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Quantitative vascular neuroimaging of the rat brain using superparamagnetic nanoparticles: New insights on vascular organization and brain function

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Abstract

A method called Quantitative Ultra-Short Time-to-Echo Contrast Enhanced (QUTE-CE) Magnetic Resonance Imaging (MRI) which utilizes superparamagnetic iron oxide nanoparticles (SPI-ONs) as a contrast agent to yield positive contrast angiograms with high clarity and definition is applied to the whole live rat brain. QUTE-CE MRI intensity data are particularly well suited for measuring quantitative cerebral blood volume (qCBV). A global map of qCBV in the awake resting-state with unprecedented detail was created via application of a 3D MRI rat brain atlas with 173 segmented and annotated brain areas. From this map we identified two distributed, integrated neural circuits showing the highest capillary densities in the brain. One is the neural circuitry involved with the primary senses of smell, hearing and vision and the other is the neural circuitry of memory. Under isoflurane anesthesia, these same circuits showed significant decreases in qCBV suggesting a role in consciousness. Neural circuits in the brainstem associated with the reticular activating system and the maintenance of respiration, body temperature and cardiovascular function showed an increase in qCBV with anesthesia. During awake CO₂ challenge, 84 regions showed significant increases relative to an awake baseline state. This CO₂ response provides a measure of cerebralvascular reactivity and regional perfusion reserve with the highest response measured in the somatosensory cortex. These results demonstrate the utility of

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QUTE-CE MRI for qCBV analysis and offer a new perspective on brain function and vascular organization.

Keywords

Cerebral Blood Volume (CBV); Magnetic Resonance Imaging (MRI); Ultra-short TE (UTE); Ferumoxytol; Anesthesia; Perfusion Reserve

1. Introduction

The mammalian brain has evolved an exceptional ability to autoregulate regional blood supply to specific anatomical regions in response to changes in function or state. While the underlying neurovascular architecture is fixed by evolutionary and developmental needs, the blood supply is highly dynamic and changes with metabolic demand. Magnetic resonance imaging (MRI) is the most common clinical imaging modality for investigating neural blood flow and vascular structure. An approach to absolutely measure and map quantitative cerebral blood volume (qCBV) would provide a proxy to measure capillary density and enable identification of brain areas critical to the evolution of function and survival. Further, deviations in this global map of capillary densities and the changes in blood volume to these areas following physiological or traumatic perturbations would greatly enhance our understanding of the distributed, integrated neural circuits underlying brain function.

Conventional MRI techniques cannot absolutely and quantitatively measure qCBV at the level of the microvasculature across the entire brain. Generally, MRI techniques rely on qualitative image assessment, relative signal changes or have high degrees of error which limit accurate quantification. Quantitative MRI measurements are hampered by susceptibility-induced spin dephasing, field inhomogeneity, spin dephasing and motion/flow artifacts. Imaging techniques that employ a time-to-echo (TE) of half a millisecond or more are particularly vulnerable to these complications (Bernstein et al., 2006; Farrar et al., 2009). Dynamic susceptibility contrast (DSC) MRI is most commonly used for measuring CBV values (Sourbron et al., 2009; Barbier et al., 2001) but fundamentally requires accurate determination of the arterial input function (AIF) (Rempp, 1994), or contrast concentration versus time curve (Yankeelov and Gore, 2009), which are typically 15–30% inaccurate (Walker-Samuel et al., 2007; Schabel and Parker, 2008). The typically employed echo-planar imaging (EPI) sequences are prone to image distortions such as warping and signal dropout due to spin dephasing. Related dynamic contrast enhanced, DCE-MRI, techniques employ TEs greater than half a millisecond and report similar error when applied to CBV (Sourbron et al., 2009). Other techniques for measuring the CBV, such as steady-state gradient echo (SSGRE) susceptibility contrast mapping, steady state CBV (SSCBV), and R_2 all utilize T_2 and T_2^* effects, which are prone to susceptibility-induced intravoxel dephasing as well as flow artifacts which necessitate a reduction in resolution to compensate for partial volume effects (Stuber et al., 2007; Troprès et al., 2001).

We have previously introduced Quantitative Ultra-short Time-to-Echo Contrast-Enhanced (QUTE-CE) MRI, a method utilizing Ultrashort-Time-to-Echo (UTE) sequences with SPIONs that produces angiographic images with positive T_1 contrast (Zhao et al., 2011;

Hoelscher et al., 2012), high clarity, high definition and the low error of quantification (Gharagouzloo et al., 2014). We utilize ferumoxytol, an FDA-approved SPION formulation already used off-label for human MRI. SPIONs have been recognized to be an alternative to nephrotoxic gadolinium-based contrast agents (CAs) (Neuwelt et al., 2009; Turkbey et al., 2015), but their use has been greatly limited clinically by the commonly employed T_2 -weighted imaging techniques which produce negative contrast.

Here we apply the QUTE-CE MRI method to find absolute measurements of qCBV across the entire awake rat brain. From these measurements, a global map of qCBV in 173 regions was created and regions of homogeneous microvasculature were identified. Next, we demonstrate that regional alterations to this qCBV can be measured during steady-state changes, here due to hypercapnia (elevated blood CO_2) and inhaled isoflurane anesthesia. Inferences about brain function and vascular organization are drawn from the spatial mapping of regional changes between these functional states.

2. Methods

2.1. Animal Model and State Changes

All animal experiments were conducted in accordance with the Northeastern University Division of Laboratory Animal Medicine and Institutional Animal Care and Use Committee. Sound-and restraint-acclimated (Ferris et al., 2015) Sprague Dawley (SD) rats (~300 g, $n=11$) were anesthetized with isoflurane (1–3%), mechanically restrained, and fit with a tail vein catheter containing heparinized saline. Animals were imaged in the anesthetized state then injected with an i.v. bolus of 6 mg/ml Fe of Ferumoxytol. The injected volume was tailored for each rat (assuming 7% blood by body weight) to produce a starting blood concentration of 200 $\mu\text{g/ml}$ Fe ($2\times$ the clinical dose approved for use in humans). Rats were allowed to awaken for 25 minutes and then 5% CO_2 was delivered via mask for 2 minutes to induce hypercapnia before commencing another scan. The CO_2 was next replaced with air at the same flow-rate, and the next scan commenced after 2 minutes to measure a standard resting steady-state. Finally, 3% isoflurane was added to the air and this concentration was used until respiration decreased to 30–40 breaths/minute, indicating anesthetization. Isoflurane percent was adjusted manually throughout the scan to maintain a steady breathing rate.

2.2. QUTE-CE Image Acquisition

In the underlying theory of QUTE-CE MRI, the intensity magnitude I_M of each voxel is a function of standard MRI parameters governed by the Spoiled Gradient Echo (SPGR) equation (Schabel and Parker, 2008; Gharagouzloo et al., 2014),

$$I_M = K \rho \cdot e^{(-TE/T_2)} \cdot \sin(\text{FA}) \frac{1 - e^{(-TR/T_1)}}{1 - e^{(-TR/T_1)} \cdot \cos(\text{FA})} \quad (1)$$

where TE is the time-to-echo, TR is the repetition time, and FA is the flip-angle. TE, TR, and FA are user-defined image acquisition parameters and their optimization is a vital

component of technique. T_1 and T_2 are relaxation time constants dependent on the local environment of each voxel, which is mutable via contrast agents, and dependent on the magnetic field strength. K is a constant determined by the properties of the receive coil and ρ is the proton density of the medium.

User-defined QUTE-CE MRI image acquisition parameters available for optimization included the TE, TR and FA. TE is chosen to be ultra-short ($<100 \mu\text{s}$) to eliminate susceptibility-induced signal modifications. The choice of a very low TR ($<5 \text{ ms}$) in a 3D volume excitation pulse minimizes effects from the extravascular water exchange (Kim et al., 2002) and eliminates signal enhancement from blood flow within the cranial space. Setting the FA at the Ernst angle maximizes the T_1 -enhanced signal and minimizes sensitivity to small perturbations. Here, QUTE-CE MRI images were obtained at ambient temperature ($\sim 25^\circ \text{C}$) using a Bruker Biospec 7.0 T/20 cm USR horizontal magnet (Bruker, Billerica, Massachusetts, USA) equipped with a 20-G/cm magnetic field gradient insert (ID=12 cm, Bruker) and a custom built 30 mm diameter 300 MHz quadrature volume coil (Animal Imaging Research, Holden, MA). The coil apparatus was equipped with restraints to hold the rat head fixed and centered during both asleep and awake imaging. Optimized acquisition parameters of TE=13 μs , TR=4 ms, and FA=20° were utilized with a high RF pulse bandwidth of 200kHz. Therefore, the pulse duration was short (6.4 μs) compared to the T_2 of the approximate ferumoxytol concentration (4.58 ms for 3.58 mM, i.e. 200 $\mu\text{g/ml}$ (Gharagouzloo et al., 2014)) to minimize signal blur and reduce the probability for a curved trajectory of the magnetization vector M_z (Tyler et al., 2007). A $3 \times 3 \times 3 \text{ cm}^3$ field-of-view was used with a matrix mesh size of $200 \times 200 \times 200$ to produce 150 μm isotropic resolution. Images were averaged over 2 scans.

2.3. Rat Brain Atlas

A 173-region rat brain atlas was developed using high resolution ($85 \mu\text{m} \times 85 \mu\text{m}$) T2-weighted anatomical MRI images acquired using a Bruker 7.0T scanner. A 2D multi-slice Turbo-RARE sequence with fat suppression was used with following parameters: TE_{eff} = 48 ms, TR=5000 ms, RARE factor=16, FA=90°, averages=15. Anatomical and functional regions were drawn on 65 contiguous axial slices of 400 micron thickness, producing 173 regions of interest. General segmentation and specific annotation were adopted from Paxinos and Watson (The Rat Brain, 6th Edition). This MRI atlas was developed to overcome the many shortcomings of existing commercial and public-domain atlases (Ekam Solutions, Boston, MA USA). The atlas more accurately reflects living structures because, unlike the histology images traditionally used in atlas development, there is no shrinkage due to preparation of the reference tissues. This greater biological accuracy improves registration and segmentation quality. Note that, while the atlas includes a region for the ventricle (#155), this was excluded from all CBV analysis.

2.4. Quantification of Cerebral Blood Volume

To extract qCBV values, the theory of partial voluming effects was applied. Use of this model is justified since ferumoxytol does not cross the blood-brain barrier and the QUTE-CE MRI image acquisition parameters chosen here minimize the flow, water exchange and susceptibility effects that would otherwise confound its application. This theory assumes that

the measured voxel intensity, I_M , is a linear combination of contributions from the blood and tissue compartments. Thus, the measured signal is given by,

$$I_M = f_B I_B + f_T I_T \quad (2)$$

where I_T is the brain tissue intensity, I_B is the blood intensity, and f_B and f_T are the fraction of blood and tissue respectively. The measured change in intensity between any two scans is given by, $\Delta I_M = f'_B I'_B - f_B I_B + f'_T I'_T - f_T I_T$. By selecting a contrast agent that is confined to the blood plasma, it is possible to vary I_B without drastically changing I_T , such that $I'_B \gg I_B$ but $I'_T \approx I_T$, where prime denotes the measured intensity after contrast agent injection.

If contrast enhancement occurs without a change in blood volume, then we can solve for the fraction of blood as,

$$qCBV = f_B = \frac{I'_M - I_M}{I'_B - I_B} \quad (3)$$

This equation can be applied to measure qCBV, as long as the pre- and post-contrast scans are collected in same animal while the animal is held in the functional steady-state. To determine changes in qCBV across different neurological states (awake vs. CO₂ challenge, awake vs. anesthetized, etc), the need for multiple pre-contrast scans is avoided by finding,

$$f'_B = \frac{I'_M - I_M + f_B (I_B - I_T)}{I'_B - I_T} \quad (4)$$

Here, a single pre-contrast scan was performed while rats were anesthetized and thus Eq. 3 was applied to determine qCBV for rats in the anesthetized state. I_T was then calculated regionally. Eq. 4 was applied to determine qCBV rats in the hypercapnic (CO₂) and resting (air) states using these I_T and f_B values while I_B values were measured in a process described in Sec. 2.5.

2.5. Image Processing

Each data set was corrected for B1 field inhomogeneity using phantom work discussed in the SI. Following inhomogeneity correction, all scans were motion-corrected and aligned spatially using the MATLAB SPM12 toolbox developed at UCL (<http://www.fil.ion.ucl.ac.uk/spm/>). Aligned scans were re-sliced using nearest neighbor interpolation. Using software developed at Northeastern University Center for Translational Neuroimaging (CTNI), in collaboration with Ekam Imaging, the 173-region atlas was custom-fit to each rat dataset, taking into account variations in brain size and positioning. Once the images were co-registered to the atlas, custom MATLAB code was used to mask individual brain regions for qCBV determination. On average, regions contained ~3200 voxels (~550,000 voxels for the whole brain). Intensity data was extracted on a per-voxel

basis for each region. Mean and median intensities per-region were found via simple MATLAB functions and their difference was used to characterize the skewness of the regional distributions.

Several steps were taken to ensure consistent measurement of the blood intensity values, I'_B and I_B . First, average blood intensity values were measured from each individual scan by drawing custom regions of interest in the superior sagittal sinus in 3D Slicer, (Fedorov et al., 2012), using the Level Tracing Effect Tool (Fig. S1). Separate ROIs were drawn for each scan. To account for ferumoxytol clearance from the blood and increase accuracy of I'_B , these average ROI blood values were fit via a weighted least-squares method to exponential decay functions (Fig. S2). The I'_B used in the qCBV calculation was extrapolated from these fits at the corresponding scan time. Application of Eq. 2 or 3 following atlas segmentation allowed the qCBV to be calculated for each scan.

Images presented in the Fig. 1 were created in Paravision 5.1 as maximum intensity projections. Additionally, 3D NIFTI images are provided with the full FOV in *QUTE-CE-FOV-angio.nii* here and just the cropped brain in *QUTE-CE-Brain-angio.nii* here. The 3D renderings presented in Figs. 2–5 were created in MIVA 2.1 [build 04/20/05] which was developed for medical imaging processing by the Center for Comparative NeuroImaging at Worcester Polytechnic Institute using custom 3D volume files developed alongside the atlas itself with custom MAT-LAB code to assist with scaling and display parameters.

2.6. Animal Variation

The minimum standard deviation in between animals was $\pm 0.62\%$ qCBV in the resting state, $\pm 0.66\%$ qCBV in the CO₂ induced state, and $\pm 0.47\%$ qCBV in the isoflurane induced state with average standard deviations of $\pm 2.0\%$, $\pm 2.0\%$, and $\pm 1.7\%$ qCBV respectively. Deviations were generally highest in regions including or bordering large vessels and visual inspection revealed noticeable variation in path and morphology of these vessels in the angiographic images. The resting state qCBV values for all animals in all regions are provided in Dataset 1 of our Data In Brief article (Gharagouzloo et al., 2017). The inter-animal variation was actually lowest on average for the isoflurane state relative to the other states which suggests that, while the dose was adjusted throughout the scan time, there was major dose dependence for the isoflurane induced state.

2.7. Distinguishing between states

Due to this inter-animal variation, it was found that the state-changes had lower error when considered for each animal separately. Thus, the difference in qCBV between states was found on a per-animal basis. This set of per-animal differences were tested for significance using a one-sample t-test with $p < 0.05$. In addition, since we are analyzing a large number of brain regions (173), the positive false discovery rate (pFDR) was also calculated and is included alongside the p-values for reference (Storey, 2002). Full lists of all changes for both states are presented in Datasets 2&3 of the Data in Brief (Gharagouzloo et al., 2017).

3. Results

3.1. Angiography and Quantitative Cerebral Blood Volume

Injection of the strictly intravascular contrast agent, ferumoxytol, and application of the QUTE-CE method creates snapshots of all cerebral arterial and venous vasculature independent of flow velocity or vessel orientation as shown Fig. 1. Pre-contrast QUTE-CE MRI images of the entire rat head are nearly uniformly dark except for Time of Flight (TOF) effects limited to the periphery, and accordingly are not required to delineate the vasculature. After bolus delivery of ferumoxytol, the blood signal intensity increases only within vessels (Fig. 1). QUTE-CE MRI done in this way and applied to the brain, is resistant to susceptibility effects, signal dropout and warping seen with other MRI methods, and allowing the signal from CBV to be isolated.

Fully awake, male Sprague Dawley rats (N=11) were imaged with QUTE-CE MRI and data from over 550,000 voxels per subject were registered to a 3D MRI rat brain atlas with 173 segmented and annotated brain areas to create a global average map of microvasculature. The mean signal intensity was used to calculate the qCBV for each brain area. Dataset 1 of (Gharagouzloo et al., 2017) lists all brain areas and the average percent volume of qCBV for each. Fig. 2a and *RestingState-qCBV-Atlas.nii* here show the regionalization of the qCBV with local variations in vasculature. To further characterize the variation in qCBV, probability distributions were generated for all the voxels within each region (Fig. 2b). In the pre-contrast scans, the measured signal intensity shows purely Gaussian distributions due to thermal noise, and relatively small difference between tissue and blood signals in the absence of contrast agent. Independent measurements collected in isotropic phantoms confirmed that homogenous structures produce Gaussian distributions (Fig. S3). From post-contrast scans, three primary types of intensity distributions were identified: Type I regions (~53%) are Gaussian ($R^2_{\text{Gauss}} > 0.9$); Type II regions (~45%) are positively skewed and less Gaussian; and Type III regions (~2%) are highly skewed. These types were separated by using the difference of the post-contrast voxel intensity distribution mean minus the median for each region. These cut-offs were defined such that Type I regions had Mean-Median ≤ 25 , Type II regions had $25 < \text{Mean-Median} \leq 250$ and Type III had Mean-Median > 250 . Representative intensity distributions are shown for the primary somatosensory cortex trunk (#135; Type I), visual 2 cortex (#157; Type II), and caudal retrosplenial cortex (#125; Type III).

3.2. Microvasculature Heterogeneity

These different intensity distributions are likely a direct consequence of the vessel architecture. Given that a Gaussian distribution implies homogeneity, combined with the observation that all Type I regions had a qCBV maximum of ~10% (Dataset 1 (Gharagouzloo et al., 2017)), suggests that Type I regions are primarily filled with capillaries of uniform size and density. For example, the somatosensory cortices (region #s 130–137), rostral piriform (#103), lateral orbital (#75), ventral orbital (#166), parietal (#116), prelimbic (#115) and secondary motor (#80) sections of the cortex all provided highly Gaussian intensity distributions with $R^2_{\text{Gauss}} > 0.9$. Non-Gaussian skewing of Type II and Type III

distributions, indicates a diversity of vasculature sizes. The variation in qCBV across animals is reasonably tight for Type I regions (Fig. S4), and is larger for Type II and Type III regions. The circumventricular organs, e.g. pineal gland (#101), anterior (#19) and posterior (#92) lobes of the pituitary, are noted for a high density of capillaries and large microvasculature (Gross, 1992) and here show Type II&III distributions. In areas like the rostral (#126) and caudal (#125) retrosplenial cortices, the Type II&III distribution may be partially due to the overlying midsagittal venous sinus embedded into the central sulcus of the brain and intimately connected with the subarachnoid space for the flow of cerebral spinal fluid into venous circulation. The mean qCBV for Type I (uniform capillary) regions is $5.65 \pm 1.81\%$, consistent with morphometric techniques that report a CBV of $\sim 5.2\%$ from capillary-rich brain tissue (Weiss et al., 1982). Averaging across all voxels ($\sim 550,000$) the whole brain yielded a mean of $9.71 \pm 0.90\%$ qCBV.

3.3. Neural Circuits with High Microvascular Density

When the qCBV is plotted against the difference between the mean and median qCBV for each region (Fig. 2c), clear differences are observed between the three types, indicating that this measurement is a robust way to characterize regional variations in skew. The variation between regions should then correlate directly with their average relative metabolic demands as fixed by evolution and development. The overall distribution of regional qCBV values is captured in Fig. 2d and each value is listed explicitly in Dataset 1 in (Gharagouzloo et al., 2017). There are 44 areas with qCBV of 10% or higher (means highlighted in yellow). These areas with highest microvasculature density presumably have high resting state energy consumption due to tonic activity. These areas primarily fall into two distributed integrated neural circuits: 1) the primary sensory systems involving smell, hearing and vision (highlighted in green), and 2) hippocampal complex and cortical areas that transition into the hippocampus, all involved in memory (highlighted in orange, displayed in Fig. 3).

In the sensory system, the regions responsible for smell, i.e. the glomerular (#57: $28 \pm 2\%$) granular (#59: $14 \pm 2\%$), and external plexiform (#53: $21 \pm 4\%$) layers of the olfactory bulbs are noted for their high metabolic activity (Lecoq et al., 2009). The cochlear nucleus (#159: $11 \pm 2\%$), inferior colliculus (#62: $20 \pm 3\%$), and its efferent connections to the medial geniculate (#85: $15 \pm 3\%$) of the thalamus and from there to the auditory cortex (#23: $8 \pm 1\%$) are the key neural nodes in the brain involved in hearing (Malmierca, 2015). Within the thalamus, the superior colliculus (#147: $14 \pm 3\%$), lateral geniculate (#72: $16 \pm 3\%$), lateral and posterior thalamus (#76: $18 \pm 3\%$) have efferent connections to the visual cortices (V1#156: $32 \pm 4\%$, V2#157: $20 \pm 3\%$), the key nodes involved in vision (Sefton et al., 2015). Further analysis was conducted by combining these sensory regions into one larger circuit and the mean qCBV of this circuit was $19 \pm 2\%$.

Many regions involved in memory had relatively high qCBV values. Along the ventral surface of the brain are contiguous cortical areas, entorhinal (#50: $52 \pm 10\%$), perirhinal (#114: $21 \pm 4\%$), entorhinal (#52: $24 \pm 2\%$), and temporal (#150: $30 \pm 6\%$) leading into the ventral subiculum (#170: $15 \pm 2\%$) and from there to the ventral dentate (#41: $19 \pm 2\%$), and ventral CA3 (#29: $12 \pm 2\%$) of the hippocampus (Cappaert et al., 2015). On the dorsal midline surface of the brain there are contiguous cortical areas starting with the anterior

cingulate (#31: $14\pm 2\%$) continuing caudally into the rostral (#126: $27\pm 5\%$) and caudal (#125: $43\pm 5\%$) retrosplenial cortices ending in the ventral subiculum noted above. The subiculum and entorhinal cortex have extensive connections to the mammillary bodies e.g. pre-mammillary (#104: $12\pm 3\%$), (#86: medial mammillary $22\pm 5\%$) and supra mammillary (#149: $21\pm 6\%$) (Vann and Aggleton, 2004) which project to the anterior thalamus (#14: $6\pm 1\%$) and from there to the anterior and retrosplenial cortices, completing a cortical, hippocampal, hypothalamic, thalamic loop as shown in Fig. 3. This neural circuit was found to have $23\pm 2\%$ qCBV as a whole.

3.4. Isoflurane Anesthesia

Sixty-six regions showed statistically significant changes from the resting state under anesthesia (Dataset 2 in (Gharagouzloo et al., 2017), Fig. 4, *IsoChangeAtlas.nii here*). Subjecting the rats to isoflurane anesthesia did not produce a uniform decrease in microvascular CBV as would be predicted by assuming a global decrease in metabolic activity. The overall change in qCBV for the whole brain was only $-0.39\pm 0.45\%$. However, the neural circuits of memory and the senses were sensitive to anesthesia. In the memory circuit (Fig. 3) there was a total decrease of $-1.43\pm 0.96\%$ ($p<0.0001$) with five of its individual component regions also showing significant decreases (Fig. 4). In the primary sensory circuit, there was a total decrease of $-1.44\pm 0.70\%$ ($p<0.0001$) with 10 individual regions also showing significant decreases. One region in this group, the cochlear nucleus (#159), showed a significant increase.

In contrast, the reticular activating system (RAS), a portion of the brainstem involved in the regulation of sleep and attention showed increased qCBV (Dataset 2 in (Gharagouzloo et al., 2017) highlighted in blue). The pontine reticular areas (#s 107,108), locus coeruleus (#70), gigantocellularis (#s 46,56), parvicellular reticular area (#97), raphe (#s 47,121–123), reticulotegmental nucleus (#129), reticular nucleus midbrain (#128), parabrachial nucleus (#96) and pedunculo pontine tegmental area (#117) (Jones, 1995), showed a significant increase of qCBV $0.58\pm 0.50\%$ ($p=0.01$) total relative to the awake state. Additionally, 9 of these areas individually showed statistically significant increases (Fig. 4) and the cochlear nucleus mentioned above is located in close proximity to these regions.

3.5. Carbon Dioxide Challenge

Autoregulation of blood flow through capillaries is sensitive to vasoactive molecules, sympathetic vascular tone and partial pressure of CO_2 . We predicted that CO_2 challenge would induce microvessel dilatation and thereby increase qCBV. When awake rats were exposed to 5% CO_2 , 84 regions showed statistically significant change with $p<0.05$ relative to the resting (awake, breathing air) state (Dataset 3 in (Gharagouzloo et al., 2017), Fig. 5, *CO2ChangeAtlas.nii here*). For the whole brain, rats exposed to 5% CO_2 showed an overall increase of $0.55\pm 0.37\%$ qCBV. Notably, not a single region with a statistically significant change had a decrease in qCBV. The distributed neural circuits for the primary senses and memory were not significantly different from the resting state. However, the primary and secondary somatosensory cortices representing the barrel field, hindlimb, forelimb, shoulder, jaw, and upper lip (#130–137) showed an overall increase of $1.08\pm 0.32\%$ ($p<0.000001$)

when grouped together and every individual region in this area showed a significant increase individually ($p < .05$) (Fig. 5).

4. Discussion

The positive correlation between the density of microvessels, cerebral metabolic activity and glucose utilization is well established (Klein et al., 1986). Areas with high resting state energy consumption require high perfusion and thus require a higher density of microvasculature to maintain function. Hence, density could be used as a proxy to identify brain areas critical to the evolution of brain function and survival. Although similar techniques have been used to produce CBV measurements for larger gross regions of the brain (Han et al., 2015), here we show for the first time that combining QUTE-CE MRI with a detailed atlas provides new insights into brain structure and function, and demonstrates that changes between states can be measured. Other studies which have attempted to absolutely measure CBV on a regional basis have used less than 15 regions (Lear et al., 1990; Julien-Dolbec et al., 2002; Cremer and Seville, 1983; Everett et al., 1956; Adam et al., 2003; Chugh et al., 2009; Wu et al., 2004; Han et al., 2015; Schwarzbauer et al., 1997; Troprès et al., 2001; Watabe et al., 2013). While ours is the first study to provide such regional detail, our findings closely fit the distributions of capillary density reported for Sprague-Dawley rats using $[14C]2$ -deoxyglucose autoradiography (Schwartz and Sharp, 1978) and various morphometric techniques for measuring capillaries (Cavaglia et al., 2001; Zeller et al., 1997; Wagman Borowsky and Collins, 1989).

In our findings, the majority of the regions with highest vascular density and presumably the highest resting state energy consumption were the neural circuits involved in memory and the primary senses of smell, hearing and vision. Indeed, these two circuits together comprise only 28 of the 173 regions in the atlas, but include 26 of the 44 regions with $qCBV > 10\%$ (Dataset 1 of (Gharagouzloo et al., 2017)). Therefore, these two circuits were consistently exceptional in blood volume measurement. The high vascular density in the olfactory, auditory and visual pathways is not unexpected considering the constant metabolic demands of the sensory system; during the awake, conscious state, these sensory modalities are always active for continuous surveillance of the external environment. The high vascular density in the memory circuit should likewise correlate with relatively continuous activity during the conscious state.

Sensory input, and the memory thereof, must be ordered in space and time for conscious experience (Kant, 1930) and the neural circuit for memory is directly connected to the sensory circuit through the entorhinal and perirhinal cortices (Burwell et al., 1995). The hippocampal complex and its connections to the entorhinal cortex, mammillary bodies and thalamus (Fig. 3) provide the physical architecture for awareness in a framework of space and time. We hypothesize that the link between memory and the senses may provide a theoretical foundation for consciousness and that the maintenance of the conscious state necessitates the high vascularization of these circuits.

In the literature on humans, the prevailing theory of consciousness is cortico-centric, both as a neurological construct characterized by the absence of responsiveness as seen in coma or

anesthesia, and consciousness as a metaphysical construct characterized by knowledge of oneself (Hudetz and Mashour, 2016). The cortico-centric theory has the neural correlates of consciousness found in the multisensory association areas of the cerebral cortex, with information integration across the frontal, parietal, temporal and visual cortices (Rees et al., 2002; Aru et al., 2012). The analogous regions in the rat would be: anterior cingulate (#31), frontal association (#55), infralimbic (#63), lateral orbital (#75), secondary motor (#80), medial orbital (#88), prelimbic (#115), parietal (#116), temporal (#150) and visual (V1 and V2 #156–157) cortices. Four of these 11 regions coincide with the sensory (visual 1 and visual 2) and memory (anterior cingulate and temporal) circuitry of the rat. These same four regions had the highest qCBV values of the 11. Further supporting their importance to the conscious state, three showed significant decreases ($p < 0.05$) in qCBV under anesthesia.

Anesthesia is a clear disruption of consciousness and, in humans, the changes in CBV under isoflurane anesthesia are variable. Studies report a decrease in regional blood flow to brainstem, cerebellum, and thalamus, while primary sensory systems like olfaction and vision and the limbic cortex show increased perfusion (Schlünzen et al., 2004, 2006; Alkire et al., 1997; Reinstrup et al., 1995). This variability is also observed in rats as region-specific increases and decreases in glucose utilization and cerebral blood flow (Ori et al., 1986; Lenz et al., 1998). The decreases in cerebral blood flow are likely due to a reduction in metabolism, yet the mechanisms for the mixture of increases and decreases are potentially more complex and undetermined.

In our data, 66 out of 173 brain areas showed significant changes in qCBV when the rat was exposed to isoflurane. Twenty-eight of these areas showed an increase that was primarily associated with brainstem and cerebellum (Dataset 2 of (Gharagouzloo et al., 2017)). The neurobiological effects of anesthesia include analgesia and akinesia on a background of sustained autonomic regulation of respiration, cardiovascular function and temperature (Brown et al., 2011). The mental states that accompany anesthesia include loss of consciousness and memory. The regional changes in qCBV observed in this study would be predictive of the physiological and mental characteristics of anesthesia. There is a significant increase in qCBV in the brainstem reticular formation necessary for maintaining autonomic regulation of respiration, heart rate and blood pressure and decrease in qCBV in the primary senses and hippocampal complex important for consciousness. Of the 27 regions with statistically significant negative qCBV changes of -1% or greater on average, 14 were associated with sensory or memory systems.

Several studies have reported continuous blood flow through capillaries dismissing the notion of intermittent flow and the potential for capillary recruitment as a means of addressing an increase in localized, metabolic activity (Göbel et al., 1989; Kuschinsky, 1996). The velocity of blood flow changes but is never discontinuous. Autoregulation of capillary blood volume to meet metabolic demand is achieved by local vasoactive molecules, sympathetic vascular tone and partial pressure of CO_2 . The study of hypercapnia is interesting because CO_2 leads to region hyperperfusion. The change in blood volume in different brain areas is taken as a measure of cerebral vascular reactivity and perfusion reserve. In this study, rats exposed to $5\% \text{ CO}_2$ during imaging showed a regional increase in CBV in the somatosensory cortex, thalamus, brainstem and cerebellum corroborating human

and animal findings (Ito et al., 2000; Bright et al., 2009; Sobczyk et al., 2014). Perfusion became more homogeneous across the brain during hypercapnic hyperemia as compared to the normocapnic condition which is consistent with the literature (Abounader et al., 1995). The general response to CO₂ challenge was a modest increase in CBV across most of the brain, an average of 0.55% qCBV change. The perfusion reserve for the primary sensory systems and memory systems were 0.44% and 0.15% respectively, and not significantly different from the whole brain response. The primary somatosensory cortex representing the barrel fields, hind and forelimbs, lips and shoulders, collectively had the highest perfusion reserve (>1%) of any brain areas. Unlike the senses of smell, hearing and vision, the peripheral sensations of touch, pain, and temperature are not tonically active but require mechanical, noxious and thermal stimulation, respectively. The tactile sensation of world gleaned from the body surface is no less important than the other sensory modalities for homeostasis but this information is episodic, on and off which might explain its high perfusion reserve.

Importantly, the QUTE-CE MRI method is readily translatable to clinical studies since ferumoxytol is FDA-approved for use in humans and is already used off-label for human MRI (Neuwelt et al., 2009; Weinstein et al., 2010; Weissleder and Nahrendorf, 2015). However, further work may be necessary to facilitate this translation due to the challenges of UTE on clinical scanners. The exact implementation of UTE sequences may vary between manufacturers, which could present a problem in itself, however some challenges will be present for all 3D UTE techniques. First, to cover the center of k-space in 3D radial UTE sequences, it is necessary to sample during gradient ramping (Tyler et al., 2007). This leads to nonlinear sampling of k-space, with oversampling near the center, and reconstruction usually necessitates regridding to a Cartesian grid (Paschal and Morris, 2004). There is a plethora of other technical considerations when designing a 3D UTE pulse sequence, including characterization of gradient imperfections to achieve a robust k-space trajectory (Zhang et al., 1998). In general, clinical machines can perform acquisition at TEs of about 70 μ s, whereas we report 13 μ s but this does not fundamentally alter the technique or its translation.

In summary, a novel imaging protocol, QUTE-CE MRI, was applied to measurement of qCBV across the entire brain. Spatial mapping of qCBV and regional changes to this metric between functional states provided insights into the neural circuits responsible for sensation, memory and consciousness. Given the strong correlation between metabolic activity, capillary density and cerebral blood flow, we used qCBV values as a proxy for microvascular density. Under the awake resting state, qCBV data from over 550,000 voxels was registered to a 3D MRI rat brain atlas with 173 segmented and annotated brain areas creating a global map of microvasculature. From this map we identified two distributed, integrated neural circuits showing the highest capillary densities in the brain. One is the neural circuitry involved with the primary senses of smell, hearing and vision and the other is the neural circuitry of memory. Under isoflurane anesthesia these neural circuits show the greatest decrease in qCBV suggesting they may have a role in consciousness. Neural circuits in the brainstem associated with the reticular activating system in the maintenance of respiration, body temperature and cardiovascular function showed an increase in qCBV with anesthesia. Because QUTE-CE MRI is non-invasive, it offers a unique opportunity for

longitudinal, prospective studies investigating changes in microvasculature that occur naturally over aging or in response to environmental or genetic risk factors as seen in drug addiction, post-traumatic stress disorder, Parkinsons and Alzheimers diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

QUTE-CE MR angiograms in SD rats. All panels show maximum intensity projections (MIPs) made in ParaVision 5.1 of one animal by applying QUTE-CE MRI. From left to right: pre-contrast images of the full field of view (FOV) focused on the rat-head, post-contrast of the full FOV, and post-contrast images of the brain itself cropped out from the adjacent scan. Before ferumoxytol injection (pre-contrast), little vasculature is visible with the exception of large vessels at the periphery, away from the brain, due to inflow TOF effects. After ferumoxytol injection (post-contrast) all vasculature on the order of the resolution is now clearly delineated in the FOV. Within the cranial space, characterized by the atlas, there are a number of large vessels visible which then branch into smaller vasculature of sub-voxel dimensions.

Figure 2.

Summary of the qCBV Atlas characterization. a) The qCBV values mapped onto the atlas in a series of axial slices. The spatial location is provided via the 3D model and slices are labeled with individual regions for ease of interpretation. b) Histograms of the pre- and post-contrast voxel intensity distributions of the three example regions with area normalized to 1. In the pre-contrast (nonshaded) histograms each region is approximately Gaussian. The post-contrast distributions are shifted positively and increase from left to right in skewness. Here, the precontrast SNRs are 3.2, 3.8 and 3.6 respectively while the post-contrast SNRs were 3.9, 10.6, and 12.7 respectively. In the SNR calculation, variance was measured from ROIs in air and corrected via the method in (Gudbjartsson and Patz, 1995). Insets: 3D renderings of each example region from the atlas. c) qCBV per-region as a function of the difference in Mean-Median of the post-contrast intensity distributions. The example regions in (b) are highlighted with separate markers and fall near the borders between the types. Type I regions are closely clustered indicating a limit in qCBV for homogeneous regions around 10%. There is great variety in the Type II regions, indicating the possibility of microvasculature of different sizes. d) histogram of all regional qCBV measurements, the clear majority of regions fall below 10%.

Figure 3.

Neural circuitry involved in memory. All views show the same collection of regions from different perspectives. On the left, views are shown from the anterior and dorsal views on the right from the posterior and ventral. The regions in this circuit had relatively high qCBV values (Dataset 1 (Gharagouzloo et al., 2017)) and had significant responses to isoflurane (Fig. 4).

Figure 4.

Steady-state changes to qCBV under isoflurane. a) qCBV changes for individual animals under anesthesia (n=11) for all regions showing statistically significant changes compared to the awake air-breathing state. b) box plot of individual animals regional qCBV changes for the aggregated neural circuitry involved with the primary senses of smell, hearing and vision followed by the statistically significant changes in component regions of the circuit in descending order of mean. c–e) equivalent figures for regions associated with memory (c), included the somatosensory cortex (d) and in reticular activating system compared to the awake state (e). While sensory and memory systems show significant decreases over all, the reticular activating system shows significant increases.

Figure 5.

Steady-State changes to qCBV under CO₂ challenge. a) qCBV changes for individual animals in the awake hypercapnic state (n=11) for all regions showing statistically significant changes compared to the awake air-breathing state. b–c) Average regional qCBV changes for neural circuitry involved in the somatosensory cortex (b) and the reticular activating system (c) compared to the awake state. In contrast to rats under isoflurane anesthesia, the entire somatosensory cortex, when treated as a whole or in its component regions, shows an increase in qCBV. The reticular activating system shows similar increases to the anesthetized state, reconfirming that there is a significant perfusion reserve which we see utilized under isoflurane and CO₂ challenge.