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Spatial and temporal heterogeneities of capillary hemodynamics and its functional coupling during neural activation

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Abstract

The cerebral vascular system provides a means to meet the constant metabolic needs of neuronal activities in the brain. Within the cerebral capillary bed, the interactions of spatial and temporal hemodynamics play a deterministic role in oxygen diffusion, however, the progression of which remains unclear. Taking the advantages of high spatiotemporal resolution of optical coherence tomography (OCT) capillary velocimetry designed with the eigen-decomposition (ED) statistical analysis, we investigated intrinsic red blood cell (RBC) velocities and their spatiotemporal adjustment within the capillaries permeating mouse cerebral cortex during electrical stimulation of contralateral hind-paw. We found that mean capillary transit velocity (mCTV) is increased and its temporal fluctuation band-width (TFB) is broadened within hind-paw somatosensory cortex. Additionally, the degree to which mCTV is increased negatively correlates with resting state mCTV, and the degree to which TFB is increased negatively correlates with both resting state mCTV and TFB. In order to confirm the changes are due to hemodynamic regulation, we performed angiographic analyses and found that the vessel density remains almost constant, suggesting the observed functional activation does not involve recruitment of reserved capillaries. To further differentiate the contributions of mCTV and TFB to the spatiotemporally-coupled hemodynamics, changes in the mCTV and TBF of capillary flow were modeled and investigated through a Monte Carlo simulation. The results suggest that neural activation evokes the spatial transit time homogenization within the capillary bed, which is regulated via both the heterogeneous acceleration of RBC flow and the heterogeneous increase of temporal RBC fluctuation, ensuring sufficient oxygenation during functional hyperemia.

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Conflicts of Interest

Dr. Wang discloses intellectual property owned by the Oregon Health and Science University and the University of Washington related to OCT angiography, and licensed to commercial entities, related to the technology and analysis methods described in part of this manuscript. The other authors have no conflicts of interest to declare.

Index Terms—

capillary hemodynamics; hind-paw electrical stimulation; mean capillary transit velocity; neural activation; optical coherence tomography angiography; spatial heterogeneity; temporal RBC fluctuation

I. Introduction

The interwoven nature of capillary network supports crucial functions in tissue metabolism through the exchange of oxygen, substrates, and metabolites with proximal cells. Such functionality can be observed in the brain, when a localized increase in blood perfusion is provoked during neural activation to serve the demands of glycolytic metabolism and oxygen consumption via a complex process termed neurovascular coupling[1]. This interactive hemodynamic response is generally summarized as functional hyperemia, with some controversy surrounding its origin; that is, whether it is regulated through arterioles and downstream capillaries[2], [3] or directly controlled by pericytes[4], [5]. In either case, the increase in cerebral blood flow (CBF) exceeds that of oxygen consumption, meaning the tissue oxygen extraction fraction (OEF) is decreased whilst CBF increases[6]–[8]. As net oxygen consumption is proportional to the product of OEF and CBF, the reduction in OEF would work to counteract the effects of augmented CBF, suggesting that other factors may be responsible for the metabolic benefits of hyperemia. Moreover, recent report indicated that during activation brain metabolism proceeds even without the increase of CBF, confirming that the metabolism of activated neurons should be attributed to a compensation mechanism in OEF[9]. One explanation suggests that recruitment of previously quiescent capillaries[10] increases the available surface area for oxygen diffusion and consequently reduces oxygen diffusion distance[11], [12]. However, increasing microscopic evidence reveals no capillary recruitment during functional hyperemia, especially in the cerebral cortex[13]–[17]. An alternative theory is established by Jepersen and Østergaard, which describes the relationship between net oxygen extraction, i.e. the product of OEF and CBF, with the use of capillary transit time heterogeneity (CTTH)[18]. In this theory, capillary transit time homogenization, i.e. decreased CTTH, is proposed as a vital mechanism to compensate inherent OEF reduction stemming from augmented CBF and to ensure sufficient capillary oxygen extraction during episodes of hyperemia.

To validate the process of capillary transit time homogenization, a two-photon microscopy-based bolus tracking technique was adopted to quantify changes in the plasma mean transit time and the CTTH during forepaw stimulation[19]. Whilst the premise of the study was positive, the imaging was directly applied to plasma rather than red blood cells (RBC), inevitably introducing measurement flaws[20]. Furthermore, the limited numbers of capillaries that can be imaged with two-photon microscopy and the long acquisition time required to sample multiple capillary segments, hinder the simultaneous investigations of both spatial hemodynamic distributions and temporal hemodynamic fluctuations. Solving this problem requires a more powerful tool capable of imaging large ensembles of capillaries with high spatiotemporal resolution within a short period of time.

In recent years, the benefits of RBC flux and speed measurements were demonstrated using optical coherence tomography (OCT) over a large field of microcirculatory bed[21]–[25], thus giving an opportunity to characterize hemodynamic response for the first time in a more quantitative manner [23]. Li *et al.* investigated the RBC flux at the baseline and during hypercapnia[26], and characterized RBC flux homogenization as the increase of lower end values while upper limit is kept constant. Moreover, RBC flux variability was shown to be proportionate to absolute mean flux. While promising, several concerns have not been fully addressed. 1) The reported relative flux change, whereas indicating the level of variation normalized to baseline flux, could not be directly used to characterize the capillary transit performance because transit heterogeneity is quantified based on the absolute transit time/velocity/flux. 2) The temporal resolution of 667 Hz that was used in the study is not fast enough for mapping temporal fluctuations against flux with the inter-B-frame scanning protocol. 3) The contributions from temporal RBC fluctuations to the OEF, other than those owing to selective flow acceleration, is uncovered.

Here, by taking the advantages of the high temporal resolution (20 kHz) of OCT velocimetry, designed with eigen-decomposition (ED) statistical analysis[27], we investigate temporal RBC fluctuation within mouse cerebral cortex, representing them as temporal fluctuation bandwidths (TFB) of RBC flow velocities. The bandwidth before and during hindpaw electrical stimulations are analyzed to resolve alterations in capillary flow patterns. The results extend our current understanding of spatial and temporal hemodynamics through presenting: 1) during electrical stimulation, both mean capillary transit velocity (mCTV) and temporal RBC fluctuation (i.e. TFB) increase; 2) the degrees of augmentation in mCTV and TFB both negatively correlate with resting mCTV, with TFB also negatively correlating with resting TFB. Furthermore, a spatiotemporal-coupled Monte Carlo model is constructed to simulate the hemodynamic behavior and the changing processes of CTTH during neural activation, highlighting the increase of mCTV and TFB as the driving force. Our results indicate that the heterogeneous acceleration of flow speed and the heterogeneous increase of temporal fluctuation synergistically diminish the spatial CTTH; therefore, ensuring sufficient oxygenation during functional hyperemia.

II. Materials and methods

A. Phase stable OCT system and imaging protocol

To acquire the complex OCT signals that carry information about mCTV and TFB, a phase stabilized spectral domain OCT system was employed[27]. Briefly, the system comprised a super luminescent diode with a central wavelength of 1340 nm and a bandwidth of 110 nm, which provided an axial resolution of $\sim 7 \mu\text{m}$. For resolving microscale tissue features, a 10X objective lens was adopted in the sample arm that provided a lateral resolution of $7 \times 7 \mu\text{m}$, the full width at half maximum (FWHM). The angiographic image was acquired using an ultrahigh sensitive optical-microangiography (OMAG) protocol[28], which consisted of 400 A-lines along the fast axis (X) at 92,000 A-lines/sec, and 3,200 B-frames along the slow axis (Y) at 180 frames/sec with 8 repeated cross-sectional scans per location, covering a field of view of $3.5 \times 3.5 \text{ mm}$. With capillary velocimetry, for balancing the trade-off between temporal sensitivity and temporal resolution[24], [29], the acquisition speed was set

to 20,000 A-lines/sec, and 50 repeated A-lines were acquired at each of the pre-assigned 200×100 lateral positions, covering a field of view of 1.5×0.75 mm (X $\times$ Y).

B. Animal preparation and neural activation

The animal experiments outlined here were approved and supervised by the IACUC committee at the University of Washington. Please refer to [30] for the detailed procedures and protocols for animal handling and neural activation. In brief, C57BL/6 mice ($n = 9$, male, ~2 months, ~25 g) were prepared via cranial surgery under isoflurane anesthesia and investigated at resting state and during functional activation. As shown in Fig. 1(a), the activation was evoked by applying an electrical stimulus (repetition rate 3 Hz, amplitude 2 mA, duration 0.3 ms) to the left hind-paw of the mouse over a period of 30 sec.

Correspondingly, the contralateral hindpaw somatosensory cortex (HSC) was imaged through our velocimetry scanning protocol, with an additional acquisition procedure being carried out on a control region (CTRL) away from the HSC. The detection and justification of these two regions were also detailed in [30] by using laser speckle contrast analysis of tissue oxygenations. To avoid potential interference from long-term stimulations, a 20-min recovery time was assigned between data-collections at HSC and CTRL regions while the animals were kept anesthetized.

C. Optical microangiography and vessel area density

The delineation of flow signals, i.e., OMAG imaging, was realized through regression filtering to remove a number of eigenvalues (first two in this work) that represent the static components[31]. This flow signal extraction method can be applied to either the typical angiography datasets through inter-B-frame analysis or the velocimetry dataset through inter-A-line analysis. *En face* angiograms were obtained by maximum intensity projection of the morphological flow signals onto the X-Y plane. We calculated vessel area density (VAD) [32] based on *en face* angiograms derived from velocimetry datasets. In brief, VAD was calculated as the percentage of area occupied by vessels with respect to the entire scanned region (200×100 pixels)[32]. For visualization purpose, the mapping of VAD was further accomplished by pixel-wise shifting a sampling window with 25×25 pixels, calculating VAD in each window, resizing the calculated map to its original size, followed by spatial Gaussian smoothing with a kernel size of 3×3 pixels. The window size and the smoothing kernel size were empirically selected to balance the lateral sample spacing and the total sample numbers.

D. Capillary velocimetry analysis

The inter-A-line capillary velocimetry analysis was achieved by representing a collective of OCT signals as eigenvalues and eigenvectors, and evaluating the frequency of moving RBCs through first lag-one autocorrelation of corresponding eigenvector pairs[27]. Briefly speaking, the mean frequency is written by a weighted normalization:

$$\bar{\omega} = \frac{\int \omega_k G(\omega_k) d\omega_k}{\int G(\omega_k) d\omega_k}, \quad (1)$$

where ω_k is the spectral moment derived from the k^{th} eigenvector and $G(\omega_k)$ is the power of corresponding spectrum component. The ω_k can be derived by lag-one autocorrelation of the eigenvector pairs as:

$$\omega_k = \frac{FPS/2}{2\pi} \arg \left\{ \frac{1}{N_D - 1} \sum_{m=0}^{N_D-2} \bar{e}_k(m) \cdot P e_k(m+1) \right\}, \quad (2)$$

in which FPS represents the sampling frequency ($FPS = 20,000$ Hz), $\arg\{\}$ is an operation that evaluates the phase angle, N_D is the number of repeated A-scans ($N_D = 50$), $e_k(m+1)$ and $\bar{e}_k(m)$ respectively represent the k^{th} eigenvector from $(m+1)^{\text{th}}$ A-scan and the complex conjugate of k^{th} eigenvector from m^{th} A-scan. Moreover, the movement of RBCs is a time-varying signal. The bandwidth of the power spectrum that is related to the temporal heterogeneity of transit RBCs can be expressed as:

$$\Delta\omega = \sqrt{\frac{\int (\omega_k - \bar{\omega})^2 G(\omega_k) d\omega_k}{\int G(\omega_k) d\omega_k}}. \quad (3)$$

On the ground of Brier's reconciliation theory that dynamic speckle signal is equivalent to laser Doppler[33], the mCTV ($\bar{v}$) and the TFB (σ) are directly proportional to the mean frequency of the dynamic OCT signal ($\bar{\omega}$) and its power spectral bandwidth ($\Delta\omega$), respectively:

$$\bar{v} = \rho \bar{\omega}, \quad (4)$$

$$\sigma = \rho \Delta\omega, \quad (5)$$

in which the scale factor ρ is empirically selected as 0.002 mm according to a phantom experiment[27]. Variations or changes in mCTV and TFB are calculated by subtracting resting state parameters from those of the stimulated, and the relative changes are obtained by further normalizing variations against the resting parameters.

E. Spatial distribution of capillary transit time

Here we adopt the theory in previous modeling study [18] to parameterize the probability density function of the spatial capillary transit time distribution, expressed as:

$$f(\bar{t}) = \frac{1}{b^a - (a)} \bar{t}^{a-1} e^{\frac{-\bar{t}}{b}}, \quad (6)$$

where $\Gamma(a)$ represents a complete gamma function; a and b represent the shape parameter and the scale parameter, respectively. Then, the averaged capillary transit time is determined as ab , and the CTTH (i.e. the heterogeneity of transit times among multiple capillary paths) is quantified by the standard deviation $\sqrt{ab}$ [18], [19].

III. Experimental findings

A. Temporal fluctuation bandwidth increases during stimulation

OMAG imaging was first performed to visualize the 3D cerebral vascular networks within cortex for each animal at resting state. A representative vascular OMAG map is shown in Fig. 1(b) that was depth-color coded to 300 μm below the cortical surface, where the responsive region (HSC) and non-responsive control region (CTRL) to the hind-paw stimulation are highlighted by white boxes for comparative purposes. Then, the animal was subjected to the electrical stimulation protocol. TFB of the HSC and CTRL at resting state and under stimulation are respectively mapped in Figs. 1(c) and 1(d). Figs. 1(e) and 1(f) display the histogram distributions of TFBs, with relative changes displayed as hollow scatters in Fig. 1(g) and 1(h), respectively. A reduction in lower TFB counts (TFB < 0.5 mm/s) and an increase in higher TFB counts (TFB > 0.5 mm/s) are visualized in the HSC region, whereas the trend in the CTRL region remains almost constant.

Figure 1(i) summarizes relative changes in temporal bandwidth at both the specified regions. Taken together, there is an average of 6% increase in TFB at the HSC region (0.72 to 0.77 mm/s) through the electrical stimulation, whereas no increase (0.72 to 0.73 mm/s) was seen at the CTRL region. Statistical analysis (paired T -test) shows a significant difference between the HSC region and the CTRL region ($P < 0.01$). A similar significant difference ($P < 0.05$) was observed for the mCTV as displayed in Figure 1(j), in which an average increase of 7.7% (1.30 to 1.40 mm/s) was obtained at the HSC region compared with a negligible change of 1.33 to 1.35 mm/s at the CTRL region.

B. Electrical stimulation-evoked functional activation involves no capillary recruitment

Although the capillary recruitment theory[11] has been known for some time now, recent microscopic evidence reveals that the role of the capillary bed is promoted without *de novo* recruitment of previously quiescent capillaries[10]. Here, we observed no change in VAD, as presented in Fig. 2(a) and (b) for the HSC and CTRL regions, respectively. Statistical analyses corroborate these findings, as shown in Fig. 2(c) and (d), and in the paired T -test: $p = 0.15$ ($>>$ critical value of 0.05) by comparing resting and stimulated states at the HSC region; $p = 0.45$ ($>>$ critical value of 0.05) by comparing relative changes in vessel density between the HSC and CTRL regions. This observation is also supported by a study conducted by Göbel *et al* [34].

C. Changes in TFB correlate linearly with those in mCTV

Figure 3(a) shows the coordinated scattering plot of the changes in TFB with the changes in mCTV at the HSC region, in which four quadrants separately stand for the characteristics of capillary flow: I, both mCTV and TFB increase; II, mCTV decreases but TFB increases; III, both mCTV and TFB decrease; IV, mCTV increases but TFB decreases, with population

probabilities of 44%, 15%, 29% and 12%, respectively. Comparatively, a scattering distribution at the CTRL region is shown in Fig. 3(b), with quadrant distribution probabilities of 38%, 12%, 37% and 13%, respectively. One noticeable phenomenon is that the population correlation coefficients (Corr) indicate a strong linear relationship between changes in mCTV and TFB in both HSC and CTRL regions (Corr = 0.64, $P < 0.01$ for both). Additionally, in accordance with Fig. 1, the changes appear to favor the HSC region with a population in quadrant I outnumbering that of III by ~15%; in contrast, the CTRL region is more homogeneous with only a difference of ~1%. With that, continued exploration into stimulation-evoked changes in TFB and mCTV via further data mining was applied to the HSC region only.

D. Changes in mCTV negatively correlate with resting state mCTV

Figure 3(c) is a histogram distribution of mCTV changes with regard to resting state mCTV (coded in orange), as well as their joint population distributions (coded in parula colormap). Consistent with the trend noted by Li *et al.* [26], during electrical stimulation, lower mCTV values in resting state increased while upper values remained relatively unchanged; thereby forming a significant negative correlation (Corr = -0.75, $P < 0.01$) between mCTV changes and resting state mCTV. These heterogeneous velocity augmentations ultimately result in spatial capillary transit velocity homogenization, contributing to the maintenance of OEF[18]. Yet, a statistically relevant connection between mCTV changes and resting state TFB (Corr = -0.25, $P > 0.05$) can not be established, as shown in Fig. 3(d).

E. Changes in TFB negatively correlate with both the mCTV and TBF at the resting state

Similar to changes in mCTV, alternations in TFB also showed strong negative correlation with the mCTV (Corr = -0.67, $P < 0.01$) at the resting state, with the majority of bandwidth broadening occurring at a speed below the averaged mCTV, as shown in Fig. 3(e). In addition, TFB alternations also show negative correlations to the TFB at the resting state (Corr = -0.49, $P < 0.05$), as shown in Fig. 3(f).

F. TFB is proportional to mCTV at the resting state and with this trend continuing during activation

Figure 3(g) displays the histogram distributions of absolute mCTV and TFB at rest (red) and during stimulation (green). Compared with the increased average TFB (0.05 mm/s, from 0.72 to 0.77 mm/s), the average mCTV across the whole capillary bed presents a twofold elevation (0.10 mm/s, from 1.30 to 1.40 mm/s). However, relative changes in mCTV and TFB are comparable. Joint distributions during resting and stimulated states are color coded into red and green channels, respectively, with the overlap coded in yellow, in which linear fittings are adopted to denote the direct proportion between mCTV and TFB either at rest (slope = 0.58, $R^2 = 0.80$) or during stimulation (slope = 0.58, $R^2 = 0.77$). The correlating changes between mCTV and TFB (Corr 0.71, $P < 0.01$) further begs for the question: in addition to the contribution of heterogeneous flow acceleration, is heterogeneous bandwidth broadening associated with oxygen extraction during functional hyperemia?

IV. Monte Carlo simulation for spatiotemporal-coupled capillary transit parameters

To characterize the synergistic behavior of mCTV and TFB during stimulation, we applied a Monte Carlo simulation to those transit parameters as an extension to the current capillary transit time homogenization model[18]. As the hemodynamic behavior is spatially and temporally coupled in *in vivo* experiments, here the simulation would be useful to separately investigate the contributions of mCTV and TFB on the spatial distributions of capillary transit time. The simulation flow chart is listed in Fig. 4. A total sample size of 200,000 positions is selected, corresponding to 10 simulated animals with 200×100 positions in each.

Initially, we derive two (spatial and temporal) universal distributions for the capillary transit speed regardless of the resting/stimulation states. First, the spatial distribution of mean capillary transit time (mCTT or $\bar{t}$) is parameterized through the gamma probability density function in equation (6). Second, we use the inverse proportion between mCTV ($\bar{v}$) and $\bar{t}$ with a constant capillary transit path $L = 0.4$ mm[18], expressed as:

$$\bar{v} = L/\bar{t}, \quad (7)$$

to describe the spatial mCTV as an inverse gamma distribution. The obtained distribution histograms of $\bar{t}$ and $\bar{v}$ are plotted in Fig. 4(a) and (b). Third, we assume a temporal Gaussian process for the instantaneous capillary transit speed (v)[35], expressed as:

$$f(v) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(v-\bar{v})^2}{2\sigma^2}}, \quad (8)$$

where σ represents the TFB. Consequently, random events of v can be generated when σ and $\bar{v}$ are known.

Subsequently, by substituting $a = 5$ and $b = 0.1$ into equations (6) and (7), a population of resting state mCTTs ($\bar{t}_r$) and mCTVs ($\bar{v}_r$) can be simulated. Those initial parameters (a and b) are selected according to previous publications [18], [30], where most of the mCTTs are around 0.5 and the CTTHs are about 0.25. Furthermore, based on our experimental results showing that σ is proportional to $\bar{v}$, while the TFB variations (σ) and mCTV variations ($\Delta\bar{v}$) are negatively correlated to $\bar{v}_r$, a series of linear approximations are added to calculate mCTV during stimulation ($\bar{v}_s$), bandwidth at resting state (σ_r) and during stimulation (σ_s) from the simulated $\bar{v}_r$, as follows:

$$\bar{v}_s = \bar{v}_r - \alpha \cdot \bar{v}_r + \beta, \quad (9)$$

$$\sigma_r = \gamma \cdot \bar{v}_r, \quad (10)$$

$$\sigma_s = \gamma \cdot \bar{v}_s, \quad (11)$$

where $\alpha = 0.2$, $\beta = 0.3$ and $\gamma = 0.5$ are assigned empirically according to the fitting lines in Figs. 3(c) and (g). It's worth noting that the same proportion coefficient γ is utilized for both rest and stimulation, and here $\gamma < 1$ guarantees that σ_r and σ_s vary slower than $\bar{v}_r$ and $\bar{v}_s$.

By gradually changing the temporal distribution parameters from $(\bar{v}_r, \sigma_r)$ to $(\bar{v}_s, \sigma_r)$ and to $(\bar{v}_s, \sigma_s)$, we obtain the instantaneous capillary transit speed and delineate the speed distribution for the resting state (Fig. 4(d)), the distribution with consideration of heterogeneous flow acceleration (Fig. 4(e)), and the distribution that considering heterogeneous bandwidth broadening (Fig. 4(f)). By re-using the inverse relation in (equation 7), the corresponding instantaneous transit time distributions were respectively calculated and displayed in Figs. 4(g) – (i). For comparison purpose, we overlay the distributions in (g) – (i) resulting in Fig. 4(j), in which a capillary transit time homogenization is achieved mainly ascribed to coordinated alterations in mCTV (pink) and TFB (yellow).

The averaged capillary transit parameters, including those of mCTV ($\bar{v}$), TFB (σ), instantaneous transit speed (v), instantaneous transit time (t) and instantaneous spatial CTTH (δ), are listed in Table 2. First, together with the 10% mCTV increase during stimulation, the instantaneous transit speed increases 10% and the instantaneous transit time decreases ~14%. Second, stemming from broadening of the temporal bandwidth, the averaged TFB increases ~10%. Consequently, the first factor contributes a 16% reduction in CTTH and the latter factor contributes an additional 13% reduction. Therefore, the functional benefits of spatiotemporal-coupled hemodynamic changes during hyperemia are interpreted through a decoupled step-by-step capillary transit time homogenization process that ensures sufficient cerebral oxygen delivery[18].

V. Discussion and Conclusions

Previous study using ED-based velocimetry analysis has revealed the phenomenon of capillary flow homogenization (spatial velocity heterogeneity reduction) during neural activation in mouse brain, which supports the important role of microcirculatory adjustment in brain oxygenation along with functional hyperemia[30]. However, temporal fluctuation patterns in capillary vessels, which is critical to achieve the flow homogenization, was not included. In this study, we investigated cerebral capillary hemodynamics and its spatiotemporal adjustment during neural activation *in vivo* through mCTV, TFB, and variations within such. Our capillary velocimetry analyses prior to and during stimulation have demonstrated a concurrent increase in mCTV and TFB due to functional hyperemia

arising from metabolically-demanding neural activities, consistent with previously published literature[36]–[40]. Here, we have taken advantage of the high spatial and temporal resolution of OCT velocimetry ($\sim 50 \mu\text{s}$), much faster than previously reported (milliseconds range) [26], [36], [41]. Consequently, one can expect more accurate velocity measurements in this study because of the higher upper limits for measurable velocities, according to the theoretical analysis discussed by Choi *et al*[42]. Additionally, total dwelling time at each spatial location was just 2.5 ms, which is short enough to exclude any possible motion artifacts due to respiration (261–750 ms) and/or heartbeat (71–194 ms)²¹.

Further correlation analyses revealed that temporal fluctuations were proportional to transit velocity during both resting and stimulated states, with growth rates for both velocity and fluctuation bandwidth negatively correlating with resting state mean transit velocity. On this foundation, linear approximations of resting TFB, stimulated TFB, and stimulated mCTV with regard to resting mCTV were used to model spatiotemporal-coupled transit parameters through a Monte Carlo simulation[43]. The linear approximations were made on solid experimental bases and the simulated results correlated well with experimental data. Although promising, the exact relationship between said parameters has not yet been fully explored. For instance, the saturation effect of high mCTV flow during activation may introduce nonlinear dependence[26], and the fact that cerebral functional connectivity regulates regional capillary blood flow in the brain[44]–[46] may add complexity with possible uncertainties. These correlations should be rigorously investigated in future studies.

It is noted that certain limitations do exist in the current experimental setup. First, due to system sensitivity fall off along the imaging depth and Rayleigh optical focusing, the OCT signal and subsequent velocimetry signal diminished along the imaging depth, degrading the lateral resolution for those capillaries that are out of the focus. This consequently limited our research scope to the superficial cortex ($\sim 300 \mu\text{m}$, corresponding to layers I – III). Second, inhalational anesthetic (e.g. isoflurane) is known to affect neuronal activity, cerebral metabolic rate and capillary blood flow speed in a dose-dependent manner[47]. Whilst the physiological parameters of all the animals were carefully monitored throughout this study, the potential effects of isoflurane may still be a confounding factor. The above-mentioned limitations might slightly bias quantification of the absolute transit parameters but are considered insignificant with regards to the relative comparisons between rest and stimulation.

In conclusion, we have innovatively employed ED-based OCT velocimetry with high spatiotemporal resolution to investigate the characteristics of capillary hemodynamics with an emphasis on temporal RBC fluctuations. A concurrent increase in mCTV and TFB has been observed during a series of electrical stimulations of the mouse somatosensory cortex. Additionally, the absolute values of mCTV and TFB were found in direct proportion with a scaling factor independent of stimulation state. Furthermore, we have observed that the changes in mCTV and TFB are both negatively correlated with resting state mCTV, with the latter too being negatively dependent on resting state TFB. Finally, we have introduced a spatiotemporal-coupled Monte Carlo model to differentiate the contributions of mCTV and TFB to the spatiotemporally-coupled hemodynamics, which provides a means to analytically investigate the biophysical implications of capillary hemodynamics, allowing for the

modeling of spatiotemporal-coupled hemodynamics, and for an understanding of the metabolic benefits of functional hyperemia. The experiments and simulations demonstrated in this work are expected to provide a more thorough and quantitative representation of capillary transit characteristics, which can be potentially used to investigate the hemodynamic responses in cerebral pathological conditions such as stroke and Alzheimer's diseases.

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Fig. 1.

Temporal fluctuation bandwidth (TFB) of the capillary blood flow within activated cortical region increases during electrical stimulation. (a) Schematic diagram of imaging during electrical stimulation. (b) A representative 3.5×3.5 mm *en face* vascular image, showing a responsive somatosensory cortex region (HSC) and a non-responsive control region (CTRL), in white boxes respectively. The image is generated by projecting the vessels onto x-y plane from 0 mm to 300 μ m in depth from the cortical surface. (c) and (d), the velocimetry maps of TFB displaying the temporal heterogeneities before (rest) and during (stimulation) neural activation in HSC and CTRL, respectively. (e) and (f), The histogram distributions of TFB from selected regions, with additional blue scatters and polynomial fitted curves indicating shifts in bandwidth distributions after stimulation as in (g) and (h), respectively. (i) Relative changes in TFB at the HSC and CTRL regions of each animal. The statistical difference reaches a significance level of $P < 0.01$. (j) Relative changes in mCTV at the HSC and CTRL regions of each animal. The statistical difference reaches a significance level of $P < 0.05$. In (i) and (j) each dot represents an individual animal, and the horizontal bar represents the mean with standard error. All scale bars represent 500 μ m. a.u.: arbitrary unit.

Fig. 2.

Electrical stimulation does not involve the capillary recruitment. (a) and (b) Representative vessel density maps, corresponding to Fig. 1 (c) and (d), showing no visible capillary recruitment in either HSC or CTRL regions. (c) Statistical analysis of vessel density comparing resting and stimulated states in the HSC regions of each animal ($p = 0.15$). (d) Statistical analysis of relative changes in vessel density for inter-region comparisons between the HSC and CTRL regions of each animal ($p = 0.45$). In (a) and (b), the colorbar represents the quantified vessel area densities. In (c) and (d), each dot represents an individual animal, and the horizontal bar represents the mean with standard error. All scale bars represent $500 \mu\text{m}$. a.u.: arbitrary unit.

Fig. 3.

Statistical analysis of correlations between temporal fluctuation bandwidth, capillary transit velocity, and the changes of such during stimulation. (a) and (b) The distributions of TFB change along with mCTV change in the HSC and CTRL regions, respectively, in which four quadrants (I, II, III, IV) denote the distribution probabilities within the region. The population correlation coefficient ($\text{Corr} 0.64$) indicates a strong linear relationship between samples with a T -test probability of $P < 0.01$. Velocimetry data from the HSC region are further mined and presented in (c) – (g). In (c) – (f), histogram distributions of mCTV changes, TFB changes, resting state mCTV, and resting state TFB are respectively plotted in orange. (c) The joint distribution of mCTV changes against resting state mCTV showing a negative correlation ($\text{Corr} = -0.75, P < 0.01$). (d) The joint distribution of mCTV changes against resting state TFB showing no significant correlation ($\text{Corr} = -0.25, P > 0.05$). (e) The joint distribution of TFB changes against resting state mCTV showing a negative correlation ($\text{Corr} = -0.67, P < 0.01$). (f) The joint distribution of TFB changes against resting state TFB showing a negative correlation ($\text{Corr} = -0.49, P < 0.05$). (g) The histogram distributions of TFB at rest (red) and during stimulation (green) against those of mCTV. The

joint distributions for rest and stimulation are color coded into red and green channels, respectively, with the yellow color indicating overlap. The linear fittings of data before and after stimulation marked by red/green perforated lines denote that TFB is proportional to mCTV for any state. R^2 represents coefficient of determination. Each scatter in (a) and (b) represents a spatial capillary sample. Colorbar in (c) – (g) represents the counts of capillary samples.

Fig. 4.

Monte Carlo simulation reveals the contributions of capillary transit velocity and temporal fluctuation bandwidth to capillary transit time homogenization during stimulation.

TABLE I

List of acronyms (alphabetic order)

Acronyms	PARAMETERS
CBF	cerebral blood flow
Corr	correlation coefficients
CTRL	control region
CTTH(δ)	capillary transit time heterogeneity
ED	eigen-decomposition
FWHM	full width at half maximum
HSC	hind-paw somatosensory cortex region
mCTT($\bar{t}$)	mean capillary transit time
mCTV($\bar{v}$)	mean capillary transit velocity
OCT	optical coherence tomography
OEF	oxygen extraction fraction
OMAG	optical-microangiography
RBC	red blood cell
TFB(σ)	temporal fluctuation bandwidth
VAD	vessel area density

TABLE II

The averaged capillary transit parameters in the Monte Carlo simulation

Capillary transit parameters	Rest	Velocity heterogeneity	Bandwidth heterogeneity
$\bar{v}$ (mm/s)	0.9987	1.0990	1.0990
σ (mm/s)	0.4994	0.4994	0.5495
v (mm/s)	0.9981	1.0986	1.1019
t (s)	0.5572	0.4809	0.4789
δ (s)	0.3401	0.2848	0.2488