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Quantification of blood flow and volume in arterioles and venules of the rat cerebral cortex using functional micro-ultrasound ☆

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ABSTRACT

Relative cerebral blood volume (rCBV), relative cerebral blood flow (rCBF), and blood flow speed are key parameters that characterize cerebral hemodynamics. We used contrast-enhanced functional micro-ultrasound (fMUS) imaging employing a disruption–replenishment imaging sequence to quantify these hemodynamic parameters in the anesthetized rat brain. The method has a spatial resolution of about 100 μm in-plane and around 600 μm through-plane, which is comparable to fMRI, and it has a superior temporal resolution of 40 ms per frame. We found no significant difference in rCBV of cortical and subcortical gray matter (0.89 ± 0.08 and 0.61 ± 0.09 times the brain-average value, respectively). The rCBV was significantly higher in the vascular regions on the pial surface (3.89 ± 0.71) and in the area of major vessels in the subcortical gray matter (2.02 ± 0.31). Parametric images of rCBV, rCBF, and blood flow speed demonstrate spatial heterogeneity of these parameters on the 100 μm scale. Segmentation of the cortex in arteriolar and venular-dominated regions identified through color Doppler imaging showed that rCBV is higher and flow speed is lower in venules than in arterioles. Finally, we show that the dependence of rCBV on rCBF was significantly different in cortical versus subcortical gray matter: the exponent α in the power law relation $\mathrm{rCBV} = s \cdot \mathrm{rCBF}^{\alpha}$ was 0.37 ± 0.13 in cortical and 0.75 ± 0.16 in subcortical gray matter. This work demonstrates that functional micro-ultrasound imaging affords quantification of hemodynamic parameters in the anesthetized rodent brain. This modality is a promising tool for neuroscientists studying these parameters in rodent models of diseases with a cerebrovascular component, such as stroke, neurodegeneration, and venous collagenosis. It is of particular import for studying conditions that selectively affect arteriolar versus venular compartments.

Introduction

The brain depends on an adequate supply of oxygen and glucose by the circulation. Changes in neuronal activity induce changes in the local blood supply, a phenomenon known as neurovascular coupling (Attwell et al., 2010). Neurovascular coupling is compromised in many neurological and neurodegenerative disorders (D'Esposito et al., 2003; Iadecola, 2004). Most imaging methods for studying of brain function rely on neurovascular coupling: hemodynamic parameters such as cerebral blood volume (CBV, in mL of blood per 100 g of tissue) and cerebral blood flow (CBF, in mL of blood per 100 g of tissue per minute) are used to infer the location(s) of neuronal activation, or identify regions with an abnormal blood supply. While the mechanisms of neurovascular coupling are being actively studied (Iadecola and Nedergaard, 2007), there is an active research in the development and optimization of methodologies to study regional differences in neurovascular coupling across the whole brain (Sloan et al., 2010). Current techniques for in vivo imaging of brain blood flow include MRI, CT, PET, and optical imaging such as laser speckle contrast imaging and two photon fluorescence microscopy. Despite their respective strengths (Calamante et al., 1999; Coles, 2006; Devor et al., 2012), these methods are challenged to provide concomitant imaging of blood flow and volume at high temporal resolution while still allowing whole brain coverage.

Functional micro-ultrasound imaging (fMUS, (van Raaij et al., 2011)) is a recent adaptation of high-frequency ultrasound imaging (Foster et al., 2002) that allows quantitative measurement of changes in CBV and CBF. The contrast mechanism used to measure hemodynamic parameters may be either power Doppler imaging of moving red blood cells (Mace et al., 2011) or nonlinear imaging of microbubble contrast agents (van Raaij et al., 2011). The term 'functional' is used here in the wider sense of measuring hemodynamic parameters of a steady-state situation, though it can also be used in

Abbreviations: fMUS, functional micro-ultrasound imaging; rCBV, relative cerebral blood volume; rCBF, relative cerebral blood flow.

☆ Disclosure: F. Stuart Foster is a consultant to VisualSonics Inc., Toronto, Canada.

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a narrower definition as referring to a hemodynamic response to a stimulus.

An important strength of high frequency ultrasound imaging is the availability of multiple contrast mechanisms. In addition to standard backscattered ultrasound images (B-mode), there are Doppler modes which measure the presence and speed of moving particles (e.g., red blood cells) in the organ of interest. The development of ultrasound contrast agents (Becher and Burns, 2000; Ferrara et al., 2007) led to another highly sensitive vascular contrast mechanism. The microbubbles are gas-filled lipid-shell particles with a diameter of 1 to 10 μm which are strictly intravascular, biologically safe (Mulvagh et al., 2008), and detectable using nonlinear imaging techniques that are very sensitive to the microbubbles while being minimally affected by signal from tissue (Powers et al., 2009).

The present work quantifies changes in CBF and CBV using microbubbles via the so-called disruption–replenishment (also: destruction–reperfusion) technique. Most of the microbubbles in the imaging plane are disrupted by a high-intensity burst of ultrasound. After the burst pulse, microbubbles from surrounding vessels gradually replenish the imaging plane. The kinetics of this replenishment process confers information on hemodynamic parameters. (The perfluorocarbon gas that is released upon disruption as shell-less bubbles circulates briefly and freely through the vasculature, eventually dissolves in the plasma, and is released by the lungs.) We use a commercially available micro-ultrasound system to image these parameters with 100 μm spatial and 40 ms temporal resolution (van Raaij et al., 2011). Additionally, we investigate hemodynamic differences in cortical arterioles and venules using color Doppler imaging, which detects whether flow is towards or away from the ultrasound transducer. We thus establish the use of disruption–replenishment contrast-enhanced high-frequency ultrasound imaging and color Doppler imaging in the quantification and characterization of the spatial heterogeneity of hemodynamic parameters in the rat brain.

Methods

Animal preparation

Male Sprague–Dawley rats (Charles River Laboratories Inc., Saint-Constant, Quebec, Canada) ($N = 13$) were anesthetized with isoflurane, tracheotomized, mechanically ventilated and placed on a heating pad as described in Lindvere et al. (2010) and van Raaij et al. (2011). The femoral artery, femoral vein and tail vein were cannulated for blood gas sampling/blood pressure monitoring, delivery of α-chloralose during imaging, and delivery of ultrasound contrast agent respectively. We monitored the breathing rate, heart rate, arterial blood oxygen saturation, body temperature, and blood gases, and made adjustments when required to ensure a normal physiological state during both the surgery and the data acquisition. A cranial window (6.5 mm × 3 mm) was opened over the forelimb representation of the primary somatosensory cortex (S1FL), leaving the dura intact. The cranial window was covered with agarose to prevent dehydration of the cortical surface. All experimental protocols reported on in this study have been approved by the Animal Care Committee of Sunnybrook Health Sciences Centre.

Functional ultrasound image acquisition

We used a Vevo 2100 microultrasound system (VisualSonics Inc., Toronto, ON, Canada) with a linear array transducer with a center frequency of 21 MHz (MS-250, VisualSonics) as described in detail in (van Raaij et al., 2011). After acquisition of B-mode and color Doppler images, a contrast agent (Vevo MicroMarker, untargeted, VisualSonics Inc.) was infused at a concentration of $6 \cdot 10^8$ bubbles/mL using an infusion pump (New Era Pump Systems Inc., NE-1000) at a constant rate of 40 μl/min for 7 min. After a 2.5 minute stabilization period, imaging for the disruption–replenishment measurement was performed in a 'nonlinear contrast' (NLC) mode. This mode employs an amplitude modulation-type ultrasound pulse sequence designed to isolate backscattered ultrasound from nonlinear scatterers (i.e., microbubbles) and reject signal from primarily linearly scattering media like tissue or red blood cells (Goertz et al., 2005; Needles et al., 2010). Furthermore, the nonlinear signal has been shown to originate from microbubbles in the 1.8 μm range, very close to the volume peak of the microbubble distribution (Sprague et al., 2010). Moreover, for the low concentrations of bubbles employed here, the signal intensity in this imaging mode is linearly dependent on bubble concentration. Therefore, the signal is effectively a relative measure of local plasma volume (Lampaskis and Averkiou, 2010), and, assuming constant hematocrit (Herman et al., 2009), of blood volume. Using the standard definition, the mechanical index was about 0.4 (van Raaij et al., 2011). The disruption pulse was a 10 cycle pulse at max power, with peak derated rarefractional pressure of 3.7 MPa, played out at the system's rate of 21 MHz, with transmit focus at 11 mm. As in prior experiments, we have found no evidence for extravasation of bubbles in these experiments. Up to five repetitions of the disruption–replenishment sequence were performed per 90-second acquisition ("cineloop"), and three cineloops were recorded within the 7-minute infusion, yielding a maximum of 15 replenishment curves per subject.

The field of view was 8 mm wide and 10 mm deep measured from the surface of the cortex. For the near-sagittal sections imaged here, the slice thickness is the extent in the lateral-to-medial direction. The slice thickness depth profile is hourglass-shaped by the acoustic lens on the transducer. The slice thickness is greatest (~ 2 mm) at the cortical surface and reduces to ~600 μm at a depth of 10 mm. The axial resolution is proportional to the physical length of the pulse at half maximum, while the lateral resolution is proportional to wavelength and f-number (focal length/diameter). The pixel resolution in the images is rather arbitrary: the system oversamples the physical resolution by a factor of about 4 axially (top-to-bottom in the current images) and about 2 laterally (left-to-right).

"Ultrasound angiograms" were created by time-averaging the replenishment cineloops.

Disruption–replenishment analysis and model fitting

The average replenishment cineloop for each subject was binned in 4 by 4 pixel bins to improve signal-to-noise ratio and reduce computation time, and a mono-exponential replenishment model was fitted to each bin: $y(t) = A \cdot (1 - \exp(-\beta \cdot t))$, where y is the signal intensity in each voxel, A is the equilibrium amplitude and β is a rate constant (Wei et al., 1998). The equilibrium signal amplitude A is taken to be a measure of CBV; the rate constant β is taken to be proportional to the blood inflow speed; and the product $A\beta$ (the slope of the tangent to the replenishment curve at the start of the replenishment process) is a measure of blood flow (Wei et al., 1998). To reduce the influence of subject-to-subject variability in signal intensity, CBV and CBF were normalized to the "whole-slice" average CBV and CBF values; the resulting ratios are labeled rCBV and rCBF (r for *relative*) in the Results section. The parametric maps were then upsampled to the original image resolution.

Two parenchymal (cortical gray matter, subcortical gray matter) and two primarily vascular (pial vessels, deep vessels) anatomical regions of interest were manually drawn on the ultrasound angiograms. The mono-exponential model was applied to region-average replenishment curves to obtain region-specific hemodynamic parameters. For every subject, color Doppler images were aligned to the nonlinear contrast images by cross-correlating the corresponding B-mode images allowing for in-plane translations. In cases where the cross-correlation algorithm did not produce satisfactory results, the alignment was adjusted manually. The aligned color Doppler images were used to identify regions dominated by arteriolar flow (blood flowing perpendicularly