NeuroImage 71 (2013) 248–259

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Cerebral microvascular network geometry changes in response to functional stimulation

Liis Lindvere [a,c], Rafal Janik [a,c], Adrienne Dorr [a], David Chartash [a], Bhupinder Sahota [a], John G. Sled [b,c], Bojana Stefanovic [a,c,*]

[a] Imaging Research, Sunnybrook Research Institute, 2075 Bayview Avenue, Toronto, ON, Canada M4N 3M5
[b] Mouse Imaging Centre, The Hospital for Sick Children, 25 University Orde Street, Toronto, ON, Canada M5T 3H7
[c] Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Accepted 8 January 2013
Available online 25 January 2013

Keywords:
Functional hyperemia
Cerebral microvasculature
Two photon fluorescence microscopy
Cerebral blood volume

ABSTRACT

The cortical microvessels are organized in an intricate, hierarchical, three-dimensional network. Superimposed on this anatomical complexity is the highly complicated signaling that drives the focal blood flow adjustments following a rise in the activity of surrounding neurons. The microvascular response to neuronal activation remains incompletely understood. We developed a custom two photon fluorescence microscopy acquisition and analysis to obtain 3D maps of neuronal activation-induced changes in the geometry of the microvascular network of the primary somatosensory cortex of anesthetized rats. An automated, model-based tracking algorithm was employed to reconstruct the 3D microvascular topology and represent it as a graph. The changes in the geometry of this network were then tracked, over time, in the course of electrical stimulation of the contralateral forepaw. Both dilatory and constrictory responses were observed across the network. Early dilatory and late constrictory responses propagated from deeper to more superficial cortical layers while the response of the vertices that showed initial constriction followed by later dilation spread from cortical surface toward increasing cortical depths. Overall, larger caliber adjustments were observed deeper inside the cortex. This work yields the first characterization of the spatiotemporal pattern of geometric changes on the level of the cortical microvascular network as a whole and provides the basis for bottom–up modeling of the hemodynamically-weighted neuroimaging signals.

Introduction

The majority of human neuroimaging techniques rely on functional hyperemia, or the rise in local cerebral blood flow following an increase in the activity of surrounding neurons. It is generally accepted that neurovascular coupling is a feedforward process: synaptic activity leads to the release of vasoactive substances, either by neurons themselves or by astrocytes, that act on the neighboring vessels causing them to dilate (Attwell et al., 2010). Although functional hyperemia is a remarkably robust phenomenon, widely exploited for human neuroimaging, and one of the major functions of the neurovascular unit that has been under intense scrutiny for decades, much uncertainty still surrounds the underlying mechanisms. Specifically, the role of the microvessels in the neurovascular coupling and the preponderance and pattern of constrictory responses remain disputed.

In a given cortical layer, maximum vessel dilation in response to a stimulus occurs at the site of greatest neuronal activity and decreases as a function of distance from the center (Blinder et al., 2010; Devor et al., 2007). In addition, the cortical hemodynamic response is not only graded in amplitude laterally but also retarded in time axially (across cortical depth) so that the dilation of penetrating arterioles in the intermediate layers (IV–V) of the somatosensory cortex precedes that in the top and bottom cortical layers (I–III and VI) (Silva and Koretsky, 2002; Tian et al., 2010). There is a large body of evidence that identifies the penetrating arterioles as the vessels driving the hemodynamic response (Devor et al., 2007; Iadecola and Nedergaard, 2007) whereby the arteriolar dilation results in increased pressure in the downstream vessels (Boas et al., 2008). Notwithstanding, the passive dilation and constriction of capillaries in response to upstream pressure changes has recently been challenged by work showing that pericytes, smooth muscle cell like cells impinging on capillaries, may allow active control of capillary diameters (Chen et al., 2011; Fernández-Klett et al., 2010; Peppiatt et al., 2006). Furthermore, mesoscopic scale evidence suggests that the hemodynamic response is initiated in the parenchyma (Kida et al., 2007; Kim and Kim, 2010; Lu et al., 2004; Mandeville and Marota, 1999; Raaij et al., 2011; Silva and Koretsky, 2002; Smirnakis et al., 2007) and not the major vessels. Stimulation induced capillary dilations on the order of 10% have been reported (Chaigneau et al., 2007; Chen et al., 2011; Devor et al., 2007; Kleinfeld et al., 1998; Stefanovic et al., 2008; Tian et al., 2010) though

* Corresponding author at: Sunnybrook Research Institute, 2075 Bayview Avenue S650, Toronto, ON, Canada M4N 3M5.
E-mail address: bojana@sri.utoronto.ca (B. Stefanovic).

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.01.011

not always (Drew et al., 2011; Fernández-Klett et al., 2010). Less frequent observations of stimulation induced microvessel constriction have been hypothesized to result from re-distribution of blood supply from dormant to active areas (Blinder et al., 2010; Kleinfeld et al., 1998). Overall, the importance of capillary volume adjustments has been controversial (Fernández-Klett et al., 2010; Krieger et al., 2012). Arteriolar constriction has also been reported during the post stimulus period (Chen et al., 2011; Devor et al., 2007; Tian et al., 2010). Finally, most (Chen et al., 2011; Kennerley et al., 2010), but not all (Drew et al., 2011) microscopic studies report no change in venous diameter in response to stimulation: in contrast to typical assumptions made in models of the hemodynamic response to functional stimulation (Buxton et al., 1998; Mandeville et al., 1999) that predicted significant dilation of veins. In addition to these discrepancies, there is a lack of data on the intermediate-sized intracortical vessels and the microvascular network as a whole (Harel et al., 2010). These data are key for understanding the vascular origins of BOLD (blood oxygenation level dependent) fMRI, the most commonly used neuroimaging modality, for estimation of its specificity, and for its quantitative interpretation (Harel et al., 2010).

In the present work, we set out to address these gaps by characterizing the spatial and temporal evolution of the response of the 3D microvascular *network*, rather than individual vessels, in vivo under physiological conditions. The microvascular topology of the forelimb representation in the primary somatosensory cortex was finely sampled under baseline conditions. Thereafter, the changes in its geometry were tracked during presentations of ultra short (single 0.3-ms pulse) and 2.4-s long (7 0.3-ms pulses at 3 Hz) electrical stimulation of the contralateral forepaw. To allow sufficient temporal resolution to track the hemodynamic response of the entire microvascular network, the acquisition protocol was designed to acquire interleaved slice over time series at progressively larger cortical depths, affording a sub-second effective sampling rate of the imaged tissue volume. Automated vessel tracking analysis was employed to represent the microvascular network as a graph and generalized linear modeling used to estimate the changes in the local vessel diameter, at ~1 μm spaced out vertices, over the course of stimulus presentation. Finally, nonlinear modeling was employed to estimate the latencies of the vascular vertexwise caliber changes in response to functional stimulation.

Methods

Animal preparation

Surgical procedures are described in detail in Lindvere et al., 2010. Briefly, adult male Sprague-Dawley rats ($n = 14$, 132 ± 26 g) were anesthetized with isoflurane, tracheotomized and mechanically ventilated. To enable 2PFM imaging of the brain, stereotaxic surgery was done to prepare a small (~5 mm in diameter) closed (1% agarose) cranial window, over the forelimb representation in the primary somatosensory cortex (S1FL). Imaging was performed under alpha-chloralose (80 mg/kg induction, 27 mg/kg/h maintenance dose) anesthetic following intravenous administration of Texas Red dextran (70 kDa, Invitrogen; 50-μL injection, 5 mg/kg b.w.) during rest and electrical stimulation of the contralateral forepaw. The excitation wavelength was 810 nm and the emission filter bandwidth spanned 570 to 620 nm. Arterial blood was sampled periodically and arterial blood gas values maintained in the normal range throughout the experiments. Across all animals, the mean (±standard deviation) blood gas values were: 7.36 ± 0.01 (pH), 40.4 ± 3.1 mm Hg (pCO_2), and 105.1 ± 5.2 mm Hg (pO_2). Rectal temperature, arterial blood pressure, end tidal respiratory pressure, heart rate, and oxygen saturation were monitored and recorded (Biopac Systems Inc.) throughout the experiment. The physiological monitoring data at the beginning/end of the experiment were: $37.6 \pm 0.1/37.5 \pm 0.1$ °C (temperature), $69 \pm 17/66 \pm 11$ mm Hg (blood pressure), $447 \pm 47/469 \pm 56$ bpm (heart rate), and $98.4 \pm 0.6/98.3 \pm 0.6\%$ (oxygen saturation). Intracranial pressure (ICP) was measured in a subset of animals ($n = 3$) to confirm the return of ICP to physiological level (3.7–4.4 mm Hg) following cranial window closure.

Image acquisition

All imaging was performed using the 25×, 1.05 NA, 2.0-mm working distance water immersion objective (Olympus):

i. Fine spatial resolution stacks (FOV: 508 μm × 508 μm × 600 μm; lateral resolution: 0.994 μm/pix, axial resolution: 3 μm/pix) were acquired for reconstruction of vascular network architecture.
ii. Functional data were acquired at coarser spatial resolution (FOV: 508 μm × 508 μm × 600 μm or 300 μm; lateral resolution: 1.59 μm/pix, axial resolution: 3 μm/pix) to enable improved temporal resolution (0.786 s/frame).

Three stimulation paradigms were presented, each employing electrical stimulation of the contralateral forepaw (0.3 ms, 2 mA pulses, played out at 3 Hz) triggered by Time Controller (Olympus, Japan). In the pilot experiments (200 slices spanning 0–600 μm of the cortex), the functional paradigm comprised 15-frame time series, with 2 OFF, 3 ON (train of 7 pulses), and 10 OFF frames, with each frame taking 0.786 s per lateral image. The next paradigm (100 slices spanning 100–400 μm) encompassed two repetitions of a 21-frame cycle, with 2 OFF/3 ON (train of 7 pulses)/16 OFF frames. In the final paradigm (100 slices spanning 100–400 μm), 40 frames were acquired, with a single 0.3 ms 2 mA electrical pulse presented at the beginning of frames 3, 11,19, 27, and 35. Image acquisition in each paradigm was leaved such that 2D frames at each cortical depth were acquired with temporal resolution of 0.786 s/frame. After the most superficial slice time series was acquired, the imaging depth was increased by 6 μm and the functional paradigm repeated. This was first done for z levels corresponding to even cortical depths, followed by z levels corresponding to odd cortical depths. This acquisition paradigm is illustrated in Fig. 1. The experimental paradigm hence comprised of the two functional acquisitions followed by the fine resolution anatomical acquisition. The functional data sets took about 100 min to complete, bringing the overall acquisition time to about 2.5 h.

Data analysis

The microvascular network was represented as a graph, specified by a set of vertices and their connection. Vertices were characterized by their location (x, y, z), radius (r), and tangent vector (θ), at each time instance. The mean distance between neighboring vertices was 0.78 ± 0.13 μm, so that a few dozen vertices made up a vessel, defined as the segment between two branch points. The processing was designed to allow the estimation of intraluminal radius at each vertex of the microvascular network at each time frame, which established vertex correspondence across time. The anatomical data were segmented using semi-automated analysis via commercially available software (Imaris, Bitplane, Zurich). Prior to segmentation, the data were subjected to edge-preserving 3D anisotropic diffusion filtering (Perona and Malik, 1990). Thereafter, the intravascular space was identified based on a range of user supplied signal intensity thresholds corresponding to the background and foreground signal intensity ranges. The labor intensive semi-automated segmentation was followed by removal of hair-like terminal branches. The resulting volumes were next skeletonized, with the network sampled roughly every 1 μm, and the aforementioned graph data structure produced. The local tangents to the vessel were evaluated at each vertex following spline interpolation to the vertices' locations.

As a part of pre-processing (Fig. 2), the coarse resolution functional slices were filtered with a 2D Gaussian kernel (σ_x ~ 1.2 μm, σ_y ~ 1.2 μm), normalized with respect to median signal intensity through the cortical depth (inormalize, MINC suite), registered to anatomical data using full affine transformation based on a set of manually identified landmarks,