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Ultra-High Spatial Resolution Basal and Evoked Cerebral Blood Flow MRI of the Rat Brain

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Abstract

Cerebral blood flow (CBF) is tightly coupled to metabolism and neural activity under normal physiological conditions, and is often perturbed in disease states. The goals of this study were to implement a high-resolution (up to 50×38 μm) CBF MRI protocol of the rat brain, create a digital CBF atlas, report CBF values for 30+ brain structures based on the atlas, and explore applications of high-resolution CBF fMRI of forepaw stimulation. Excellent blood-flow contrasts were observed among different cortical and subcortical structures. CBF MRI showed column-like alternating bright and dark bands in the neocortices, reflecting the layout of descending arterioles and ascending venules, respectively. CBF MRI also showed lamina-like alternating bright and dark layers across the cortical thicknesses, consistent with the underlying vascular density. CBF profiles across the cortical thickness showed two peaks in layer IV and VI and a shallow trough in layer V. Whole-brain CBF was about 0.89ml/g/min, with the highest CBF values found amongst the neocortical structures (1ml/g/min, range: 0.89–1.16ml/g/min) and the lowest CBF values in the corpus callosum (0.32ml/g/min), yielding a gray:white matter CBF ratio of 3.1. CBF fMRI responses peaked across layers IV–V, whereas the BOLD fMRI responses showed a peak in the superficial layers II–III. High-resolution basal CBF MRI, evoked CBF fMRI, and CBF brain atlas can be used to study neurological disorders (such as ischemic stroke).

Keywords

High resolution imaging; columnar resolution; layer specificity; arterial spin labeling

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1. Introduction

Cerebral blood flow (CBF) is an important physiological parameter. Under normal physiological conditions, CBF is tightly coupled to basal metabolism and neural activity. Basal CBF and its regulation are perturbed in many acquired brain injuries and neurological disorders, such as ischemic stroke, traumatic brain injury, Alzheimer's diseases, and cancer, among others.

The most commonly used CBF MRI techniques are based on bolus tracking (Rosen et al., 1990) and arterial spin labeling (ASL) (Detre et al., 1992; Kwong et al., 1992). Bolus tracking techniques rely on the first passage of an exogenous MRI contrast agent and thus require high temporal resolution, which could limit spatial resolution and/or the signal-to-noise ratio. Importantly, some subjects experience severe negative side effects with exogenous MRI contrast agents. By contrast, ASL techniques use an endogenous tracer and can be repeated every few seconds (on the order of water T_1), which can be used to augment spatial resolution and/or the signal-to-noise ratio.

Functional MRI based on CBF change is spatially more specific to the site of increased neural activity, is easier to interpret than the more commonly used blood-oxygenation-level-dependent (BOLD) fMRI signals, is less susceptible to pathological perturbations to baseline signal, and has less inter-subject and across-day variability (Barbier et al., 2001). Combined CBF and BOLD fMRI measurements offer a means to estimate stimulus-evoked changes in the cerebral metabolic rate of oxygen in a totally non-invasive manner (Davis et al., 1998; Hoge et al., 1999; Kim and Ugurbil, 1997; Liu et al., 2004). The spatial resolution of CBF MRI in animal studies is typically on the order of $\sim 500\ \mu\text{m}$ (Bardutzky et al., 2005; Lee et al., 2002; Shen et al., 2003; Thomas et al., 2006), and $\sim 200\ \mu\text{m}$ in recent studies (Baskerville et al., 2012; Ren et al., 2004; Shen et al., 2011; Shen et al., 2013). High-resolution CBF MRI could provide important information on CBF supply to the brain at the columnar and laminar levels.

The goals of the current study were: i) to improve high-resolution CBF MRI of the rat brain, targeting up to $50\times 38\ \mu\text{m}$ in-plane resolution, ii) to create a high-resolution digital CBF atlas, iii) to report CBF values of 30+ brain structures, iv) to evaluate lamina-specific CBF fMRI responses associated with forepaw stimulation with comparison to BOLD fMRI.

2. Results

Multislice CBF images of a rat brain (Figure 1A, $75\times 56\times 1000\ \mu\text{m}$, PLD = 250 ms) showed excellent blood-flow contrast among different cortical and subcortical structures in the brain. In the neocortex, columns of alternating hypointense and hyperintense CBF contrast were observed. Layers of alternating bright and dark bands of CBF contrast across the cortical thickness were also observed. CBF contrast of the corpus callosum showed markedly lower CBF compared to the surrounding structures. The anterior commissure (AC), composed of white matter, also exhibited low CBF values. The subcortical structures showed heterogeneous CBF contrast with many distinctive structures, corresponding to known anatomy. CBF in the middle of the brain (i.e., slide #5) showed significantly lower CBF. While the hippocampus showed some bright pixels, it was relatively hypointense on CBF

images compared to neighboring structures. The globus pallidus was also relatively hypointense compared to its neighboring structures. There were bright pixels along the interhemisphere fissure, on the cortical surface, and some pixels in the hippocampus and ventricles. These were the result of cerebrospinal fluid contamination. The corresponding T2-weighted EPI images are shown in Figure 1B. For detailed comparison between CBF and T2-weighted EPI, we color-coded the CBF image, thresholded and overlaid it on the raw EPI (Figure 2). Most of the bright CBF bands are localized between the hypointense bands (venules).

To further characterize the ASL signals, CBF measurements were made at different PLDs (Figure 3A). With increasing PLD, the ASL signals decreased and the contrasts between bright and dark bands decreased only slightly compared to 250 ms PLD as expected. The plot of ASL signal as a function of PLD for the bright and dark bands showed that the ASL signal of bright bands decreased monotonically with PLD and that of the dark bands increased then decreased monotonically with PLD (Figure 3B).

The digital atlas showed the major color-coded brain structures (Figure 4). Quantitation of CBF excluded these cerebrospinal fluid pixels by using a threshold of 2 ml/g/min (Figure 5). The CBF values and volumes were tabulated for different brain structures (Table 1, $n = 6$). The major structures in the neocerebral cortex were the retrosplenial, limbic, cingulate, motor, somatosensory, auditory, visual, insular, temporal association, perirhinal, entorhinal, parietal association, and orbital cortex. For some smaller structures, neighboring structures were grouped together to improve signal-to-noise ratio, as indicated by ROIs 1, 2 and 3. The group-averaged whole-brain CBF was 0.89 ± 0.16 ml/g/min. The highest CBF was found in the neocortices (0.89 – 1.16 ml/g/min) and the lowest in the corpus callosum (0.32 ml/g/min), yielding gray to white matter (GM:WM) CBF ratios ranging from 2.8 to 3.6. The limbic, cingulate, orbital and entorhinal cortices showed slightly higher CBF among the cortices. With the exception of the caudate putamen and nucleus accumbens, most subcortical structures showed relatively lower CBF compared to neocortical structures. The cerebellum also showed lower CBF compared to neocortical structures.

With the smaller surface coil, higher resolution CBF images were obtained (Figure 6, $50 \times 38 \times 1000$ μm). The neocortex within the ROI was linearized showing CBF values at different layers. The profiles were plotted from the cortical surface to the corpus callosum. Group-averaged CBF profiles across depths showed that CBF peaked at layers IV and VI with a trough in layer V. Cortical layers (I, II/III, IV, V and VI) were assigned according to cortical thicknesses. Although the cortical thicknesses of each of 6 cortical layers varied depending on location from anterior-posterior and lateral-medial directions, it is evident that CBF profiles generally did not peak in layer IV; rather CBF showed consistent multiple layers of alternating hypo- and hyperintense bands.

High-resolution fMRI studies of forepaw stimulation were performed using the smaller surface coil. The CBF fMRI responses peaked in layers IV–V, dropped off in layers I–II, and VI (Figure 7), whereas the BOLD fMRI responses peaked in the superficial layers II–III.

3. Discussion

This study reports a high-resolution CBF MRI study of the rat brain up to $50 \times 38 \mu\text{m}$ and establishes a CBF atlas of the rat brain. A novel finding is that CBF MRI at high resolution showed column-like alternating bright and dark bands in the neocortices, consistent with the layout of alternating descending arterioles and ascending venules. CBF MRI also showed lamina-like alternating bright and dark layers across the cortical thickness, consistent with the underlying vascular density. The high-resolution CBF images also showed rich and heterogeneous CBF contrast in the subcortical structures. Another novel finding is that the CBF profiles across the cortical thickness showed two peaks in layers IV and VI and a shallow trough in layer V. CBF fMRI responses peaked across layers IV–V, whereas the BOLD fMRI responses showed a peak in the superficial layers II–III. High-resolution CBF MRI and CBF atlas can be applied to study neurological disorders.

3.1. Basal blood flow

MRI of basal CBF at $100 \times 100 \mu\text{m}$ in rats (Ren et al., 2004) and evoked CBF fMRI at $500 \times 500 \mu\text{m}$ to resolve orientation columns in cats (Duong et al., 2001b) have been reported. The current study demonstrated substantial improvement in spatial resolution in CBF MRI, providing remarkable blood-flow contrasts among different cortical and subcortical structures. The continuous arterial spin-labeling technique enabled signal averaging to improve signal-to-noise ratio and spatial resolution, in contrast to dynamic-susceptibility-enhanced CBF techniques. A separate labeling neck radiofrequency coil also offered improved CBF sensitivity.

There were some bright pixels in the interhemispheric fissure and lateral ventricles on the CBF images. We attributed this to contamination by cerebrospinal fluid because we assumed a single T_1 for the whole brain in the CBF calculation instead of using T_1 map. Nonetheless, the pixels with CSF can be readily identified and were excluded by using a threshold of $>2 \text{ ml/g/min}$ for quantitation. On the T_2 -weighted EPI, a few large veins (hypointense lines) were visible which could potentially contaminate CBF signals. T_2 and T_2^* effects should have been subtracted out in the CBF calculation. Some animals were also euthanized while in the scanner, and their CBF signals were within noise levels, demonstrating the CBF signals are genuine (data not shown).

The group-averaged whole-brain CBF ($0.89 \pm 0.16 \text{ ml/g/min}$, excluding bright cerebrospinal fluid pixels) is consistent with previous reports using MRI under similar experimental conditions (Duong et al., 2001a; Shen et al., 2003; Shen et al., 2004; Shen et al., 2005; Sicard et al., 2003; Sicard and Duong, 2005) and autoradiographic techniques (Baskerville et al., 2012; Engel et al., 2008; Lear, 1988). With the high-resolution MRI, CBF of the corpus callosum can be clearly resolved. CBF in the entire corpus callosum was 0.32 ml/g/min , compared to cortical gray mater CBF of 0.89 to 1.16 ml/g/min , with the GM:WM CBF ratio ranging from 2.8 to 3.6. By comparison, previous GM:WM CBF ratios have been reported to be 2.3 in baboon (Wey et al., 2011), 2.3 in rhesus by MRI (Zhang et al., 2007), and 2.0 by PET (Ye et al., 2000). A high-resolution autoradiographic CBF study also reported a GM:WM ratio of 2.5~3.2 (calculated based on their data) (Iadecola and Xu, 1994). Our ratios were among the highest, likely because high-resolution CBF MRI allowed imaging of

the corpus callosum without significant partial volume effects. Differences in species and anesthetics could be potential contributing factors to the differences in these ratios.

Columnar structures—A novel finding is that CBF MRI at high resolution showed column-like alternating bright and dark bands in the neocortices, consistent with the known vascular layout of the descending arterioles and ascending venules, respectively (Nakai et al., 1981). Note that the bright and dark CBF bands were not arterioles and venules *per se*, respectively. Rather they are predominantly the extravascular signals associated with these vessels. The majority of the ASL signals have already left the large vessels with the post labeling delay of 250 ms. Moreover, the ASL bands are a lot thicker than vessels, supporting extravascular contributions. The overlaid of CBF images on EPI showed that most of the bright CBF bands are localized between the hypointense bands (venules), supporting the notion that the bright CBF bands are associated with descending arterioles and the dark CBF bands are associated with the ascending venules. The radial-oriented vessel signals decreased with post labeling delay as expected and the contrast between arteriole- and venule-like bands decreased, but nonetheless still showed the bright and dark pattern. The plot of ASL signal as a function of PLD for the bright and dark bands showed that the bright band decreased monotonically with PLD and the dark bands increased then decreased monotonically with PLD, consistent with the notion of signals associated arterioles and venules, respectively. The initial rise of the dark CBF veins suggest transit time effect and signal have not reached the venule at 0 s PLD. Such transit time effect could have accentuated the differences between the bright and dark bands. Such PLD-dependent decrease is likely the limitation of using a single compartment model that does not consider intra-arterial and tissue signal separately. It is possible that the observed pattern could be the result of intraluminal signal (transit artifact) from descending arterioles. Much of the arterial transit time is likely to be in the microvasculature, and an alternative conclusion could be that the optimal PLD for imaging tissue perfusion at ultra-high resolution is much longer than previously thought. Performing experiments with and without crushing gradients could help to further elaborate the ASL signal sources. Nevertheless, these data together suggest that the ASL signals are associated with the arterioles and venules.

It is worth noting that CBF measurements by autoradiography generally did not show similar column-like patterns (Baskerville et al., 2012; Engel et al., 2008; Vaucher et al., 1997). Although the intrinsic physical spatial resolution of autoradiography is very high, the actual contrast (and resolution) in autoradiography could be significantly lower, depending on the autoradiographic exposure protocol and the mean free path and the energy of the emitter. While the majority of CBF autoradiography did not show column-like patterns, we found a high-resolution CBF measurement by [^{201}Tl]diethyldithiocarbamate and $^{99\text{m}}\text{Tc-D}$, L-hexamethylpropyleneamine oxime complex (Lear, 1988) to exhibit hints of column-like CBF pattern as ours. It is worth noting that 2-deoxyglucose autoradiography also did not show column-like patterns (Sokoloff et al., 1977).

Laminar structures—Cortical layers (I, II/III, IV, V and VI) were assigned based on depth from the cortical surface (Masamoto K, et al., 2003; Silva AC, et al., 2008). A key finding is that CBF profiles showed clear laminar CBF structures across the cortical

thickness, consistent with the underlying anatomical layers. Moreover, CBF profiles across depth exhibited double peaks, at layers IV and VI with a shallow trough in layer V. Layers IV and VI showed higher CBF, likely associated with higher basal metabolic needs. The relative anatomical thickness of each of the six cortical layers also varied along the anterior-posterior and along the medial-lateral directions, as expected. The relative individual CBF layer thickness and spacing and density of metabolism scaled with vascular density (Sokoloff et al., 1977).

Our finding of the double-CBF peaks in layers IV and VI with a shallow trough in layer V is consistent with the vascular density distribution previously reported using confocal laser scanning technique showing double peaks at layers IV and VI (Masamoto et al., 2004). Most previous CBF MRI studies, however, showed a single peak with the highest vascular density around layer IV (Herman et al., 2013; Shen et al., 2008; Shih et al., 2013; Silva and Kim, 1999), likely as a result of comparatively lower spatial resolution in these previous studies. Close examination of a few previous studies (Masamoto et al., 2004; Nakai et al., 1981; Zheng et al., 1991) showed hints of a possible double CBF peaks across the cortical thickness, although such observation was not previously emphasized.

Corpus callosum—The CBF contrast around the corpus callosum and other subcortical structures showed markedly lower CBF compared to the surrounding structures. This study shows the extent of vasculature around and permeating the corpus callosum for the first time. CBF of the corpus callosum appeared significantly bigger than those based on T₂ or diffusion contrast. The anterior commissure (AC), composed of white matter, also exhibited low CBF values relative to the neocortices.

Subcortical structures—Subcortical structures also showed remarkable CBF contrast and its patterns were markedly different from those of the neocortices. The striatum and the caudate putamen in particular showed rich contrasts that are consistent with the underlying anatomical contrast by histology. CBF contrasts are adequate for visualizing the cerebellum, hippocampus, amygdala and thalamus. They showed comparatively lower CBF. With few exceptions (caudate putamen and accumbens nucleus), subcortical CBF was lower than cortical CBF. The cerebellum CBF was also low compared to neocortical CBF.

Some of the subcortical structures around the ear canals were not visible because of susceptibility induced signal losses. This was the result of gradient-echo planar imaging acquisition although it is already much improved over lower resolution data because of reduced intravoxel dephasing and the shorter echo time of the CBF acquisition compared to BOLD acquisition. Such susceptibility induced signal losses can be further improved using spin-echo acquisition or parallel imaging albeit at reduced sensitivity. Filling the ear canals with susceptibility neutral gels could also reduce susceptibility induced signal losses.

In stroke imaging, partial-volume effect could hamper proper delineation of normal, ischemic, and at-risk tissues by blurring the boundaries among different tissue types and tissue viability. Visual delineation of ischemic lesions by manually drawing regions of interest on the diffusion- and perfusion-weighted images is common and the presence of partial-volume effect could lead to significant errors in identifying ischemic tissue fates. In

addition, it is conceivable that a substantial number of pixels with mild apparent diffusion coefficient (ADC) or CBF reduction could arise simply from the physical effect of partial voluming, thereby confounding the interpretation of the operationally defined ischemic penumbra. High-resolution CBF imaging could minimize such tissue classification errors.

3.2. CBF atlas

While anatomical and diffusion atlases of the rodent brain have been developed (Aggarwal et al., 2011; Dorr et al., 2008; Johnson et al., 2012; Mori et al., 1999), this study is the first attempt to create a digital CBF atlas of the rat brain to our knowledge and to report CBF values of many regions. We reported CBF values for 30+ brain structures. The brain atlas was re-constructed using an in-house written Matlab program to semi-automatically identify brain structures based on the PDF files of the Praximos anatomical digital atlas. This approach is susceptible to errors as with other studies involving co-registration to anatomical brain atlas, including mis-registration between the atlas and our rat brain MRI data, particularly for structures that are small and close to the skull. From the anatomical brain atlas, many small brain structures can be readily identified. However, in our CBF atlas some of the small brain structures were grouped together for reporting CBF values (labeled as ROI 1, 2, 3 in Table 1) due to the constraint of poor signal-to-noise ratio. Future studies will focus on further improving spatial resolution and signal-to-noise ratio.

3.3. Evoked fMRI

Considerable efforts have been made to improve the spatial resolution and contrast of the fMRI signals (Duong et al., 2001b; Herman et al., 2013; Shen et al., 2008; Shih et al., 2013; Silva and Kim, 1999). We found that CBF fMRI responses peaked in layers IV–V, whereas the BOLD fMRI responses peaked in the superficial layers II–III. The majority of the studies of forepaw stimulation (Duong et al., 2001b; Herman et al., 2013; Shen et al., 2008; Shih et al., 2013; Silva and Kim, 1999) and visual stimuli (Goense et al., 2012; Jin and Kim, 2008; Smirnakis et al., 2007; Zhao et al., 2006) found that the CBF and cerebral blood volume fMRI responses peaked in the middle of the cortical layer. By contrast, BOLD fMRI signal were also generally found to peak in the superficial layers. Another important observation is that the CBF activation maps across the cortical layers were relatively flat compared to BOLD activation maps. Herman et al. reported that CBF fMRI responses across cortical layers were not significantly different (Herman et al., 2013). Similar to our current study, Goense et al. also reported a double-peak functional cerebral blood volume response pattern in excitatory regions in monkeys (Goense et al., 2012). Differences in CBF activation profile across cortical depths could depend on the nature of the stimuli, which could result in different synaptic activities to different cortical layers. It may also depend on the type and level of anesthetic.

4. Conclusions

This study demonstrated very high-spatial resolution CBF MRI of the rat brain and built a digital CBF MRI atlas of rat brain. This approach sets the stage for investigating basal CBF perturbation and CBF fMRI dysregulation in neurological disorders at very high spatial resolution.

5. Materials and methods

5.1. Animal Preparation

Fourteen male Sprague-Dawley rats (250 to 350 g, Taconic Farms, NY, USA) were studied with approval by the Institutional of Animal Care and Utilization Committee, University of Texas Health Science Center at San Antonio. Rectal temperature was maintained at $37.0 \pm 0.5^\circ\text{C}$ via a circulating warm pad with feedback control. Heart rate (320 to 390 bpm) and arterial oxygenation saturation ($>90\%$) were recorded continuously using the MouseOx system (STARR Life Science Corp., Oakmont, PA, USA) onto a computer via the Biopac system (Santa Barbara, CA, USA). All recorded physiological parameters were within normal physiological ranges.

In Group 1 ($n=6$), whole-brain basal CBF data at relatively low spatial resolution ($75 \times 56 \times 1000 \mu\text{m}$) were obtained in spontaneous breathing rats under 1.2–1.3% isoflurane.

In Group 2 ($n=4$), basal CBF data of the cortex at high spatial resolution ($50 \times 38 \times 1000 \mu\text{m}$) were obtained in spontaneous breathing rats under 1.2–1.3% isoflurane.

In Group 3 ($n=4$), BOLD and CBF fMRI of forepaw stimulation was performed using a small surface coil over the cortex ($75 \times 56 \times 1000 \mu\text{m}$). Rats were initially anesthetized with 3% isoflurane and orally intubated for mechanical ventilation (Harvard Model 683 Small Animal Ventilator, Holliston, MA, USA). End-tidal CO_2 was continuously monitored using a capnometer (Surgivet, Smith Medical, Waukesha, WI, USA). The lateral tail vein was catheterized for subsequent drug administration. After the animal was secured in a custom-made headset, isoflurane was discontinued and α -chloralose (60 mg/kg first dose, followed by 30 mg/kg/hr, intravenous infusion) was administered for anesthesia. The rat was then paralyzed with pancuronium bromide (3 mg/kg first dose, followed by 1.5 mg/kg per h, iv). Two needle electrodes were inserted under the skin of a forepaw: one between the first and second digits and the other between the third and fourth digits. Electrical stimulation at 2 mA, 3-Hz square wave, and 1.0-ms pulse duration was applied to the same rat by a constant-current stimulator (model 2100, AM Systems, Carlsburg, WA, USA). Typically, 3 repeats of the stimulation trials were performed on each animal.

5.2. MRI Experiments

MRI experiments were performed on a Bruker 11.7-Tesla/16-cm scanner (Billerica, MA). Two different sized transceiver surface coils (inner diameters: 2.1-cm and 0.9-cm) with active decoupling were used for brain imaging. A butterfly neck coil was used for perfusion labeling.

Quantitative CBF was measured using the continuous arterial spin-labeling technique with four-shot, gradient-echo echo-planar image acquisition (Shen et al., 2005). Labeling used a 2.65-s square radiofrequency pulse applied to the neck coil in the presence of a 1.0-G/cm gradient. Imaging parameters were repetition time = 3 s, echo time = 9 ms.

In Group 1, whole brain basal CBF of $75 \times 56 \times 1000 \mu\text{m}$ spatial resolution was acquired using the larger surface coil of 2.1-cm inner diameter, gradient-echo planar imaging (EPI), matrix

=192×144 and reconstructed to 256×256, field of view (FOV) = 19.2 × 14.4 mm, and fifteen 1-mm slices acquired in three separate blocks to cover the whole brain. The post-labeling delay (PLD) was 250 ms. Addition experiments were also performed with multiple PLDs.

In Group 2, high-resolution protocol was used to acquire basal CBF of cortex at 50×38×1000 μm using the smaller surface coil of 0.9-cm inner diameter, matrix =192×144 and reconstructed to 256×256, FOV=12.8 × 9.6 mm and three 1-mm slices in the center brain. The PLD was 250 ms.

In Group 3, fMRI of the cortex at 100×75×1000 μm was acquired using the smaller surface coil (0.9-cm inner diameter), single shot gradient-echo EPI, FOV = 12.8 × 9.6 mm, matrix size = 96×72, reconstructed to 128×128. The electrical stimulation paradigm was OFF–ON–OFF–ON–OFF, where OFF = 4 mins and ON = 2 mins. Typically, the stimulation paradigm was repeated three times per animals. The PLD was 250 ms.

5.3. Digital Atlas of rat brain

A rat brain digital atlas was created by digitizing PDF files of the rat brain atlas (Paxinos and Watson, 1998). Images were set to transparent color to remove the background color. Different anatomical structures were assigned different colors. The figures were converted to gray scale images. Each anatomical structure of the brain atlas was given a unique number as an index. Fifteen slices from slice No.7 (interaural 12.70 mm) to slice No. 65 (interaural –2.30 mm) with 1mm gap were identified and used to co-register with CBF images.

5.4. Data analysis

CBF images, in units of milliliters per gram per minute, were calculated (Duong et al., 2000) pixel-by-pixel using, $S_{CBF} = \lambda/T_1 \cdot (S_C - S_L)/(S_L + (2\alpha - 1) S_C)$, where S_C and S_L are signal intensities of the control and labeled images, respectively and λ (0.9) is the water brain-blood partition coefficient. Tissue T_1 of 2.0 seconds and labeling efficiency (α) of 0.75 were measured using a method described elsewhere (Duong et al., 2000; Shen et al., 2003; Shen et al., 2004).

To better understand the signal sources of the hyper- and hypointensity on CBF maps, the bright bands with CBF values between 1.3 and 2 ml/gram/min were color-coded and overlaid on T_2 -weighted EPI. The ASL signals ($\Delta S/S$, where ΔS is the difference between control and labeled amplitude) were also evaluated as a function of PLD for the bright (0.06~0.11 of $\Delta S/S$ at 1 ms PLD) and dark (0.02~0.059 of $\Delta S/S$ at 1 ms PLD) bands.

CBF images were co-registered with digital atlas using custom-designed registration software (<http://www.quickvol.com>). CBF value and volume of 259 structures were analyzed. The structures included cortex, caudate putamen, cerebellum, hippocampus, amygdala, thalamus, hypothalamus, corpus callosum, colliculus, geniculate nucleus, accumbens nucleus, septal nucleus and periaqueductal gray. Large vessel and CSF pixels (CBF > 2ml/gram/min) were excluded from the tabulation of quantitative values.

The cortex was flattened using a custom-designed Matlab code (Cheng et al., 2006; Muir and Duong, 2011a; Muir and Duong, 2011b). CBF profiles across the cortical depth were obtained by averaging along the length of the cortex. Cortical layers (I, II/III, IV, V and VI) were assigned according to cortical thickness based on literature (Masamoto et al., 2004; Silva et al., 2008).

For fMRI analysis, cross correlation was performed to obtain percent change maps associated with the forepaw primary somatosensory cortices. Response maps were overlaid on basal CBF maps. Basal CBFs and percent changes at different cortical layers were derived.

All data were reported as mean $\pm$ standard deviation (SD).

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A digital MRI atlas of cerebral blood flow at $50 \times 38 \times 1000 \mu\text{m}$ is created for rat brain.

Cortical blood flow profiles show two peaks in layer IV & VI and a trough in layer V.

Blood flow MRI shows column-like alternating arterioles and venules in the cortex.

Blood flow MRI clearly delineates perfusion territory in ischemic stroke.

Blood flow fMRI peaked in layer IV–V, whereas BOLD fMRI peaked in layer II–III.

Figure 1A

Figure 1B

Figure 1.
(A) Multislice CBF images of a rat brain at $75 \times 56 \times 1000 \mu\text{m}$ from one animal. (B) Corresponding echo-planar image images from the same animal.

Figure 2. Overlay of CBF image on EPI. The CBF images were color-coded, thresholded and overlaid on the raw EPI. The bright band of the CBF images between 1.3 and 2 ml/gram/min were color-coded.

Figure 3A

Figure 3B

Figure 3.

(A) ASL images (S/S') at different post-labeling delays (PLDs). (B) ASL signals from the bright (0.06~ 0.11 of S/S' at 1 ms PLD) and dark (0.02~0.059 of S/S' at 1 ms PLD) bands as a function of post-labeling delays.

Figure 4.

The digital atlas shows the major color-coded brain structures. The digital CBF atlas was created via co-registering with the digital anatomical atlas.

Figure 5. CBF images from Figure 1A with a threshold of CBF < 2 ml/g/min. These images were used to tabulate CBF values of different structures.

Figure 6.

Higher resolution CBF (A) images, (B) 2-D map, and (C) profiles at $50 \times 38 \times 1000 \mu\text{m}$ obtained using the smaller surface coil. ROI in (A) indicates the expansion for (B). In C, the distance from 0 to $2000 \mu\text{m}$ is from cortical surface to corpus callosum boundary. The assignment of layers is based on (Masamoto et al., 2004; Silva et al., 2008). Data were obtained from 4 animals (mean $\pm$ SD). The unit for (B) is ml/gram/min.

Figure 7.

The CBF and BOLD fMRI responses at $100 \times 76 \times 1000 \mu\text{m}$ obtained using the smaller surface coil. The ROIs indicate the typical regions used for each of the animals. Data were obtained from 2 animals with 3 repeated fMRI trials for each animal (mean $\pm$ SD).

Table 1

CBF and volumes of brain structures

structures		CBF (ml/gram/min)	volume (mm ³)
Cortex CBF: 0.95±0.16 volume: 347.4	retrosplenial	0.91±0.22	22.9
	limbic	1.16±0.13	5.7
	cingulate	1.14±0.13	9.4
	motor	0.94±0.12	51.3
	somatosensory	0.95±0.14	110.6
	auditory	0.94±0.16	16.7
	visual	0.91±0.22	55.6
	insular	1.00±0.19	20.9
	temporal association	0.92±0.20	11.4
	perirhinal	0.89±0.22	12.5
	entorhinal	1.05±0.20	18.2
	parietal association	1.03±0.19	5.7
	orbital	1.09±0.15	6.5
caudate putamen		1.13±0.15	66.6
cerebellum		0.72±0.24	83.4
hippocampus		0.82±0.13	35.9
amygdala		0.79±0.20	7.2
thalamus		0.85±0.16	22.0
hypothalamus		0.76±0.13	8.8
corpus callosum		0.32±0.04	47.2
geniculate nucleus		0.86±0.18	3.9
periaqueductal gray		0.77±0.22	8.9
colliculus		0.92±0.22	23.9
ROI1 ↗		0.98±0.22	18.5
accumbens nucleus		1.04±0.18	9.6
septal nucleus		0.75±0.10	8.7
ROI2 ↗		0.84±0.16	5.7
globus pallidus		0.78±0.12	10.7
deep mesencephalic nucleus		0.77±0.22	14.4
ROI3 ↗		0.85±0.31	5.4

ROI1: tenia tecta, endopiriform nucleus, piriform cortex and olfactory nucleus

ROI2: forebrain bundle, interstitial nucleus of the posterior limb of the anterior commissure, bed nucleus of the stria terminalis, substantia innominata, preoptic nucleus

ROI3: interstitial nucleus of the medial longitudinal fasciculus, parabrachial nucleus, red nucleus, retrorubral field