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A comparison study between the saturation-recovery- T_1 and CASL MRI methods for quantitative CBF imaging

Xiao Wang^{a,b}, Xiao-Hong Zhu^a, Yi Zhang^a, Afshin A. Divani^c, Amanda J. Murphy^c, and Wei Chen^{a,*}

^aCenter for Magnetic Resonance Research, Department of Radiology, University of Minnesota Medical School, Minneapolis, MN, USA

^bDiagnostic Radiology Residency Program, Department of Radiology, University of Minnesota Medical School, Minneapolis, MN, USA

^cDepartment of Neurology, University of Minnesota Medical School, Minneapolis, MN, USA

Abstract

The saturation-recovery (SR)- T_1 MRI method for quantitatively imaging cerebral blood flow (CBF) change (CBF) concurrently with the blood oxygenation level dependence (BOLD) alteration has been recently developed and validated by simultaneous measurement of relative CBF change using laser Doppler flowmetry (LDF) in rats at 9.4T. In this study, CBF induced by mildly transient hypercapnia and measured by the SR- T_1 MRI method was rigorously compared with an established perfusion MRI method—continuous arterial spin labeling (CASL) approach in normal and preclinical middle cerebral artery occlusion (MCAo) rat models. The results show an excellent agreement between CBF values measured with these two imaging methods. Moreover, the intrinsic longitudinal relaxation rate (R_1^{int}) was experimentally determined in vivo in normal rat brains at 9.4T by comparing two independent measures of the apparent longitudinal relaxation rate (R_1^{app}) and CBF measured by the CSAL approach across a wide range of perfusion. In turn, the R_1^{int} constant can be employed to calculate the CBF value based on the R_1^{app} measurement in healthy brain. This comparison study validates the fundamental relationship for linking brain tissue water R_1^{app} and cerebral perfusion, demonstrates the feasibility of imaging and quantifying both CBF and its change using the SR- T_1 MRI method in vivo.

Keywords

Perfusion; Cerebral blood flow; Saturation-recovery- T_1 MRI; CASL MRI; Rat brain; Middle cerebral artery occlusion

1. Introduction

Cerebral blood flow (CBF) and its change closely reflect the energy metabolism and perfusion demands of neuronal activity, and cerebral vascular diseases may cause severe

*Correspondence author at: Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota Medical School, 2021 6th St. SE, Minneapolis, MN 55455, USA. wei@cmrr.umn.edu (W. Chen).

neurological impairment. The apparent longitudinal relaxation rate (R_1^{app}) or time ($T_1^{\text{app}} = 1/R_1^{\text{app}}$) is not only a basic magnetic resonance imaging (MRI) parameter for brain tissue contrast, but also plays an important role in quantifying and imaging CBF [1–3]. One hallmark at an early stage of ischemia shows an increase T_1^{app} in the injured brain regions, indicating a possible link between the brain tissue T_1^{app} change and the perfusion impairment caused by the ischemia [4–7]. We have developed the saturation-recovery (SR)- T_1 MRI method for concurrently imaging CBF change (ΔCBF) and blood oxygenation level dependence (BOLD), and have demonstrated that the R_1^{app} change caused by global CBF reduction using an acute four-vessel occlusion rat model was tightly linked to ΔCBF [8].

The mathematical derivation for the underlying theory of the SR- T_1 MRI approach for measuring ΔCBF and BOLD has been described in details previously [8]. Briefly, the water spins within the saturated brain tissue exchange with the fully relaxed and flowing water spins from the outside of the saturation region through blood circulation, resulting in an alteration on the R_1^{app} or T_1^{app} . The difference of R_1^{app} (ΔR_1^{app}) measured under two physiopathological conditions (e.g., normocapnia versus hypercapnia) can be used to quantify the corresponding CBF change. The functional MRI (fMRI) BOLD signal measured by the SR- T_1 MRI acquired under near fully relaxed condition of tissue water proton spins (i.e., with a relatively long saturation recovery time) provides “true” BOLD contrast without the confounding effect associated with the perfusion alternation [9]. We have validated this MRI method by comparing the ΔCBF value based on the R_1^{app} measurement with simultaneous monitoring of relative CBF change using laser Doppler flowmetry (LDF) with both physiology (hypercapnia induced CBF increase) and pathology (acute global brain ischemia-induced CBF reduction) perturbations, and the estimated baseline CBF values from these two complementary measurements coincided with the results of the majority of literature reported for the rat brain under similar animal conditions [9–14].

In this study, we first compared the SR- T_1 MRI method for imaging ΔCBF with an established perfusion MRI technique—continuous arterial spin labeling (CASL) method on both normal and unilateral middle cerebral artery occlusion (MCAo) rats to further validate the SR- T_1 MRI method and exploit its utility of studying perfusion changes associated with brain physiopathology at 9.4 Tesla (T). Secondly, by comparing the experimentally measured R_1^{app} (using the SR- T_1 MRI method) and CBF (using the CASL MRI method) values across a wide range of perfusion, we were able to quantitatively determine the intrinsic longitudinal relaxation rate (R_1^{int}) at 9.4T; validate the fundamental relationship of linking R_1^{app} and CBF; and demonstrate the feasibility of quantifying and imaging absolute CBF value at a given physiological condition using the SR- T_1 MRI method.

2. Methods

2.1. SR- T_1 MRI method for quantitatively imaging CBF change

The SR- T_1 MRI pulse sequence consists of a global brain saturation preparation achieved by a radiofrequency (RF) surface coil associated with an adiabatic RF pulse followed by

magnetization acquisition with fast echo-planar imaging (EPI) [15] sampling after waiting a serial of saturation recovery time (T_{SR}). The fully relaxed arterial blood water spins flowing into the saturated brain region and rapidly exchanging with the saturated brain tissue water spins result in an enhanced R_1^{app} (or a shortened T_1^{app}) of brain tissue [8]. The T_1^{app} value regressed from the saturation recovery magnetizations with various T_{SR} values can be linked to CBF according to the following equation [2,16]:

$$\frac{1}{T_1^{app}} = \frac{1}{T_1^{int}} + \frac{CBF}{C \cdot \lambda} \quad \text{or} \quad R_1^{app} = R_1^{int} + \frac{CBF}{C \cdot \lambda} \quad (1)$$

where T_1^{int} (R_1^{int}) is the intrinsic longitudinal relaxation time (rate) of brain tissue water, which is an intrinsic characteristic of the tissue and can be treated as a CBF-independent constant in healthy brain; λ is the tissue blood partition coefficient for water with a value of 0.9 ml/g commonly used in the literatures [17–19]; C is a unit conversion factor (60 s/min) and leads to the conventional CBF unit as ml/g/min. An increased CBF, for instance, caused by hypercapnia or brain stimulation leads to an accelerated apparent longitudinal relaxation rate ($R_1^{app'}$). Therefore, the CBF increase under hypercapnia or stimulation can be calculated from the R_1^{app} difference (ΔR_1^{app}) between the normocapnia (or control) and hypercapnia (or stimulation) conditions according to the following equation:

$$\Delta R_1^{app} = R_1^{app'} - R_1^{app} = \frac{\Delta CBF}{C \cdot \lambda} \quad \text{or} \quad \Delta CBF = C \cdot \lambda \cdot \Delta R_1^{app} \quad (2)$$

Note that both Eqs. (1) and (2) are also valid for the case of CBF reduction, for instance, induced by global or regional brain ischemia [8].

2.2. Calculation of baseline CBF and CBF change using the CASL MRI method

The CASL imaging method was first introduced in 1990s [16,20–23], and it enables the measurement of CBF under a steady-state condition using a long RF pulse to label and invert the magnetizations of arterial blood water spins with a high contrast to noise ratio (CNR) as compared with the pulsed ASL (PASL) method [1,24–26]. Since the magnetization transfer effect between brain water and macromolecules is negligible with a two-coil system configuration [27,28], the CBF value in an absolute quantification scale and unit of ml/g/min can be determined from a pairwise subtraction of the labeled and control image on a pixel-by-pixel basis, according to the following equation [23,28,29]:

$$CBF = \frac{\lambda}{T_1^{int}} \cdot \frac{S_C - S_L}{S_L - (2 \cdot \alpha - 1) \cdot S_C} = \lambda \cdot R_1^{int} \cdot \frac{S_C - S_L}{S_L - (2 \cdot \alpha - 1) \cdot S_C} \quad (3)$$

where S_C and S_L are signal intensity of the image without and with the RF spin labeling respectively; α is the effective efficiency coefficient of the arterial spin labeling and it is determined by:

$$\alpha = \alpha_1 \cdot \frac{TL}{TR} \quad (4)$$

where α_1 is the initial degree of spin labeling measured at the labeling plane, which equals to 1 for perfect inversion magnetization preparation; TL/TR is the duty cycle of the labeling, where TL is the length of labeling RF pulse, TR is the repetition time of acquisition. The

CBF value simply equals the difference between the CBF values measured under two pathophysiological conditions according to Eq. (3).

Eq. (3) can be further modified as the following, CBF

$$\frac{CBF}{\lambda} = R_1^{int} \cdot \frac{S_C - S_L}{S_L - (2 \cdot \alpha - 1) \cdot S_C} \quad (5)$$

by combining Eqs. (1) and (5)

$$R_1^{app} = R_1^{int} + \frac{R_1^{int}}{C} \cdot \frac{S_C - S_L}{S_L - (2 \cdot \alpha - 1) \cdot S_C} \quad (6)$$

or

$$R_1^{app} = R_1^{int} (1 + S) \quad (7)$$

where

$$S = \frac{1}{C} \cdot \frac{S_C - S_L}{S_L - (2 \cdot \alpha - 1) \cdot S_C}$$

then

$$R_1^{int} = \frac{R_1^{app}}{(1 + S)} \quad (8)$$

Theoretically, R_1^{int} is independent on cerebral perfusion or its change, and can be treated as a constant under physiology condition. This relationship was tested in the present study using

independent measures of the R_1^{app} values using the SR- T_1 MRI method and the S values associated with the CBF measurements using the CASL MRI method across a wide range of CBF.

2.3. Animal preparation and experiment procedures

All animal experiments were conducted according to the National Research Council's Guide for the Care and Use of Laboratory Animals and under the protocols approved by the Institutional Animal Care and Use Committee of University of Minnesota. There were nine male normal Sprague-Dawley rats weighing 299 ± 59 g and ten MCAo rats with one-hour occlusion using a newly developed transfemoral approach [30] weighing 384 ± 45 g included in the present study. Initially, each rat was anesthetized and intubated using 5% (v/v) isoflurane in $N_2O:O_2$ (60/40) gas mixture. The animal was placed in a custom-built cradle incorporating ear bars and a bite bar to minimize head movement and to ensure proper positioning inside the MRI scanner. Anesthesia was maintained at 1.8–2% isoflurane level during MRI experiments.

The mild transient hypercapnia was introduced by switching to a gasbag filled with a combination of 6% (or 3%) CO_2 , 34% O_2 , 58% N_2O and 2% isoflurane for about 10 min. The hypercapnia studies were repeated two to four occurrences in seven of the normal rats to test the response extent and reproducibility of the CBF increase measured with two CBF imaging methods. The MCAo rats were scanned at 1 day post MCAo, whereas the hypercapnia experiment (6% CO_2) was performed only once in this animal group. Rectal temperature was monitored and maintained at 37.0 ± 0.5 °C with a homemade circulating/heating water blanket, and the inspired and expired gases were monitored during the entire MRI experiments.

2.4. MRI experiment and acquisition parameters

MRI experiments were conducted in a 9.4T horizontal animal magnet (MagneX Scientific, Abingdon, UK) interfaced to a Varian INOVA console (Palo Alto, CA, USA). A butterfly-shape surface coil (long axis of 2.8 cm and a short axis of 2.0 cm that is parallel to the animal spine) was used to collect the MRI data using both the SR- T_1 and CASL imaging methods. A separate single loop coil (2.5 cm $\times$ 1.2 cm) was used for carotid arterial spin labeling to eliminate the magnetization transfer effect between tissue water and macromolecules [23,27]. The distance between labeling plane and the brain image was adjusted to about 2 cm to reduce the interaction between the two RF coils.

Control rat brain scout images were acquired using a turbo fast low angle shot (TurboFLASH) imaging sequence [31] with the following acquisition parameters: TR=10 ms, echo time (TE)=3.1 ms, image slice thickness = 2 mm, field of view (FOV) = 3.0 cm $\times$ 3.0 cm and image matrix size = 128 $\times$ 128. A modified TurboFLASH sequence was used for the CASL MRI measurement. The labeling efficiency α_1 was measured using a 500 ms RF inversion preparation at 0.5 cm upstream of the carotid labeling plane followed by the TurboFLASH sequence at the carotid arterial labeling plane with TR = 530 ms and TE = 4 ms. Specifically, the labeling efficiency was calculated from the signal intensity in the two-side carotid arteries with and without turning on the inversion RF pulse power. The duration

of the RF labeling pulse (TL) was 2.2 s and TR was 3 s, resulting in a duty cycle of 0.73. The other acquisition parameters for perfusion imaging were: 30 ms TE, 3.0 cm $\times$ 3.0 cm FOV, 64 $\times$ 64 image matrix size resulting in a 0.47 mm $\times$ 0.47 mm in-plane image resolution. In addition, the labeling pulse was also applied at a plane equilateral symmetry to the brain image plane to balance the off-resonance effect of the static spins in the brain image plane, serving as a control image. The labeled and control brain images were acquired in an interleaved fashion.

For the SR-T₁ MRI experiment, the global brain saturation was achieved with an adiabatic 90° pulse (4 ms pulse width) followed by three orthogonal crush gradients with 3 ms length. Gradient-echo (GE) EPI (TE=21 ms; variable TRs; FOV=3.0 cm $\times$ 3.0 cm; image matrix size = 64 $\times$ 64; single slice coronal image with 2 mm thickness) combined with the saturation-recovery preparation was used to image R_1^{app} with nine T_{SR} values of 0.008, 0.1, 0.2, 0.3, 0.4, 0.5, 1.4, 3 and 10 s [8].

The T₂-weighted images were acquired with a fast spin echo sequence (TE = 10 ms; TR = 4 s; FOV = 3.2 cm $\times$ 3.2 cm; matrix = 256 $\times$ 256; thickness = 1 mm; 8 echoes train length) and served as the anatomic images of the MCAo rats. Both the CASL and the SR-T₁ MRI measurements were acquired under stable normocapnia or hypercapnia condition for about 2 min, corresponding to 10 CASL image volumes (1 CASL image volume resulted from the subtraction of 2 nonlabeling images and 2 labeling images) and 8 SR-T₁ image volumes, respectively.

2.5. Data analysis

MRI data analysis was performed using the STIMULATE software package (Stimulate, Center for Magnetic Resonance Research, University of Minnesota, USA) [32] and the Matlab software package (The Mathworks Inc., Natick, MA, USA). The least-square nonlinear curve-fitting program using the Matlab software was applied to perform the R_1^{app} regression analysis [8]. The regression accuracy was estimated by the sum squared error (SSE) and the coefficient of determination (R^2). Region of interest (ROI) taken from the rat somatosensory cortex was used to perform the R_1^{app} regression analysis and to calculate ΔR_1^{app} , and the CBF value according to Eq. (2) for quantitative CBF comparison with the CASL MRI method. Two additional subcortical ROIs (with different lesion severity) in MCAo rats were identified to quantitatively evaluate the R_1^{app} , ΔR_1^{app} and CBF based on the SR-T₁ MRI method. Identical ROIs were used to calculate the CBF and CBF with the CASL MRI method according to Eq. (3).

The CBF maps between hypercapnia and normocapnia were created on a pixel-by-pixel basis (pixel size 0.23 $\times$ 0.23 $\times$ 2 mm³ for normal rats, 0.25 $\times$ 0.25 $\times$ 1 mm³ for MCAo rats with the nearest neighbor interpolation) with two-dimensional median filtering and then overlapped on their corresponding anatomic images. Correlation coefficient and its associated p value of ROI based CBF values between the SR-T₁ MRI method and the CASL MRI method were calculated. The coefficient of determination R^2 was also used to judge the linear regression accuracy of R_1^{app} (measured with the SR-T₁ MRI method) versus baseline CBF (measured with the CASL MRI method), and CBF calculated with the SR-T₁

MRI method versus that calibrated with the CASL MRI method. Paired t -test was used to compare CBF calculated with the SR-T₁ MRI method and with the CASL MRI method in the same ROI, and to compare CBF in the homologous regions at the lesion side and the control side of MCAO rats. A p value of <0.05 is considered as statistically significant.

3. Results

The measured initial spin inversion efficiency (α_1) for the CASL MRI measurements was 0.76 ± 0.02 ($n=9$), which leads to the effective efficiency of the arterial spin labeling α as 0.56 according to Eq. (4). The degree of α_1 measured in this study is consistent with previously reported values, ranging from 0.71 to 0.82 [4,21,23]. It has been demonstrated that the degree of inversion efficiency does not affect the outcome of CBF quantification as long as it is determined and counted in the perfusion calculating model [33].

Fig. 1 shows the linear regression results of CBF measured with the SR-T₁ MRI method (CBF_{SR-T1}) versus the CASL MRI method (CBF_{CASL}) in the somatosensory cortex regions of a representative normal rat for four occurrences of hypercapnia during the same MRI session. Both regressed results based on the pixel by pixel analysis (Fig. 1a) and mean value (Fig. 1b) within the ROI under four occurrences of hypercapnia reveal a strong ($p < 0.01$), positive correlation between CBF values imaged with the SR-T₁ and CASL MRI methods across a wide range of CBF change approximately with a unity slope and near zero intercept. It also demonstrates that both imaging methods are sensitive to the CBF change induced by varied level of inhaled CO₂ concentration, for instance, the CBF increase was more than double under 6% CO₂ hypercapnia compared to 3% CO₂ of hypercapnia.

Fig. 2 demonstrates a coronal anatomical image and corresponding CBF maps imaged by the SR-T₁ MRI method (top row) and the CASL MRI method (bottom row), respectively, for four occurrences of hypercapnia from a representative (normal) rat. Repeatable, similar spatial pattern and magnitude of CBF increase were observed with these two imaging methods with the similar degree of CO₂ inhalation performed at different time.

Fig. 3a shows an excellent positive correlation between CBF measured with the SR-T₁ MRI method and those values obtained with the CASL MRI method in somatosensory cortex from 9 rats with 20 occurrences of different levels of hypercapnia. The consistency of the CBF measurements between the two imaging methods was quantitatively evaluated and the Bland-Altman plot between the difference and the mean of CBF calculated with these two techniques is shown in Fig. 3b. The mean (black line in Fig. 3b) and the standard deviation (SD) of the difference between the CBF values measured with the two imaging techniques were 0.087 ml/g/min and 0.12 ml/g/min, respectively. The gray lines in Fig. 3b represent the limits of consistency where 95% of the difference values are expected to be less than two SD, and all of the data points were within the range, demonstrating an excellent consistency between the CBF results measured with these two imaging techniques.

Fig. 4a shows the independence of R_1^{int} on various R_1^{app} induced by CBF changes based on the results of linear regression according to Eq. (8) ($R^2 = 0.061$; $p = 0.16$). This

demonstrates that R_1^{int} indeed can be treated as a perfusion-independent constant under normal physiology condition. The averaged R_1^{int} value based on 34 measurements from 7 rats was $0.46 \pm 0.01 \text{ s}^{-1}$ (SD) at 9.4 T. These results provide critical experimental evidence in supporting the theoretical relationship of connecting R_1^{app} and CBF (Eq. (1)), as well as the CASL CBF quantification method as described by Eq. (3). Importantly, the R_1^{int} value derived in this study can be employed to quantify the absolute CBF value based on the R_1^{app} value measured by the SR-T₁ MRI method according to Eq. (9) (modified from Eq. (1)):

$$\text{CBF} = C \cdot \lambda \cdot (R_1^{\text{app}} - R_1^{\text{int}}) \quad (9)$$

Fig. 4b displays the linear correlation between the CBF values measured by the SR-T₁ MRI method ($\text{CBF}_{\text{SR-T}_1}$) according to Eq. (9) and the CASL MRI approach (CBF_{CASL}) according to Eq. (3) with an excellent correlation coefficient and high significance ($R^2 = 0.822$; $p = 9.7 \times 10^{-14}$) as expected. This result indicates the feasibility of the SR-T₁ MRI method for measuring and imaging the absolute CBF value in addition to the CBF change.

Fig. 5 shows three contiguous coronal brain slices of the T₂-weighted anatomic images (top row), CBF images induced by hypercapnia and measured by the CASL MRI method (middle row) and with the SR-T₁ MRI method (bottom row), respectively, from a representative MCAo rat scanned on 1 day after 1-hour occlusion. The stroke lesion areas show hyper-intensity in T₂-weighted images, mostly localized in the right-hemisphere sub-cortex region of the rat brain (i.e., the left side of brain image in Fig. 5). Impaired vascular response to a CO₂ vascular challenge was observed clearly in the lesion side of the rat brain in the CBF maps imaged by both the CBF imaging methods. Interestingly, the areas with compromised vascular reactivity in response to a CO₂ challenge, as delineated by the white lines shown in Fig. 5, spread beyond the regions of the sub-cortical lesions seen in the corresponding T₂-weighted images. This result indicates the extensive involvement of the impaired vascular response to the ischemic attack induced by the MCA occlusion. Table 1 summarizes the mean and SD of CBF induced by hypercapnia in different ROIs identified in Fig. 5 and measured by the SR-T₁ MRI method and with the CASL MRI method in ten MCAo rats on day 1 of post-occlusion. The CBF values in three ROIs selected from somatosensory cortex, periphery and core brain regions in subcortex at the lesion side were significantly smaller than those in their homologous ROIs on the control side ($p < 0.01$). Importantly, the CBF results measured with these two imaging methods were highly consistent in different ROIs with varied lesion severity at the lesion side and from their corresponding ROIs at the control side.

4. Discussion

ASL is a noninvasive MRI technique for imaging perfusion in which the moving water spins in the flowing arterial blood are labeled by RF pulses as an endogenous perfusion contrast [16,20,24,25]. The magnetization difference between the paired-images (one with “spin tagging” and one without) is proportional to its perfusion and can be used to quantify CBF. Instead of focusing the magnetization change by delivering the tagging spins to the image

slice as applied in the ASL technique, the T_1 perfusion model [2,3] views CBF as an enhanced longitudinal relaxation through $R_1^{\text{app}}/T_1^{\text{app}}$ change as defined in Eq. (1). In the SR- T_1 MRI method, the R_1^{app} difference measured between two steady-state physiology and/or pathology conditions enables a quantitative link of ΔR_1^{app} to CBF [8].

We have validated the SR- T_1 MRI method for quantitatively imaging CBF with simultaneous LDF measurement (relative CBF change) under both the hypercapnia and global brain ischemia using a rat model at 9.4T [8]. This quantitative CBF imaging method was further evaluated with the two-coil CASL MRI method by directly comparing the perfusion change in an absolute scale in both normal and MCAo rat brain within the same MRI session in the present study. An excellent agreement of rat brain CBF increase induced by hypercapnia was observed between the two imaging measurements using the SR- T_1 and CASL MRI methods. Firstly, an excellent pixel-wised CBF correlation (Fig. 1a) between these two methods was shown in the rat brain somatosensory cortex with different levels of transient hypercapnia. Secondly, the spatial pattern of the whole brain CBF maps imaged using the SR- T_1 and CASL MRI methods was also highly similar for varied degrees of hypercapnia although the deep brain region on the CBF maps measured with the SR- T_1 MRI method was less uniform (Fig. 2), probably due to the high demand of homogeneousness of the RF field (B_1) and static field (B_0) for achieving high quality of EPI compared to the CASL MRI approach based on the TurboFLASH MRI scheme. Comparable imaging spatial pattern and magnitude of CBF in response to hypercapnia were also observed in the MCAo rats (Fig. 5 and Table 1). Furthermore, the amount of increased CBF correlated well with the concentration of CO_2 of the hypercapnia being used, i.e., the higher the CO_2 concentration the more the corresponding CBF increased. These results indicate that both the SR- T_1 MRI method and the CASL MRI method are sensitive for CBF change and are able to provide reliable measures. It is also interesting to note that the degree of CBF increase was close but not exactly identical for the repeated measurements at the same level of mild hypercapnia. For instance, the perfusion increased more for the second occurrence of 6% hypercapnia, while it increased less for the second occurrence of 3% hypercapnia when compared with the first occurrence of same level CO_2 concentration hypercapnia (Figs. 1 and 2). This observation shows the complicated whereas delicate cerebral perfusion regulation ability of living brains when the multiple vascular challenges are encountered.

Based on the results of R_1^{app} measured with the SR- T_1 MRI method and CBF measured independently with the CASL MRI method, we were able to test and validate the relationships linking R_1^{int} , R_1^{app} and CBF as described by Eqs. (1) and (8). The regression result shown in Fig. 4a clearly indicates the independence of R_1^{int} on R_1^{app} and the excellent consistence of the relationship across a wide range of physiology condition. The averaged R_1^{int} value of 0.46 s^{-1} is coincident with the previously reported R_1^{int} value as $\sim 0.5 \text{ s}^{-1}$ measured with various MRI techniques at 9.4T [34,35]. The R_1^{int} value was employed to quantify the absolute CBF values based on the R_1^{app} measurement using the SR- T_1 MRI method and derived Eq. (9). These values show a linear correlation to the CBF results measured by the CASL approach with excellent correlation coefficient and statistical significance, unity of slope and near zero interception (Fig. 4b). These findings corroborates

that the T_1 perfusion model is valid for CBF quantification and the CBF change indeed could be viewed as a modulator of T_1 process. In addition, aside from CBF change, the baseline CBF value can also be estimated according to Eq. (1) or Eq. (9) using the SR- T_1 MRI method once the R_1^{int} value is determined at a given magnetic field strength (9.4T as demonstrated in this study). R_1^{int} is an intrinsic property of tissue and can be treated as a constant, both from theoretical and experimental perspective (Fig. 4a). Therefore, the SR- T_1 MRI method could offer a simple and noninvasive tool to measure both CBF in normal rat brain (when the R_1^{int} is known) and CBF change in response to physiological and pathological changes. Nevertheless, caution needs to be exercised as the R_1^{int} obtained in the present study is only valid at 9.4T since R_1^{int} is a magnetic field dependent parameter just as R_1^{app} . On the other hand, the similar approach could be applied to other field strengths to determine the R_1^{int} at any given field strength.

Under diseased conditions such as the preclinical MCAo rat stroke model in this study, the relationship between R_1^{app} and CBF could become more complicated and may not simply follow Eq. (1). This is because the brain underwent stroke experienced sophisticated hemodynamic (elongated transit time, CBF decrease initially and reperfusion, etc.) and pathophysiological evolution (edema, infarction, inflammation, etc.) [36–39]. Consequently, the biophysical property of the brain tissue water might be changed chronically which could lead to a deviation of R_1^{int} , for instance, caused by cell swelling and tissue water density changes. The pathology related R_1^{int} change that is independent from perfusion change is very intriguing and can provide great clinical value due to the fact that it may serve as a noninvasive biomarker to quantitatively monitor the pathological evolution and assess the efficacy of treatment [40]. Combined MRI and histological studies are helpful for quantitatively understanding the pathology evolution associated R_1 changes in MCAo rats.

The SR- T_1 MRI method is suitable for imaging CBF and BOLD changes in response to pathophysiology challenges, and parametric R_1^{app} simultaneously. Parametric R_1/T_1 image reflects the inherent biophysical property of tissue and is of great importance to tissue characterization, imaging parameter optimization, CBF measurement and quantification. In addition, one of the serial SR- T_1 image sets with the longest saturation-recovery time could be useful to calculate the “true” BOLD contrast without the confounding effect from the CBF change [9]. Compared with the CASL technique, the SR- T_1 MRI method has the advantages of simple experiment setup using a single RF surface coil similar with PASL methods. A key merit of the SR- T_1 MRI method is less sensitive to the variation of artery transition time which could become an issue for human brain application with a much longer artery transition time as compared to rodents. However, one unsolved question is if the SR- T_1 MRI method could provide a comparable contrast-to-noise ratio (CNR) for imaging CBF as the CASL MRI approach. Since two different MRI schemes were employed in this study for acquiring SR- T_1 and CASL imaging datasets, it was challenging to quantify and compare the CNR difference between the two MRI methods.

5. Conclusion

In summary, the SR-T₁ MRI method for imaging CBF change is further validated by direct comparison with two-coil system CASL MRI method for both normal and preclinical MCAo rats in the present study. The results are statistically identical between the two imaging methods. We also demonstrate the feasibility of the SR-T₁ MRI method for quantifying the baseline CBF in healthy brain. The overall findings from the serial studies of the SR-T₁ MRI method collectively demonstrate that the SR-T₁ MRI method should provide a valid, effective and noninvasive neuroimaging tool to quantitatively image baseline CBF in normal brain, CBF and BOLD changes under both physiological conditions and pathological perturbations.

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Fig. 1.

Linear regression results of rat brain somatosensory cortex CBF measured with the SR-T₁ MRI method (ΔCBF_{SR-T1}) versus the CASL MRI method (ΔCBF_{CASL}) from a representative normal rat for four occurrences of hypercapnia. (a) Pixel-wised linear regression results of CBF measured with the two imaging methods in the brain cortex ROI (delineated with the green line) shown in the anatomic brain image. (b) Linear regression results of the mean of CBF in ROI (shown in a) measured with the SR-T₁ MRI method versus that measured with the CASL MRI method. SD bars are also shown. Different colors represent four occurrences of hypercapnia: 6% CO₂ was used in the 1st (blue) and 2nd (red) occurrences of hypercapnia; 3% CO₂ was used in the 3rd (black) and 4th (green) occurrences of hypercapnia. The p and R^2 values of linear regression correlation coefficient of CBF measured with the SR-T₁ MRI method versus the CASL MRI method are indicated in (a) (pixel by pixel wise) and (b) (mean CBF), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Anatomic image (left panel) and CBF maps imaged by the SR-T₁ MRI method (top row) and the CASL MRI method (bottom row) from a representative normal rat for four occurrences of hypercapnia. 6% CO₂ was used in the 1st and 2nd occurrences of hypercapnia; 3% CO₂ was used in the 3rd and 4th occurrences of hypercapnia.

Fig. 3.

(a) Linear regression results of rat brain somatosensory cortex CBF measured with the SR-T₁ MRI method versus the CASL MRI method from 9 rats with 20 occurrences of hypercapnia. The p and R^2 values of linear regression correlation coefficient of CBF measured with the SR-T₁ MRI method versus the CASL MRI method are indicated. (b) The Bland-Altman plot of the difference versus the mean values of the CBF measured with the SR-T₁ MRI method and with the CASL MRI method. The black line indicates the mean (0.087 ml/g/min) of the difference of the CBF measured with these two methods and the gray lines define the limits of agreement where 95% of the difference values are expected to be less than two SD (0.12 ml/g/min).

Fig. 4.

(a) Linear regression to test the relationship between R_1^{int} and R_1^{app} , and to determine the R_1^{int} value at 9.4T. (b) Linear regression between the CBF values imaged by the SR-T₁ MRI method ($\text{CBF}_{\text{SR-T1}}$) and the CASL MRI method (CBF_{CASL}) across a wide range of CBF change induced by hypercapnia.

Fig. 5.

Three contiguous coronal brain slices of the T₂-weighted images, CBF maps induced by hypercapnia and imaged with the SR-T₁^{MRI} method and the CASL MRI method independently from one representative rat scanned on day 1 after 1-hour MCA occlusion. The color squares overlapped on the T₂-weighted images are identified ROIs at cortex (green), peripheral lesion area (red) and core lesion area (blue) at ipsilateral (lesion) side and corresponding ROIs of the contralateral (control) side. The quantitative CBF in different ROIs measured with both imaging methods are summarized in Table 1. L: left hemisphere; R: right hemisphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Summary of mean $\pm$ SD of the CBF induced by hypercapnia in different ROIs calculated with the SR-T₁ MRI method and with the CASL MRI method, respectively. The color indicator shown in the ROI column matches the ROI indicated in Fig. 5. Paired *t*-test was used to compare CBF measured with two imaging methods in the same ROI, *p* value ranges from 0.25 to 0.74 (*n* = 10, mean $\pm$ SD), indicating no statistically difference between them.

ROI (pixels)	Lesion side		Control side	
	CBF (ml/g/min) (SR-T ₁ method)	CBF (ml/g/min) (CASL method)	CBF (ml/g/min) (SR-T ₁ method)	CBF (ml/g/min) (CASL method)
Cortex (17 $\pm$ 8); green	0.65 $\pm$ 0.35	0.61 $\pm$ 0.36	1.00 $\pm$ 0.44	0.96 $\pm$ 0.32
Peripheral (12 $\pm$ 8); red	0.04 $\pm$ 0.47	0.09 $\pm$ 0.14	0.80 $\pm$ 0.35	0.76 $\pm$ 0.24
Core (8 $\pm$ 4); blue	0.00 $\pm$ 0.29	0.00 $\pm$ 0.09	0.82 $\pm$ 0.49	0.60 $\pm$ 0.25