygenation dynamics (Fig. 3e), a cocktail of ionotropic glutamate receptor antagonists 6-cyano-7-nitroquinazoline-2,3-dione (CNOX, 0.6 mM), NMDA receptor antagonist (2R)-amino-5-phosphonopentanoic acid (AP5, 2.5 mM) and GABA receptor antagonist muscimol (10 mM) were applied to suppress neural activity. All drugs were applied topically over the craniotomy and were allowed to diffuse into the cortical tissue for at least 90 min before the oxygen measurements. The efficacy of the CNOX/AP5/muscimol cocktail was monitored with simultaneously recorded neural activity. Neural data were amplified 1000x and filtered (0.1–10 kHz, DAM80, World Precision Instruments) and then sampled at 30 kHz (PCI-6259, National Instruments). The oxygen signal in these experiments was recorded at a depth of ~100–200 µm.

At the end of the experiment, the mouse was deeply anesthetized, and a fiduciary mark was made by advancing an electrode (0.005” stainless steel wire, catalog #794800, A-M systems) into the brain with a micro-manipulator to mark the oxygen measurement site.

Respiration measurements using thermocouple. We conducted simultaneous respiration recordings in a subset of mice (n = 28) along with cortical oxygen measurements. Measurements of breathing were taken using 40-gauge K-type thermocouples (TC- TT K-40-36, Omega Engineering) placed near the mouse’s nose (1 mm lateral from bregma) with care taken to not contact the skin. The thermocouple was identified 2000x, filtered below 30 Hz (Model 440, Brownlee Precision), and sampled at 1000 Hz (PCI-6259, National Instruments). Downward and upward deflections in respiration recordings correspond to inspiratory and expiratory phases of the respiratory cycle, respectively (Fig. 4a). We identified the time of each expiration peak in the entire record as the zero-crossing point of the first derivative of the thermocouple signal.

Laminar electrophysiology. Laminar electrophysiology recordings were performed in a separate set of mice (n = 7, Fig. 1e). On the day of measurement, the mouse was anesthetized using isoflurane (in oxygen, 5% for induction and 2% for maintenance). Two small (1 x 1 mm²) craniotomies were performed over FC (1.0–2.5 mm rostral and 1.0–2.5 mm lateral from bregma) and FL/HL (0.5–1.0 mm caudal and 1.0–2.5 mm lateral from bregma) over the contralateral hemisphere (Fig. 1e), and the dura was carefully removed. The craniotomies were then moistened with warm artificial cerebrospinal fluid (aCSF) and porcine gelatin (Vetbond). The microelectrode was then moved to and head-fixed on the spherical treadmill. Oxygen measurements started at least one hour after the mouse woke up from anesthesia to minimize the effects of anesthesia.

Cerebral tissue oxygenation was recorded with a Clark-type oxygen microelectrode (Oxarx, Uden, The Netherlands). A total of nine probes were used in this study, with an average response time of 0.33 ± 0.11 s (n = 9 probes, Supplementary Fig. 3a, b). No compensation for the delay was performed. The oxygen electrodes were calibrated in air-saturated 0.9% sodium chloride (at 37°C) and oxygen-free standard solution [0.1 M sodium hydroxide (SIX0607H-6, Sigma-Aldrich) and 0.1 M sodium ascorbate (A7673, Sigma-Aldrich) in 0.9% sodium chloride] before and after each experiment. The linear drift of the oxygen electrode signal (1.86 ± 1.19% during each hour, Supplementary Fig. 3c, d) was corrected by linearly interpolating between pre- and post-experiment calibrations. The oxygen electrode was connected to a high-impedance picoammeter (OXYMeter, Unisense A/S, Aarhus, Denmark), whose output signals were digitized at 1000 Hz (PCI-6259, National Instruments). Current recordings were transformed to millimeters of mercury (mmHg) using the calibrations with air-saturated and oxygen-free solutions.

For oxygen polarography measurements, the oxygen microelectrode was positioned perpendicularly to the brain surface and advanced into the cortex with a micro-manipulator (MP-285, Sutter Instrument). The zero depth was defined as when the tip of the oxygen microelectrode touches the brain surface under visual inspection. The probe was then advanced to depth of 100, 300, 500, and 800 µm below the pia at the rate of 0.2 µm for each step, and 30–40 min data were recorded for each depth. The tissue was allowed to recover for at least 5 min before the start of the next recording.

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Neural activity signals were recorded using two linear microelectrode arrays (A1x16-3mm-100-703-A16, NeuroNexus Technologies). The electrode array consisted of a single Shank with 16 individual electrodes with 100 µm inter-electrode spacing. The signals were digitalized and streamed to SmartBox™ via a custom-written LabVIEW™ software (National Instruments). The array was positioned perpendicular to the cortical surface, one was in FL/HL and the other one was in FC on the contralateral side. Recording depth was taken from the manipulator.
CBF measurements using laser Doppler flowmetry. We measured CBF responses to voluntary locomotion in a separate set of mice (n = 5) using laser Doppler flowmetry (OxyLab, Oxford Optronix). The probe was fixed 0.3 mm above the PoRTS window at a 45° angle. Data were sampled at 1000 Hz (PCi-6259, National Instruments).

Brain oxygen measurements using spectroscopy. We mapped the spatio-temporal dynamics of oxyhemoglobin and deoxyhemoglobin concentrations using their oxygen-dependent optical absorption spectra. Reflectance images were collected during periods of green LED light illumination at 530 nm (equally absorbed by oxygenated and deoxygenated hemoglobin, M503L3, Thorlabs) or blue LED light illumination at 470 nm (absorbed by oxygenated but not by deoxy-

Drugs. All drugs were purchased from Sigma-Aldrich except asCFs (#3525, Tocris) and sterile water (USP). Muscimol (M1523, 10 mM), CNQX (C127, 0.6 mM), AP5 (A5282, 2.5 mM) and L-NNAME (N5751, 100 mM) were diluted in aCSF. Atenolol (A7655, 2 mg kg⁻¹ body weight) and glycopyrrolate (SMI0029, 0.5 mg kg⁻¹ body weight) were diluted in sterile water. All drug solution was stored at 20 °C and warmed up using a water bath (WB605A12E, Polyscience) immediately before application. Oxyphor ZP was kindly provided by Sergei Vinogradov.

Histology. At the conclusion of the experiment, mice were deeply anesthetized with 5% isoflurane, transcardially perfused with heparinized saline, and then fixed with 4% paraformaldehyde. The brains were extracted and sunk in a 4% paraformaldehyde with 30% sucrose solution. The flattened cortices were sectioned tangentially (60 µm thick sections) using a freezing microtome and stained for the presence of cytochrome-oxidase. The anatomical locations of the oxygen measurement sites were then reconstructed using a combination of vascular images taken during surgery and the stained brain slices using Adobe Illustrator CS6 (Adobe Systems).

Data analysis. All data analyses were performed in Matlab (R2015b, MathWorks) using custom code (by Q.Z., K.W.G., and P.J.D.).

Locomotion events identification. Locomotion events identified in the spherical treadmill were identified by first applying a low-pass filter (10 Hz, 5th order Butterworth) to the velocity signal from the optical rotary encoder, and then comparing the absolute value of acceleration (first derivative of the velocity signal) to a threshold of 3 cm s⁻². Periods of locomotion were categorized based on the binarized detection of the treadmill acceleration:

where \( \delta(t) \) is the acceleration at time t, and \( \alpha \) is the treadmill acceleration threshold.

Spontaneous and evoked activity. To characterize spontaneous (non-locomotion-evoked) activity, we defined resting periods as periods after the end of previous locomotion event lasting more than 10 s. Locomotion-evoked events were defined as segments with at least 3 s of resting period prior to the onset of locomotion and followed by at least 5 s of locomotion. For oxygen measurements using polarographic electrode and 2PLM, the locomotion segments need to last at least 10 s in duration.

Oxygen data preprocessing. Oxygen data from polarographic electrodes were first low-pass filtered (1 Hz, 5th order Butterworth). The oxygen data were then down-sampled to 30 Hz to align with binarized locomotion events for calculation of locomotion-triggered average and HRF.

Laminar neural activity. The neural signal was first digital filtered to obtain the local field potential (LFP, 0.1–300 Hz, 5th order Butterworth) and multiunit activity (MUA, 300–3000 Hz, 5th order Butterworth)11,25. Time-frequency analysis of LFP signal was conducted using multi-taper techniques (Chronux toolbox version 2.11, http://chronux.org)77. The power spectrum was estimated on a 1 s window with 1 Hz bandwidth averaged over nine tapers. MUA signals were low-pass filtered (5 Hz, Bessel filter). The locomotion-evoked LFP power spectrum was converted into relative power spectrum by normalizing to the 3 s resting period prior to the onset of locomotion. Spike rate was obtained by counting the number of events that exceed an amplitude threshold (three SDs above background) in each 1 ms bin.

Spike sorting. Sortable spike waveforms were extracted from MUA recordings using spike times identified from threshold crossings at four SDs of the mean. Spike waveforms were interpolated using a cubic spline function (MATLAB function: interp1) and were normalized by the amplitude of the peak of the action potential. We classified waveforms as fast spiking (FS) or regular spiking (RS) neurons based on the peak-to-trough-duration of the normalized waveform of each spike.
Peak-to-trough times of all spikes across all layers were binned at 0.05 ms intervals (the minimal temporal resolution at 20 kHz sampling rate). A histogram of peak-to-trough times was fitted as a sum of two Gaussian distributions (Supplemental Fig. 2a, f), and a receiver operator characteristic curve was used to segregate spikes in a given bin as either FS or RS waveforms using a 95% probability of belonging to a group as the inclusion threshold. Spikes not reaching the inclusion threshold for either group were not included in the analysis. Fast spiking waveforms (Supplementary Fig. 2b, g) were characterized by short durations between action potential in a given bin as either FS or RS waveforms using a 95% probability of belonging to the signals, and $S_g(f)$ is the cross-spectrum. For ordinary coherence analysis between two signals ($x$ and $y$), highly coherent oscillations can occur if they are functionally connected or because they share a common input. To differentiate between these possibilities, we also computed the partial coherence, i.e., coherence between two signals ($x$ and $y$) after the removal of the components from each signal that are predictable from the third signal ($z$). The partial coherence function measuring the relationship of $x$ and $y$ at frequency $f$ after removal of $z$ is defined as

$$\text{PC}_{xy|z}^2(f) = \frac{S_{xy|z}(f)}{S_{xz}(f)S_{yz}(f)} \tag{5}$$

where $S_{xy|z}$ and $S_{xz}$ is the auto-spectra associated with the residual part of $x$ and $y$ after removing the part coherent with $z$, respectively. $S_{xz}$ is the cross-spectrum between the residual part of $x$ and $y$ after removing the part coherent with $z$. If all the networks are connected, partial coherence will be between zero and the level of the ordinary coherence. If the connection behaves in an asymmetric manner, i.e., signal $z$ affects $x$ and $y$ differentially, the coherence between two signals may increase after partialization (Supplementary Fig. 9a).

### Statistical analysis

Statistical analysis was performed using Matlab (R2015b, Mathworks). All summary data were reported as the mean ± SD unless stated otherwise. Normality of the samples were tested before statistical testing using Anderson-Darling test (adtest). For comparison of multiple populations, the assumption of equal variance for parametric statistical method was also tested (vartest2 and varstestn). If criteria of normality and equal variance were not met, non-parametric methods (Kruskal-Wallis H-test, Wilcoxon signed-rank test, Kruskal-Wallis ANOVA). All p values were Bonferroni corrected for multiple comparisons. Significance was accepted at $p<0.05$.

### Computational modeling

We used two-dimensional, axisymmetric, time-dependent finite element models to simulate the coupled physics of blood flow and oxygen diffusion/consumption. All the simulations were performed in COMSOL (COMSOL Inc.) using the weak form partial differential equation module. The model was divided into two domains: the fluid domain and the solid domain. The fluid domain, which represents the arterial lumen, was assumed to be a cylinder of radius $R_2$ and a length of $L_2$. The solid domain, which represents the surrounding tissue that is oxygenated by the arteriole, was assumed to be a cylinder of radius $R_1$ and a length of $L_1$. To account for the dilation of the blood vessel, the equations of transport in the fluid domain were written in an arbitrary Lagrangian-Eulerian formulation. Therefore, the computational mesh inside the fluid domain can move arbitrarily to optimize the shapes of the elements. The equations in the solid domain were implemented in their Lagrangian formulation, which is more appropriate for simulating solid mechanics. We presented all the equations in their most familiar physical (Eulerian) coordinates for simplicity.

In the fluid domain, the blood flow was modeled by Stokes law (Eq. 6) to simulate the low Reynolds’ number flow78 in the arterioles.

$$\nabla \cdot \mathbf{v} = 0 \tag{6}$$

where $\mathbf{v}$ is the fluid velocity, $p$ is the fluid pressure and $\mu$ is the dynamic viscosity of blood.

The oxygen transport in blood was modeled by the advection-diffusion equation (Eq. 7), and subject to the equilibrium of free and hemoglobin-bound oxygen.

$$\frac{\partial C_f}{\partial t} = -\mathbf{v} \cdot \nabla C_f + \nabla \cdot (D_{O2} \nabla C_f) \tag{7}$$

In Eq. 7, $C_f$ is the total concentration of oxygen in blood and can be represented by the sum of concentration of free ($c_f$) and hemoglobin-bound ($c_B$) oxygen, and $D_{O2}$ is the diffusion coefficient of oxygen. The equilibrium relation between free and bound oxygen (Eq. 8) is given by the oxygen saturation-dissociation curve79 ($SO_2$, a function of free oxygen) and the hemoglobin concentration $C_{Hb}$.

$$c_f = 4C_{Hb}SO_2(c_f) \tag{8}$$

The mesh movement in the dilating arteriole was modeled using a linear model (Eq. 9) since the dilation occurs in only one direction (radially).

$$u_{rad} = \frac{r}{R_0} \text{dilation}(t) \text{ and } u_{max} = 0 \tag{9}$$

where, $u_{rad}$ and $u_{max}$ is the radial and axial components of the mesh displacement, respectively.

In the solid domain, we calculate the tissue displacement, $u$, and the concentration of free oxygen $c_f$. The transport of oxygen in the tissue was modeled

$$\frac{\partial C_t}{\partial t} = \nabla \cdot (D_T \nabla C_t) \tag{10}$$
by Fick’s law of diffusion (Eq. 10).

$$\frac{dc}{dt} = \nabla \cdot (D_o \nabla c_f) - CMRO_2$$ (10)

where, $\frac{dc}{dt}$ is the material time derivative of $c_f$, which is different from the partial time derivative, $c_f$, seen in Eq. 7. The time-profile of CMRO2 used in the model was given in Fig. 5c.

The deformation of brain tissue was modeled by a nearly incompressible (Poisson’s ratio = 0.45) linear elastic model. Although the volume of the brain tissue in the model will decrease slightly, we kept the integrated CMRO2 of the tissue unchanged, so these deformations will not change CMRO2. We calculated the displacement of the tissue ($u_0$) based on the equations for linear elasticity (Eq. 11).

$$\nabla \cdot \sigma = 0$$ (11)

where, $\sigma$ is the Cauchy stress, and is given by the elastic coefficients $\mu$ and $\lambda$, and the linearized elastic strain $\epsilon$, using Eqs. 12 and 13.

$$\sigma_{ij} = 2\mu\epsilon_{ij} + \lambda \epsilon_{kk} \delta_{ij}$$ (12)

$$\epsilon_{ij} = \frac{1}{2} (\nabla u_i + \nabla u_j^T)$$ (13)

All the calculations were performed for an initial stationary (steady-state) case and the solution was used as the initial condition for the dynamic simulation that followed. $I$ is the identity tensor. $T$ is the trace.

We modeled a cylinder with length $L_0$ of 100 µm to simulate measurements in layer I. As arterial dilation can cause a decrease in the flow resistance and result in an increase in blood flow velocity, we dictated the fluid flow by a pressure difference across the two ends of the arteriole. This was achieved by applying a traction equal to $p_1$, mmHg at the inlet (Fig. 5a, top) of the arteriole and zero traction at the outlet (Fig. 5a, bottom). The pressure difference $p_1$ was chosen so that the blood velocity was approximately 2 mm s$^{-1}$. The concentration of free oxygen (and consequently the hemoglobin-bound oxygen) was fixed at the inlet of the arteriole. A no-flux boundary condition was used at the three open ends ($z = 0$; $z = L_0$ and $r = R_0$) of the tissue for symmetry. The displacement at the tissue-artery interface followed the same time course as the arterial dilation (Eq. 9).

In this model, we used a value of the diffusion coefficient for $O_2$ of 2000 µm² s$^{-1}$. Resting arterial oxygen tension in the penetrating vessels was taken to be 35 mmHg. The arterial and tissue radius was assumed to be 9 and 50 µm, respectively. The arterial oxygen tension in the penetrating vessels was taken to be 35 mmHg. For further information on research design, see the Nature Research Reporting Summary linked to this article.

Data availability

The datasets generated and analyzed during the current study are available at https://psu.app.box.com/v/Zhang-O2-during-behavior.

Code availability

The code used to generate the figures in this paper is available at https://psu.app.box.com/v/Zhang-O2-during-behavior.

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Author contributions

Q.Z. and P.J.D. designed the project. Q.Z. performed experiments and analyzed data using polarographic electrode, intrinsic optical imaging, optical imaging of spectroscopy, and laser Doppler. M.R. performed experiments and analyzed data using 2PLM. K.W.G. performed experiments and analyzed laminar electrophysiology data. E.C. analyzed 2PLM data. R.T.K. and W.D.H. implemented the computational modeling. S.C. supervised experiments and data analysis using 2PLM. P.J.D. supervised experiments, modeling, data analysis, and preparation of the manuscript. Q.Z. and P.J.D. wrote the manuscript.
Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to P.J.D.

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