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Cerebral tissue pO₂ response to stimulation is preserved with age in awake mice

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Abstract

Compromised oxygen supply to cerebral tissue could be an important mechanism contributing to age-related cognition decline. We recently showed in awake mice that resting cerebral tissue pO₂ decreases with age, a phenomenon that manifests mainly after middle-age. To extend these findings, here we aimed to study how tissue pO₂ response to neuronal stimulation is affected by aging. We used two-photon phosphorescence lifetime microscopy to directly measure the brain tissue pO₂ response to whisker stimulation in healthy awake young, middle-aged and old mice. We show that despite a decrease in baseline tissue pO₂, the amplitude of the tissue pO₂ response to stimulation is well preserved with age. However, the response dynamics are altered towards a slower response with reduced post-stimulus undershoot in older ages, possibly due to stiffer vessel wall among other factors. An estimation of the net oxygen consumption rate using a modified Krogh model suggests that the O₂ overshoot during stimulation may be necessary to secure a higher capillary O₂ delivery to the tissue proportional to increased CMRO₂ to maintain the capillary tissue pO₂. It was observed that the coupling between the CMRO₂ and capillary O₂ delivery is preserved with age.

Keywords

Cerebral tissue oxygenation; Neural stimulation; Two-photon microscopy; Aging; Awake mice

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Author contributions F.L conceived and supervised the study. M.M and F.L designed the experiments and analyzed the data, with contributions from S.B and D.B. M.M and X.L performed the experiments. M.M synthesized the PtP-C343 dye under the supervision of A.K. D.B helped in confirming the performance of the dye. F.P provided the aged mice. M.M, F.L, D.B, and X.L were involved in interpreting the findings. M.M prepared the manuscript and figures. All authors edited and approved the manuscript.

Conflict of interest

Dr. Lesage reports a minority ownership in LabeoTech Inc.

1. Introduction

It has been established that cognitive function declines even with healthy aging [1–4]. With clinical observations showing a correlation between cognitive impairments and vascular disorders [5–7] and with cerebral blood flow (CBF) [3,8], it is hypothesized that impaired cerebral tissue oxygenation in old age may contribute to cognitive impairment.

In a previous study in awake mice, we revealed an age-related decrease in resting cerebral tissue pO_2 which manifested itself mainly after 15 months of age. In 27-month old mice, the substantial decrease in brain tissue pO_2 was accompanied by the presence of pockets of hypoxia in some regions of the cortex [9]. We anticipate that these tissue regions, with low pO_2 , are more susceptible to demand-induced hypoxia. Given the reductions in cerebral tissue pO_2 with aging, it is thus important to understand if the pO_2 response to neuronal stimulation is affected by aging. Most previous works studying oxygenation changes during neural stimulation were performed mainly with functional magnetic resonance imaging (fMRI) and near-infrared spectroscopy (NIRS), methods that measure the changes in blood oxygenation during neural activation. There are a few studies investigating tissue pO_2 response to stimulation, but none of these studies addressed potential aging effects and furthermore were done in anesthetized conditions [10–13]. Although fMRI and NIRS studies have described an altered hemodynamic and blood oxygenation response with age [14–18], interpretation of these observations to make inferences about the tissue pO_2 response is challenging, because the latter is affected by a complex interaction between vascular hemoglobin concentration, oxygen saturation of hemoglobin (SO_2), CBF, the geometry and morphology of the vascular network, capillary density, and the cerebral metabolic rate of oxygen consumption ($CMRO_2$); all potentially modulated by age.

Here, using two-photon phosphorescence lifetime microscopy [19] and the O_2 -sensitive two-photon enhanced phosphorescent dye PtP-C343 [20], we aimed to directly measure the brain tissue pO_2 response to neuronal activation in awake mice and investigate its change with aging.

2. Methods

2.1. Synthesis of the PtP-C343 probe

The O_2 -sensitive phosphorescent molecule PtP-C343 [20] was synthesized as described in Moeini et al. (2018) [9] by adaptation and modification of a procedure described in the literature [20]. It has been reported that the brain interstitial fluid is slightly more acidic than blood, with a pH of 7.3 or even lower [21,22]. The dye was then calibrated at pH 7.2 and 37 °C, following previous protocols [19,23]. However, it should be noted that the pO_2 dye PtP-C343 does not exhibit pH dependence between pH 6 and 8.5 [20]. Therefore, a slight deviation of the brain tissue pH from 7.2 will not affect our pO_2 measurement.

2.2. Animal preparation

Animal handling and surgical procedures were approved by the ethics committee of the research center of the Montreal Heart Institute. All experiments were performed in

accordance with the ARRIVE guidelines and the recommendations of the Canadian Council on Animal Care. Mice were randomly assigned to experimental groups. Young adult ($n = 7$), middle-aged ($n = 6$) and old ($n = 7$) C57BL/6 J healthy male mice were obtained from the colony of aged mice of the Quebec Network for Aging Research (RQRV) and housed in 12-hr light/dark cycle until imaging. In our study, in addition to the young and old groups, we also included a middle-age group to determine if potential age-related changes are progressive or they only happen in old ages. Indeed, in a previous study [9] we observed that the changes in resting cerebral tissue oxygenation are exacerbated after middle-age. Young adult mice were 8–9 months old, which ensured the absence of maturational growth and related anatomical and biological changes observed in younger ages [24]. Middle-aged mice were 15–16 months old, according to the recommended age range of 10–15 months for the middle-aged groups [24]. Old mice were 26–28 months old, well above 18 months after which almost all aging biomarkers show age-related changes [24]. No reduction in mice survival was observed until this age. 8–10 days before measurements, a thinned skull window was created over the left barrel cortex under isoflurane anesthesia (2.0% in pure oxygen) as described in Shih et al. [25]. Briefly, the scalp was removed and the exposed skull was covered with a thin layer of tissue adhesive (Vetbond). A custom head-plate made from titanium was fixed on the skull using dental cement. The skull was then slowly thinned to translucency with a micro-drill (OmniDrill 35, World Precision, USA). A 150 μm -thick cover glass was glued to the dried window using cyanoacrylate glue and the edges were sealed with dental cement to form a 3 mm diameter cranial window. A small thinned region at the edge of the cover glass (0.5 mm) was left uncovered with dental cement to allow the injection of the PtP-C343 dye into tissue through the soft thinned membrane. During the surgery, animals were fixed on a controlled physiological monitoring system (LabeoTech, Canada) which enabled continuous monitoring of the rectal temperature, respiration and heart rate. Ketoprofen (s.c., 5 mg/Kg, Merial, Canada) and buprenorphine (s.c., 0.05 mg/Kg, Reckitt Benckiser Healthcare, UK) were injected before the surgery and baytril (i.p., 5 mg/Kg, Bayer, Germany) was injected after the surgery. Animals were returned to the cages and injections were repeated 24 h after the surgery. Although this study was not blinded, the analysis of microscopic data was automated and the same parameters and algorithms were used to analyze all images limiting investigator bias. In addition, no data was discarded and care was taken to treat all groups equally during surgery, handling, training and imaging.

2.3. Awake imaging

All measurements were performed in awake animals to avoid potential age-related anesthesia confounds. During the imaging sessions, animals were fixed on an angled treadmill wheel which allowed free movement of the limbs while the head was restrained by a custom titanium bar, as described in Moeini et al. [9]. They were trained on the wheel over four fixation training sessions (starting after 3 days recovery following surgery) to habituate to head restraint and minimize stress during the imaging sessions. The duration of the restraint was gradually increased from 10 min to 45 min over 4 sessions.

2.4. Whisker stimulation

Air puffs, generated from pulses of compressed air (pressure: 25 psi, frequency: 5 Hz, pulse width: 100 ms) from a pressure microinjection system (PICOSPRITZER III, Parker

Hannifin, USA) were used to stimulate the right whiskers (nozzle to whisker distance: 150–200 mm). We used stimulus duration of 5 s with interstimulus intervals of 30–35 s.

2.5. Tissue pO₂ response to whisker stimulation measured by two-photon phosphorescence lifetime microscopy

Two-photon imaging was performed using a custom-built laser scanning microscope, as described by Moeini et al. [9]. For tissue pO₂ imaging, the PtP-C343 dye solution (~150 μM in ACSF) was slowly injected into the brain tissue ~300 μm below the surface with a glass micropipette using a microsyringe injector (UMP3, World Precision Instruments, USA). The injection was done through the thinned skull next to the cover glass. The diffusion of the injected dye to the surrounding tissue allowed imaging of cerebral tissue beneath the cover glass. It is assumed that the dye is mainly distributed in the interstitial and perivascular space. Since we measured the phosphorescence lifetime rather than intensity (see below), our pO₂ measurements are not affected even if the injected dye slightly concentrates near the surface or in the perivascular space. ~200 μl 2 M Da FITC-Dextran (50 mg/ml in saline, Sigma) was also injected through the tail vein to visualize the vasculature. Each measurement contained 10 points around diving arterioles, up to 200 μm from the arteriole. At each point 150 decays were recorded before moving to the next point. This was repeated 600 times, covering 13 stimulus blocks, to increase the signal to noise ratio. Each excitation cycle consisted of 25 μs excitation period in which the laser pulse was “on” followed by 275 μs “off” period in which the phosphorescence emission was allowed to decay. The number of the points and decays were adjusted in a way to allow a temporal resolution below 1 s. For each point, all decays (from all 13 stimulation blocks and interstimulus periods) were sorted based on their time from the stimulation onset and were averaged in 0.9 s intervals to obtain the pO₂ response for individual points. Averaged phosphorescence decay at each point/time was fitted with a single-exponential curve to determine the phosphorescence lifetime. The lifetimes were then converted to pO₂ using a calibration curve.

For each measurement point, the time course of obtained pO₂ values yielded the tissue pO₂ response to stimulation. From this response, the baseline was estimated as the averaged pO₂ within a 4 s window before stimulus onset. The post-stimulus pO₂ was estimated as the average of values between 8–25 s following the stimulus onset while the peak response was estimated as the average response between 2–5 s following the stimulation onset. All sampled points in each age group were pooled for age comparisons.

Finally, tissue pO₂ responses for all point measurements in each age group were averaged. The average responses were then used to parameterize the dynamics of tissue pO₂ response to whisker stimulation. Two temporal parameters (T_{rise} and T_{fall}) were obtained from the average response curves of age groups. T_{rise} and T_{fall} were defined as the response times during the rise or fall periods in which tissue pO₂ was at half maximum. It should be noted that since two-photon measures are very localized (compared to confocal microscopy, for example), the response curves for individual sampled points were more noisy which made it difficult to extract reliable T_{rise} and T_{fall} for individual curves.

2.6. Estimation of net oxygen consumption rate (OC) in cerebral tissue

Previous studies on rats have observed a marked absence of capillaries in the vicinity of penetrating arterioles, forming a capillary depleted cylindrical region with a radius of $\sim 50 \mu\text{m}$ [26]. This has formed the basis of some efforts [27] to calculate the CMRO_2 by fitting the tissue pO_2 gradients around arterioles with the Krogh cylinder model [28] of O_2 diffusion from a vessel to surrounding tissue. In our experiments on mice, however, we found that the use of the ideal Krogh model is not accurate because in most cases either tissue pO_2 profiles from arterioles do not plateau within the capillary depleted region or the capillary depleted regions are absent. Therefore, for mice the oxygen consumption term in the Krogh model includes both CMRO_2 and capillary O_2 supply [9]. Assuming a uniform capillary O_2 supply in tissue (for simplicity), a modification of the Krogh model was used to estimate the net oxygen consumption rate in the tissue (OC), defined as CMRO_2 minus capillary O_2 supply per unit volume of the tissue (Please note the difference between the CMRO_2 and OC: CMRO_2 is the oxygen consumption rate in tissue by cells, while OC is the same but after subtracting the rate of oxygen supply by cerebral capillaries. Therefore, OC represents the balance between the oxygen consumption and supply in the tissue.) [9]:

$$p\text{O}_2(r) = p\text{O}_{2\text{art}} + \frac{\text{OC}}{4\alpha D} \left[\left(r^2 - R_{\text{art}}^2 \right) - 2R_t^2 \ln(r/R_{\text{art}}) \right] \quad (1)$$

In Eq. (1) $p\text{O}_{2\text{art}}$ is the arterial pO_2 , R_{art} is the mean arterial radius, R_t is the radius of the Krogh cylinder, α is oxygen solubility ($1.27 \times 10^{-3} \mu\text{moleO}_2/\text{ml}/\text{mmHg}$ [27]), and D is oxygen diffusivity in tissue (assumed to be $\sim 4000 \mu\text{m}^2/\text{s}$ [27]). Tissue pO_2 profiles around arterioles, obtained from tissue pO_2 maps (at baseline and during stimulation or post-stimulation periods), were fitted with the Krogh model to find OC and $p\text{O}_{2,\text{art}}$. R_t was determined as the distance at which the curve reached a plateau.

2.7. Non-capillary blood flow measurements with Doppler-OCT

We also measured blood flow in non-capillary vessels (diameter $> 10 \mu\text{m}$) at rest using a custom optical coherence tomography (OCT) setup, as described by Moeini et al. (2018) [9]. For each mouse, an OCT angiogram and a 3D Doppler OCT volume were acquired over a cortical surface of $\sim 700 \mu\text{m} \times 700 \mu\text{m}$ with the procedure explained in Moeini et al. (2018) [9]. The 3D Doppler OCT datasets consisted of *en face* slices of z-projection blood velocity maps at cortical depth intervals of $\sim 3.8 \mu\text{m}$. Pial vessels generated both positive and negative Doppler shifts because of their undulating shape, but diving arterioles and ascending venules exhibited pure positive or negative Doppler shifts. To avoid the pial vessels and focus only on the penetrating vessels, we excluded the top $100 \mu\text{m}$ of the OCT datasets in our analyses. Arterioles and venules were labeled on *en face* slices over the depth of $100\text{--}650 \mu\text{m}$ and their diameter, blood velocity, and mean blood flow were obtained. The relationship between the baseline flow and vessel diameter was then calculated by linear regression analysis between the arterial/venular velocity and the vessel diameter and also between the logarithmic arterial/venular flow and the logarithmic vessel diameter.

2.8. Statistical analysis

The results are presented as either box-and-whiskers plots (in which the box represents the 75th and 25th percentiles, the central line indicates the median, the whiskers represent the most extreme data not considered outliers ($> \pm 2.7SD$), and the '+' symbols represent the outliers) or bar plots (representing mean $\pm$ 95% confidence intervals, c.i.). Statistical significance was calculated using ANOVA followed by Tukey HSD post hoc test. Statistical significance was assigned at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ and ***** $p < 0.00001$. The sample sizes are described in the corresponding figure legends. The sample sizes were chosen empirically based on our previous experience.

3. Results

3.1. Preserved magnitude, but altered dynamics of tissue pO_2 response to whisker stimulation with aging

Tissue pO_2 responses to whisker stimulation for young, middle-aged and old mice are shown in Fig. 1a. We observed a substantial decrease in baseline tissue pO_2 after middle-age, which is consistent with our previous observations at rest [9]. When converting the tissue pO_2 responses to ΔpO_2 ($pO_2 - \text{baseline } pO_2$), we found no significant difference in the response delay (~ 1 s) (Fig. 1b) and the peak tissue ΔpO_2 response between age groups (Fig. 1b and c). However, the response in young animals had faster dynamics (faster rise and fall, Table 1) and showed a clear post-stimulus undershoot (Fig. 1b and c), in agreement with previous BOLD fMRI [29–31] and NIRS [16] studies. On the other hand, no undershoot was observed in middle-age and old animals (Fig. 1b and c).

3.2. The balance between the $CMRO_2$ increase and capillary O_2 supply increase during stimulation is preserved with aging

Estimation of OC and arterial pO_2 at baseline and during stimulation and post-stimulation periods (by fitting the tissue pO_2 profiles from arterioles with Krogh model) showed a slight increase in arterial pO_2 and OC during stimulation which returned to baseline values after the stimulation, but differences were subtle and did not reach statistical significance (Fig. 1d). This trend and the magnitude of changes were not affected by age. This shows that for all age groups, both $CMRO_2$ and capillary O_2 supply increase proportionally during stimulation such that the net oxygen consumption rate ($OC = CMRO_2 - \text{capillary } O_2 \text{ supply}$) does not significantly change during stimulation.

3.3. Age-related increases in the slope of flow-diameter and speed-diameter correlations for venules

To investigate whether the change in response undershoot could have a biomechanical component, we performed a linear regression analysis of baseline flow-diameter (on a logarithmic scale) and baseline speed-diameter from Doppler OCT data (at rest). Data showed an age-related increase in the slope of linear fits for venules, while the slopes for arterioles were unchanged with age (Fig. 2). This could indicate a reduced ability of venules and veins for passive dilation due to decreased compliance with aging (see below).

4. Discussion

In this study, we found a substantial decrease in average baseline tissue pO_2 in the cortex of old mice, compared with young and middle-aged groups (Fig. 1a). This is in agreement with our previous observations in awake mice at rest, indicating impaired resting cerebral tissue oxygenation in old mice [9]. Regarding the response to whisker stimulation, we observed that the peak magnitude of the tissue pO_2 response was not changed significantly with age, but the response dynamics were altered towards a slower response with reduced post-stimulus undershoot in older ages (Fig. 1b and c).

All measurements were performed in awake behaving mice, removing the confounding effects of anesthesia. This is a very important aspect of this study since anesthetized experiments often need mechanical ventilation and/or adjustment of physiological parameters which confounds the results as many of these parameters normally change with aging. In addition, anesthesia may affect neuronal activation and corresponding flow and pO_2 responses. To minimize animal stress during imaging sessions, a custom-built treadmill wheel was used in which the animal was able to freely walk or run, while the head was fixed, similar to that of Lyons et al. [32], and animals were trained on the wheel for habituation before the main experiments. To our knowledge this is the first study on direct measurement of the tissue pO_2 response to neural stimulation under awake conditions and the changes in the response with aging.

The observed changes in the dynamics of tissue pO_2 response to whisker stimulation are in agreement with previous studies. A slower hemodynamic response and a reduced post-stimulus undershoot in older subjects have also been described using BOLD fMRI [14,15,17] and NIRS [16] studies. Hemodynamic response modeling using the arteriolar compliance model coupled with the passive Balloon model [33] also predicted a slower response and no undershoot in aged subjects, mainly due to reduced arteriolar wall elasticity [34,35]. The Balloon model itself [29] also predicts a slower response for stiffer vessel wall. One observation in our study in support of these findings is that flow-diameter and speed-diameter correlations for venules from our Doppler OCT data showed age-related increase in the slope of linear correlations (Fig. 2). Considering the general relationship between flow and the pressure drop (ΔP) across a vessel section ($\text{flow} = \Delta P / \text{resistance}$), and since resistance varies with diameter and vessel length, the slope of the flow-diameter correlation (across different vessels) quantifies either changes in ΔP across vessel segments of similar size with age or changes in vascular segment length. Changes in upstream network with age (for example, reduced capillary density [36]) will increase the upstream resistance, and reduce the input pressure to the venule (assuming no change of systemic pressure with age and that pressure goes to zero on the other end of the venule, and keeping all other variables constant). Our results then rather support another possibility of reduced venule compliance associated with a higher pressure drop. The latter is in agreement with previous reports of decreased elasticity of vessels with age [36,37], which reduces the ability of venules and veins for passive dilation. Other mechanisms have also been proposed for the post-stimulus undershoot, including prolonged elevation in $CMRO_2$ and post-stimulus CBF undershoots [30] associated with a post-stimulus decrease in neuronal activity [38]. In addition, CO_2 washout during the stimulation period has also been suggested to play a role by producing a

brief post-stimulus CBF undershoot by hypocapnia induced vasoconstriction [39]. Further studies need to be performed to investigate the role of these proposed mechanisms in the observed age-related changes in the post-stimulation response. For example, possible age-related changes in the baseline $p\text{CO}_2$ or post-stimulus neuronal inhibition may play a role.

There has been considerable debate over the underlying mechanisms for the O_2 overshoot observed during the hemodynamic response and some investigators have perceived it as a paradox or uncoupling [40,41]. Prior measurements of tissue $p\text{O}_2$ changes during brain activation suggest that the O_2 overshoot occurs to prevent tissue $p\text{O}_2$ decrease in the capillary bed during stimulation [10]. Having an O_2 overshoot with constant tissue $p\text{O}_2$ would result in a greater O_2 concentration gradient from capillaries to tissue, which supports greater oxygen extraction from the capillaries and oxygen consumption by tissue. Indeed, our observation that OC (net oxygen consumption rate in tissue = $\text{CMRO}_2 - \text{capillary } \text{O}_2 \text{ supply}$) remains unchanged or tends to increase slightly during neuronal activation, means that despite an O_2 overshoot, the increase in capillary O_2 supply during stimulation is still less than or at most equal to the CMRO_2 increase. Thus, the O_2 overshoot may be necessary to secure a higher capillary O_2 delivery to the tissue proportional to increased CMRO_2 to maintain the capillary tissue $p\text{O}_2$. Our data shows that the coupling between the CMRO_2 and capillary O_2 delivery during neuronal activation is well preserved with age (Fig. 1d).

Given the reductions in baseline cerebral tissue $p\text{O}_2$ with aging and the presence of hypoxic regions in the cortex of old mice [9], it is important to understand if neuronal stimulation can also dynamically create regions of tissue hypoxia and further compromise the cerebral tissue oxygenation in old ages. However, the limitations of the present study did not allow the investigation of the presence of hypoxic regions during stimulation. To reach a high temporal resolution as a requirement accurate measurement of tissue $p\text{O}_2$ response, we had to measure limited number of sampling points which does not allow the spatial identification of sparse hypoxic pockets. With the development of brighter $p\text{O}_2$ probes [42] which allow faster measurements, future studies may be able to investigate the hypoxia potential during stimulation in aged animals. One other limitation of the present study is that the OCT data (Fig. 2) were obtained only at rest. Although the findings at rest could also help in better understanding of the mechanisms behind altered $p\text{O}_2$ response dynamics with age (as explained above), in future, fast repetitive OCT scans on single slices crossing the arterioles and venules, similar to the work done by Baraghis et al. [43], could be performed to estimate the vascular compliance by measuring the changes in the vessel diameter and blood flow during the stimulation. Specifically, the OCT and two-photon setups could be integrated to allow the measurement of vascular reactivity and tissue $p\text{O}_2$ response for the same vessels.

Overall, our study reveals that while baseline cerebral tissue $p\text{O}_2$ decreases with age in conscious mice, tissue $p\text{O}_2$ response to stimulation is well preserved with age. However, the response dynamics are altered towards a slower response with reduced post-stimulus undershoot in older ages. In addition, our data suggests that the O_2 overshoot during stimulation may be necessary to sustain capillary O_2 supply, and that the coupling between the CMRO_2 and capillary O_2 delivery is preserved with age.

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Fig. 1.

Tissue pO_2 response to whisker stimulation. (a) Averaged time courses (mean $\pm$ s.e.m.) of absolute tissue pO_2 changes in response to 5 s whisker stimulation (dark area). (b) The same data as in (a) but plotted as ΔpO_2 (pO_2 - baseline pO_2). (c) Peak response magnitude (average response through 2–5 s from the stimulation onset) and average response during post-stimulation period (8–25 s from the stimulation onset, PostStim). The box represents interquartile range, the central line indicates the median and the whiskers extend to the most extreme data points not considered outliers ($> \pm 2.7SD$); the outliers are plotted individually

using the '+' symbol. (d) Each stimulation test consisted of a gridline point measurement from an arteriole (up to 200 μm from the arteriole). Average pO_2 values at baseline (BL) and during the stimulation (Stim) and pos-stimulation (PostStim) periods were found for all sampled points and were plotted against the distance from the arteriole. Net O_2 consumption rates (OC) (top) and pO_2 at arterial wall ($\text{pO}_{2,\text{art}}$) (bottom) were obtained by fitting the data to the Krogh model, Eq. (1). Bar plots represent mean $\pm$ c.i.(95%). Statistical significance was calculated using ANOVA followed by Tukey HSD post hoc test. *****: $p < 0.00001$ ***: $p < 0.001$. Y: young (n = 195 points); M: middle-aged (n = 188 points); O: old (n = 222 points).

Fig. 2.

Flow-diameter correlations. (a) Correlation between the blood flow and diameter in a log-log scale (top) and linear correlation between the blood velocity and diameter (bottom) for arterioles (left) and venules (right). The points show the experimental data and the solid lines show the best linear fits. (b) The slope of the linear fits in (a) for arterioles and venules (V). Top: flow-diameter correlation in log-log scale. Bottom: velocity-diameter correlation. Bar plots represent mean $\pm$ c.i.(95%). *****: $p < 0.00001$ *****: $p < 0.0001$. *: $p < 0.05$, #: p -value approaches significance; statistical significance was calculated using ANOVA followed by Tukey HSD post hoc test. Y: young ($n = 7$ mice); M: middle-aged ($n = 6$ mice); O: old ($n = 7$ mice).

Table 1

Parameterization of tissue pO_2 response to whisker stimulation (obtained from the average response curves of age groups). T_{rise} and T_{fall} were defined as the response times during the rise or fall periods in which tissue pO_2 was half maximum.

	T_{rise} (s)	T_{fall} (s)
Young	1.43	5.75
Middle-aged	1.70	6.41
Old	1.46	6.72