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Quantitative separation of arterial and venous cerebral blood volume increases during voluntary locomotion

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ABSTRACT

Voluntary locomotion is accompanied by large increases in cortical activity and localized increases in cerebral blood volume (CBV). We sought to quantitatively determine the spatial and temporal dynamics of voluntary locomotion-evoked cerebral hemodynamic changes. We measured single vessel dilations using two-photon microscopy and cortex-wide changes in CBV-related signal using intrinsic optical signal (IOS) imaging in head-fixed mice freely locomoting on a spherical treadmill. During bouts of locomotion, arteries dilated rapidly, while veins distended slightly and recovered slowly. The dynamics of diameter changes of both vessel types could be captured using a simple linear convolution model. Using these single vessel measurements, we developed a novel analysis approach to separate out spatially and temporally distinct arterial and venous components of the location-specific hemodynamic response functions (HRF) for IOS. The HRF of each pixel of was well fit by a sum of a fast arterial and a slow venous component. The HRFs of pixels in the limb representations of somatosensory cortex had a large arterial contribution, while in the frontal cortex the arterial contribution to the HRF was negligible. The venous contribution was much less localized, and was substantial in the frontal cortex. The spatial pattern and amplitude of these HRFs in response to locomotion in the cortex were robust across imaging sessions. Separating the more localized arterial component from the diffuse venous signals will be useful for dealing with the dynamic signals generated by naturalistic stimuli.

Introduction

Changes in cerebral blood volume, flow, and oxygenation are widely used to infer neural activity (Logothetis, 2008). Because increases in neural activity, which can last for only a few milliseconds, are usually much briefer than the hemodynamic response, which evolves over seconds, it is necessary to use a quantitative model to relate the two. The hemodynamic signal is usually assumed to be a convolution of neural activity or sensory stimulus with the hemodynamic response function (HRF, also known as a kernel) (Boynton et al., 1996; Friston et al., 1994; Glover, 1999; Vazquez and Noll, 1998; Hirano et al., 2011; Logothetis et al., 2001; Martindale et al., 2003). Typically, the HRF is fit with a gamma distribution function (Boynton et al., 1996; Hirano et al., 2011), but there is evidence that the CBV HRF may be the sum of multiple components (Silva et al., 2007). Because HRFs differ across cortical location (Handwerker et al., 2004) and layer (Hirano et al., 2011), potentially due to neural and vascular differences (Tsai et al., 2009), it is useful to mechanistically understand their vascular origin.

The CBV HRF will be determined by the dynamics of volume changes in various vascular compartments. Arterial dilation, which is mediated by smooth muscle relaxation and is thought to be under the control of neural activity, follows stimulation within a second (Hillman et al., 2007; Kim et al., 2007; Drew et al., 2011; Tian et al., 2010). The distention of veins takes place over tens of seconds in response to prolonged stimuli (Drew et al., 2011; Kim and Kim, 2010a, 2010b), and is thought to reflect the passive mechanical properties of the vessel wall (Clark, 1933; Edvinsson et al., 1983). Theoretical models incorporating the fast arterial and slow venous components reproduce the observed cerebral hemodynamic response well (Barrett et al., 2012; Kim et al., 2013). However, there is spatial variation in HRFs across the brain (Aquino et al., 2014; Bießmann et al., 2012; Handwerker et al., 2004), which may underlie the diverse temporal profiles of hemodynamic responses across the entire cortex (Gonzalez-Castillo et al., 2012; Vickery et al., 2011).

The arterial and venous CBV changes induced by long sensory stimulation have different spatial extents (Kim and Kim, 2010a, 2010b), with the arterial component being more spatially restricted. This

Abbreviations: CBV, cerebral blood volume; CBF, cerebral blood flow; HRF, hemodynamic response function; IOS, intrinsic optical signal; 2PLSM, two-photon laser scanning microscopy; LDF, laser Doppler flowmetry; LTI, linear, time-invariant; MSE, mean-squared error; cc, correlation coefficient; ROI, region of interest; FOV, field of view; FL, forelimb; HL, hindlimb.

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suggests that the spatial variation (Handwerker et al., 2004) in HRFs might be due to differences in arterial and venous contributions to the HRF. Since the spatial pattern of the arterial response is thought to be more closely related to the spatial pattern of neural activity (Moon et al., 2013), brief, temporally isolated stimuli will give a hemodynamic response that more precisely reflects the underlying neural activity. However, using impulse-like stimuli is not always possible, particularly when using 'naturalistic' stimuli, which have multiple time-scales (Kay et al., 2008; Ben-Yakov et al., 2012; Honey et al., 2012; Naselaris et al., 2011). A method of separating the more localized arterial response from the less specific venous response to temporally extended stimuli would be very helpful for these stimulation paradigms.

Here, we investigated the vascular mechanisms underlying the hemodynamic response function in the superficial layers of the cortex in response to exercise. Previous work on the existence, localization and even direction of cerebral blood flow and volume changes during exercise are contentious (reviewed in (Ide and Secher, 2000)), with some studies showing no changes (Globus et al., 1983), some showing global, non-specific increases (Herholz et al., 1987), and other showing localized increases (Jørgensen et al., 1992; Linkis et al., 1995). One explanation for the discrepancies among these studies, which use different methodologies and durations of exercise, is that different techniques have different sensitivity to arterial versus venous changes. We have previously shown, using intrinsic optical signal (IOS) imaging in head-fixed, voluntarily locomoting mice, that in the first few seconds of locomotion, the frontal cortex shows little to no change in blood volume, while there are substantial increases in blood volume in the limb representations of sensory cortex (Huo et al., 2014). Because arteries and veins have very different temporal (Kim and Kim, 2010a, 2010b; Drew et al., 2011; Gao and Drew, 2014) and spatial (Moon et al., 2013) dynamics, we wanted to determine if the discrepancies in the spatial extent of the hemodynamic response to exercise could be due to different spatial extents of arterial and venous changes.

Using two-photon laser scanning microscopy (2PLSM) (Drew et al., 2011; Shih et al., 2012a, 2012b), we measured individual vessel dilation dynamics during voluntary locomotion in awake, head-fixed mice (Dombeck et al., 2007; Nimmerjahn et al., 2009; Huo et al., 2014). Based on these single vessel measurements, we developed a HRF model to quantify the spatiotemporal characteristics of optically measured pixel-wise CBV changes in the superficial layers. We demonstrated that using the temporal dynamics of the blood volume signal, distinct spatial maps of the localized arterial and diffuse venous responses could be extracted. The arterial and venous responses to locomotion were linear, and repeatable across trials. Using our linear convolution model to separate the more spatially localized arterial changes from the less localized venous responses should prove useful for experiments using dynamic stimuli (Hasson et al., 2010).

A subset of the IOS data presented here has been previously presented (Huo et al., 2014), and is reanalyzed below.

Methods

Animals

All care and experimental manipulation of animals were done in accordance with the Institutional Animal Care and Use Committee of Pennsylvania State University, University Park. A total of 18 male C57BL/6J mice (Jackson Laboratory) were used. Mice were maintained on a 12-hour light/dark cycle in isolated cages.

Surgery

Mice were 2–10 months old (25–40 g) at the time of surgery. All surgical procedures were performed under isoflurane anesthesia. For 2PLSM imaging, polished and reinforced thinned-skull (PoRTS) windows were implanted (Drew et al., 2010; Shih et al., 2012a, 2012b; Gao and Drew, 2014) in the right parietal cortex ($N = 10$). Under anesthesia, a custom-machined titanium head-bolt was attached to the skull and 3 self-tapping, 3/32′ #000 (J.I. Morris) screws were implanted into the skull and connected to the head-bolt with dental cement. Using a hand drill (Foredome), the skull over the right parietal cortex was carefully thinned to ~30 μm with a #7 bit (Fine Science Tools). It was then polished with 3f and 4f lapidary polish (Convington Engineering, Redlands, CA, USA), and a #0 coverslip was attached to the skull with cyanoacrylate glue (Vibra-Tite, 32002). A meniscus-holding well was made with dental cement around the window. For IOS imaging, larger reinforced thinned-skull windows (Huo et al., 2014) that spanned frontal and parietal cortices were made either bilaterally ($N = 6$) or unilaterally ($N = 2$). For animals used for IOS imaging, the skull over frontal and parietal cortices in each hemisphere was thinned to remove skull vessels using a Foredom drill with the frontal–parietal suture sealed with cyanoacrylate glue and dental cement. Then the frontal–parietal suture was exposed and carefully thinned until the suture was flush with the frontal and parietal bones. Successful suture thinning was identified by absence of dural or pial vasculature remodeling after surgery. A #1 coverslip cut to the window size in each hemisphere was mounted using cyanoacrylate glue (Vibra-Tite, 32002). A custom machined titanium head-bolt was glued to the skull, centered on the midline suture, posterior to lambda. One self-tapping, 3/32″ #000 (J.I. Morris) screw was implanted into the skull over the olfactory bulb and connected to the head-bolt via midline suture using cyanoacrylate glue and black dental acrylic resin (Lang Dental Mfg. Co., REF 1530) to minimize skull movement, and to absorb reflected light. Keeping the skull intact is important for accurate measures of hemodynamic signals, as craniotomies are known to cause inflammation (Cole et al., 2011; Xu et al., 2007), changes in brain tissue mechanical properties (Hatashita and Hoff, 1987), and angiogenesis (Arieli et al., 2002; Drew et al., 2010; Sohler et al., 1941), all of which are likely to disrupt the normal hemodynamic response.

Experiments

The treadmill (60 mm diameter) had one degree of freedom, and was covered with nonabrasive antislip tape. An optical rotary encoder (US Digital, E7PD-720-118) (Nimmerjahn et al., 2009) was attached to the treadmill axel to quantify the mouse's velocity. Mice were habituated to being head-fixed on the spherical treadmill over 3–5 days. All experiments were performed within 8 months of surgery in sound attenuating boxes.

Before each 2PLSM imaging session, the mouse was briefly anesthetized with isoflurane and infraorbitally injected with 50 μL 5% (weight/volume) fluorescein conjugated dextran (70 kDa; Sigma-Aldrich). The two-photon microscope consists of a Sutter Movable Objective Microscope and a MaiTai HP laser, controlled by MPScan software (Nguyen et al., 2006). Images were acquired at 6–9 Hz for ~20 min/trial using water dipping objectives (Olympus, 10× 0.3 N.A. 20× 0.5 N.A., or 20× 1.0 N.A.). For imaging of pial vessels, power exiting the objective was typically 15–20 mW at 800 nm. We selected a rectangular field of view containing both arteries and veins (Fig. 2A). A total of 151 arteries and 104 veins in 10 mice were imaged.

IOS and laser Doppler flowmetry (LDF) data were collected using custom-written software in LabView 8.6 (National Instruments). For IOS imaging, four 530 nm LEDs (Thorlabs, M530L2-C1) (Bouchard et al., 2009) passed through a ±10 nm filter (Thorlabs, FB530-10) were used to uniformly illuminate the cortical surface. A CCD camera (Dalsa, Pantera 1 M60) was used to acquire 12-bit images (Drew and Feldman, 2009). Each 256 × 256 pixel image had a resolution of 27–37 μm/pixel. A second camera (Microsoft, LifeCam Cinema) was used to observe the mouse's behavior. After the animal was habituated, we collected IOS data at a frame rate of 3 Hz (~33 min/trial). In order to measure the power spectrum of the intrinsic signal (Fig. 1), some trials were collected at 30 Hz, but these were not used for fitting of the