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Optimization of Phase-contrast MRI for the Estimation of Global Cerebral Blood Flow of Mice at 11.7T

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Abstract

Purpose: To optimize phase-contrast (PC) MRI for the measurement of global cerebral blood flow (CBF) in mouse at 11.7T.

Methods: We determined proper encoding velocity (VENC) for internal carotid arteries (ICAs) and vertebral arteries (VAs). Next, we optimized spatial resolution of the sequence. To shorten scan time without compromising data quality, we further optimized repetition time and developed a reduced FOV scheme for ICA and VA PC MRI. Whole-brain volume was determined with T₂-weighted image to obtain unit-volume CBF.

Results: Peak flow velocities were 13.8 ± 1.7 , 14.4 ± 0.6 , 6.5 ± 1.7 , and 6.7 ± 1.3 cm/s for left ICA, right ICA, left VA, and right VA, respectively. Thus, VENC values of 20 cm/s and 10 cm/s were chosen for ICA and VA PC MRI, respectively. An in-plane spatial resolution of $50 \times 50 \mu\text{m}^2$ was found to provide a reasonable tradeoff between reducing partial-volume effects and maintaining signal-to-noise ratio. Due to the fact that saturated spins in the imaging slice are rapidly replaced by fresh spins, TR of the sequence can be decreased to as short as 15 ms without reducing signal intensity, thereby substantially lowering scan time. Moreover, reduced FOV along the phase-encoding direction was able to shorten scan time by 33.3% while maintaining measurement accuracy. With these optimizations, it took 96 sec to evaluate CBF with a test-retest variability of approximately 5% and an inter-rater correlation of >0.95 . Global unit-volume CBF was found to be 279.5 ± 11.1 ml blood/100 ml tissue/min.

Conclusion: We have optimized PC MRI for non-invasive quantification of blood flow in mice at 11.7T.

Keywords

cerebral blood flow; internal carotid artery; vertebral artery; maximum blood velocity; spatial resolution; partial volume effect; spatial folding

INTRODUCTION

Cerebral blood flow (CBF), which denotes the amount of blood supplying the brain per unit time (1,2), is one of the most fundamental physiological parameters of the brain. Measurement of CBF in mouse models has broad potential applications. For example, CBF may provide an important biomarker in many neurological and psychiatric diseases (3–9). CBF is also important in quantitative interpretation of functional MRI results (10–12). Furthermore, since virtually all contrast agents and drugs are delivered to the brain by blood, evaluation of the efficacy of such agents should be conducted in the context of CBF. CBF may also provide an early index of toxicity in that it will inform us about the extent to which the agent affects physiology.

The gold standard for in vivo CBF quantification is positron emission tomography (PET), which estimates CBF by measurement of ^{15}O -labeled radiotracer in the brain while simultaneously determining tracer concentration by arterial blood sampling (13,14). However, continuous blood sampling is not feasible for mouse studies because there is simply not enough blood to be sampled due to its limited total blood volume of 1–2 ml (15). For MRI-based techniques, arterial-spin-labeling (ASL) has been used as a non-invasive approach to measure CBF in mice (16). However, its reliability for absolute quantification of CBF has not been established in mouse imaging, due to several confounding factors such as labeling efficiency, bolus arrival time, and T_1 relaxation rate of the labeled spins. Velocity-encoded phase-contrast (PC) MRI is a technique complementary to ASL, in which flow velocity is assessed through phase images. When combined with brain volume measures using anatomic scans, global CBF can be measured by applying PC MRI in main feeding arteries. In humans, it has received increasing interest for absolute quantification of CBF in physiological and pathophysiological studies (17,18), and has also been used to calibrate ASL signals (19). To our best knowledge, mouse CBF imaging using PC MRI has not been demonstrated.

In this study, we sought to evaluate the feasibility of CBF measurement using PC MRI in mice. We optimized several key imaging parameters, including encoding velocity (VENC) and spatial resolution, for major feeding arteries, i.e. internal carotid artery (ICA) and vertebral artery (VA), of the brain. We also optimized repetition time (TR) and implemented reduced field-of-view (FOV) scheme in an effort to minimize scan time while preserving image quality. With the optimized protocol, absolute quantification of CBF in mice can be obtained in 96 sec. Unit-volume CBF was obtained by normalizing the BF value by whole-brain volume measured with T_2 -weighted scans.

METHODS

Animal preparation

The local institutional animal care and use committee approved the experimental protocol. A total of 14 mice were studied, with some of them used in multiple studies. Of these, nine BALB/c mice (20~26 g) were used for spatial resolution and repetition time studies; five C57BL/6 mice (21~26 g) were used for velocity and reduced FOV studies. Anesthesia was administered by using 1.5% vaporized isoflurane for 15 min, then switching to 1.0% for maintenance until the end of experiments. Respiration rate was monitored during the experiment to ensure the survival of the mouse. In case that a mouse breathed at a rate >150 breaths per minute, the maintenance isoflurane dosage was increased slightly to ~1.2%. At the 10th minute under 1.5% isoflurane inhalation, the mouse was immobilized with a bite bar and ear pins, and then placed on a water-heated animal bed with temperature control and the bed was placed in the magnet.

MRI Experiments

All MRI experiments were performed on an 11.7T Bruker Biospec system (Bruker, Ettlingen, Germany) equipped with a horizontal bore and actively shielded pulsed field gradients (maximum intensity of 0.74 T/m).

Images were acquired using a 72-mm quadrature volume resonator as transmitter, and a four-element (2×2) phased array coil as receiver. The B_0 field over the mouse brain was homogenized by a global shimming (up to 2nd order) based on a subject-specific pre-acquired field map. Several sub-studies were conducted to optimize different aspects of the PC MRI pulse sequence, including VENC, in-plane spatial resolution, TR, and reduced FOV (as detailed below). These studies were performed sequentially on different mouse groups, and the optimized parameter from a previous study was used in latter studies.

Placement of PC MRI imaging slice: Similar to humans, the blood supply to the mouse brain primarily originates from four major feeding arteries, i.e., left ICA (LICA), right ICA (RICA), left VA (LVA), and right VA (RVA). ICAs provide perfusion to cortical and subcortical regions whereas VAs supply blood to brainstem, cerebellum, and part of the cerebral cortex (20). Once blood flows (BF) in these four arteries (i.e. LICA, RICA, LVA, and RVA) are quantified, the sum of them then yields global BF. One can further normalize global BF by total brain volume to calculate unit-volume CBF.

We developed the following procedure to ensure correct identification of the target arteries and positioning of PC MRI slices (Figure 1), which was found to be robust in all mice studied. Based on the localizer image, we first performed a coronal time-of-flight (TOF) angiogram (7 slices, slice thickness 0.5 mm, no gap, TR=45 ms, TE=2.6 ms, scan duration 2 min). The center of the imaging slab was placed to be along the ventral margin of the brain (Figure 1b). Maximum-intensity-projection (MIP) display of the TOF allows the visualization of all four arteries of interest (Figure 1c). To better localize each artery, the TOF images are inspected in a 2D mode to identify the slice that contains the target artery (see Figures 1d and e for ICA and VA, respectively). Next, a sagittal TOF (single slice, tilted to contain the artery, thickness 0.5 mm, TR=60 ms, TE=2.5 ms, scan duration=0.4 min) is

performed to visualize the target artery from another direction (see Figures 1f–i, for ICA and VA, respectively). Then, using both coronal and sagittal TOF images, the imaging slice PC MRI was placed to be perpendicular to vessel trajectories (see yellow box in Figure 1f for an example for LICA). For easy identification of the target vessel in post-processing, we placed the target artery to be at the center of the PC MRI's FOV, although this is not a requirement.

Study 1: Determination of typical blood flow velocity in mouse brain: Encoding velocity (VENC) is a crucial parameter in PC MRI. VENC should be chosen to be slightly higher than the peak velocity of the vessel. We therefore conducted a study to measure blood flow velocity in the mouse brain. PC MRIs were performed for each of the left/right ICA and left/right VA, respectively. A VENC of 20.0 cm/s was used for both ICA and VA to ensure the acquisition of aliasing-free PC images. The other parameters were: TR/TE = 60.0/3.2 ms, FOV = 16×16 mm², matrix size = 256×256, slice thickness = 0.5 mm, number of average = 4, dummy scan = 8, receiver bandwidth = 100 kHz, FA = 25°, and scan duration = 2.0 min. A partial Fourier acquisition (factor=0.7) was applied along the reading-out direction to shorten the TE and obtain a higher sensitivity. Three mice were scanned for this sub-study.

Study 2: Dependence of BF measurement on spatial resolution: High spatial resolution is associated with longer scan duration and low signal-to-noise ratio (SNR), whereas low spatial resolution suffers from partial-volume effects. We therefore conducted a sub-study to investigate the resolution-dependence of BF results. Four in-plane spatial resolutions of 80×80, 60×60, 50×50, and 40×40 μm² were examined, corresponding to scan durations of 1.5, 2.0, 2.4, and 3.0 min, respectively. Other parameters were similar to the ones described above, except that TE was chosen to be as short as possible for given resolution to maximize SNR. Each measurement was repeated 3 times to allow the calculation of coefficient of variation (CoV). Five mice were scanned for this sub-study.

Study 3: Optimization of TR: To further shorten the scan duration of PC MRI, two schemes were considered. One is to reduce the TR while avoiding signal loss. This is feasible because spins in the vessel are quickly replaced by fresh spins and, as long as TR is sufficient to allow complete refreshing of saturated spins, shortening TR should not negatively impact the signal intensity in the vessel. Therefore, in this sub-study, four TR values (60, 45, 30, and 15 ms) were used, which corresponded to scan durations of 2.4, 1.8, 1.2, and 0.6 min, respectively. Measurements were repeated 3 times. Four mice were scanned for this sub-study.

Study 4: PC MRI scan with reduced FOV: The other scheme to shorten scan duration was to employ reduced FOV along the phase-encoding direction. Since ICA and VA are located in the middle of the head rather than on the edge, a certain degree of spatial folding is tolerable as long as the foldover tissue does not overlap with the targeted vessel. Based on data collected in earlier sub-studies, we found that a FOV of 10 mm (2/3 of the full FOV) can be used without introducing foldover tissue signals to the artery of interest. Experiments were then performed in four mice to compare the reduced FOV (10×15 mm²) to the full FOV (15×15 mm²) scan. The dataset from reduced FOV experiments was also used for exploring inter-rater consistency in terms of region-of-interest (ROI) drawing. To normalize

the BF value into unit-volume CBF, we also used a T₂-weighted scan (35 slices, thickness 0.5 mm, TR=4000 ms, TE=15 ms, FOV=15×15 mm², matrix size=128×128, scan duration=1 min) to measure whole-brain volume of the mice. Four mice were scanned for this sub-study.

Data Processing

All data processing was performed with custom-written MATLAB scripts (MathWorks, Natick, Massachusetts). Similar to processing strategies applied for human PC MRI data, the artery of interest was first manually delineated on the complex-difference image, which usually shows an excellent contrast between vessel and surrounding tissue. The mask was then applied to the phase velocity map and the integration of all voxels yields flow through the artery in ml/min.

To evaluate the rater-dependence of the manual drawing, two raters with more than 1 year of experience in processing PC data analyzed the same datasets independently and the differences in their results were quantified.

T₂-weighted anatomic scans were processed with a semi-automatic algorithm to estimate the brain volumes. In this algorithm, an empirical thresholding level (15% of averaged whole-brain signal intensity) was first applied to get a coarse mask covering the brain region. Then, the mask was manually checked and modified to correct any mis-identified brain boundaries.

Student t-test was used to compare results across scan protocols and a $p < 0.05$ is considered significant. CoV was calculated as standard deviation across repetitions divided by the mean.

RESULTS

Study 1:

Blood flow velocity in the mouse brain: Figure 2 shows typical images from a PC MRI scan, including unsubtracted images (magnitude images I and II), complex difference image, and velocity map. These images were acquired at a relatively low resolution of $62.5 \times 62.5 \mu\text{m}^2$ with an identical VENC of 20 cm/s. Figure 3 summarizes peak (across voxels) and mean blood flow velocities (across voxels) in these major arteries. Peak flow velocities were 13.8 ± 1.7 (mean $\pm$ standard deviation, SD, across mice), 14.4 ± 0.6 , 6.5 ± 1.7 , and 6.7 ± 1.3 cm/s for LICA, RICA, LVA, and RVA, respectively. There was not a significant difference in peak velocity between LICA and RICA ($p = 0.68$) or between LVA and RVA ($p = 0.94$). However, ICA was found to have a significantly higher peak velocity than VA ($p < 0.05$ for both the left and right side). Based on these observations, a VENC value of 20 cm/s and 10 cm/s were used for ICA and VA PC MRI, respectively, in the remainder of the study.

Study 2:

Dependence of PC MRI results on spatial resolution: Figure 4a shows representative complex-difference MR images using four different spatial resolutions. Figures 4b-4e show a summary of results from all animals ($N = 5$). It can be seen that low resolution acquisitions are associated with an over-estimation of BF (Figure 4b for ICA and Figure 4d for VA), because voxels on the edge of the vessel containing partial blood and partial tissue were

treated as whole-blood voxels by the PC MRI. On the other hand, high resolution acquisitions tend to take longer time and also appear noisier, as shown by a higher CoV (Figure 4c for ICA and Figure 4e for VA). Collectively, a resolution of 50 μm was concluded to be a reasonable tradeoff for both ICA and VA and was used in the remaining sub-studies.

Study 3:

Optimization of TR: Figures 5a and b show PC MR images as functions of TR. It can be seen that the vessel intensities in the complex difference image are minimally affected by TR, due to rapid refreshing of flowing spins in the vessel. Similarly, velocity values in the vessel voxels are comparable across TR. Velocity values in tissue voxels become noisier as TR decreases, because tissue signal is diminished with a shorter TR. Figures 5c–f reveal mean blood flow and CoV as functions of TR, showing relatively constant values. Based on these results and the benefit of shorter scan time, we concluded a TR of 15 ms to be optimal.

Study 4:

PC MRI scan with reduced FOV: Figure 6a displays a representative dataset from the reduced FOV study. As can be seen, Regions I and II in the full FOV scan are folded to a different location in the reduced FOV scan. However, these folded tissue signals present no overlap with the arteries of interest, thus do not affect the flow quantification. Figure 6b presents the BF values of LICA, RICA, LVA, and RVA obtained with full FOV and reduced FOV methods. No significant differences were found between these two methods for any of the arteries ($p=0.34$ for LICA, $p=0.92$ for RICA, $p=0.83$ for LVA, and $p=0.70$ for RVA). Similarly, the global BF did not exhibit significant difference between these two methods ($p=0.83$). BF values in the right arteries were slightly higher than the left side for both ICA and VA. However, the differences did not reach statistical significance ($p=0.21$ and 0.31 for ICA and VA, respectively).

Brain volume in these mice as measured by T_2 -weighted anatomic scans was 0.494 ± 0.013 (mean $\pm$ SD) mm^3 . Combining these volume measures and global BF, unit-volume CBF was found to be 279.5 ± 11.1 ml blood/100 ml tissue/min ($N=4$).

Inter-rater consistency: The Dice indices between ROIs drawn by two raters were 0.97 ± 0.02 (mean $\pm$ SD) and 0.92 ± 0.06 for ICA and VA, respectively. In terms of BF, CoV between two raters' values were $2.2 \pm 1.6\%$ and $7.6 \pm 8.2\%$ for ICA and VA, respectively. Cross-correlation coefficients between two measures were 0.96 and 0.97, respectively.

DISCUSSION

Phase-contrast MRI is a simple yet powerful technique to quantitatively measure blood flow velocity. However, the application of this method to measure blood flow in mouse brain has been scarce. There have been a few cardiac studies of PC MRI in mice (21,22); but those applications were more focused on PC angiography than on flow quantification. The present study aimed to optimize PC MRI flow quantification method in mouse brain so that this technique can be more widely used in physiological studies of mouse models.

Utility of measuring CBF includes many conditions with cerebrovascular abnormalities such as atherosclerosis (3), ischemia (4), and vascular stenosis (5). Moreover, neurodegenerative diseases such as Alzheimer's disease (7) and Parkinson's disease (8) can be evaluated by CBF due to the presence of coupling between CBF and cerebral metabolism. Furthermore, measurement of CBF can serve as a building block of probing more advanced physiological parameters such as cerebral metabolic rate of oxygen (CMRO₂), by combining CBF information with oxygenation methods, e.g. T₂-Relaxation-Under-Spin-Tagging (TRUST) (23,24). Additionally, CBF can be used to evaluate the effects of drug or treatment in molecular imaging or intervention studies.

This study optimized a number of imaging parameters for PC MRI in mouse brain. One of the most important parameters in PC MRI is VENC. Our study suggested that peak flow velocity in ICA was approximately 14 cm/s and that in VA was about half of that value, i.e. 7 cm/s. Therefore, to account for some degrees of intersubject variations in flow velocity, we chose a VENC of 20 cm/s and 10 cm/s for ICA and VA, respectively. Using these VENC values, we did not observe any phase aliasing throughout our studies. However, under certain circumstances such as CBF measured under hypercapnic condition or in certain disease models, peak flow velocity might exceed VENC, which would result in velocity aliasing in the flow map. Under these circumstances, one approach would be to set the VENC on an individual mouse basis. To do this, a "scout" phase-contrast scan can be performed and analyzed in real-time to determine the peak velocity of the vessel-of-interest. The VENC can then be set to be 10–20% higher than the peak velocity. However, a somewhat circular problem is that the VENC of the "scout" scan needs to be chosen properly, which in turn depends on the flow velocity. An alternative approach is to use post-processing methods, in which phase-unwrapping can be performed to recover the correct velocity information, as has been reported for human PC MRI studies (25,26). It should also be pointed out that blood flow velocity can be affected by anesthesia, thus different VENC values may be needed for different types or levels of anesthesia. The experiments in the present study were performed under inhalational isoflurane, which is a vasodilator and will increase blood flow (27). If a vasoconstrictive anesthetic is used (e.g., dexmedetomidine), we expect that a lower VENC should be used.

Partial-volume effects and scan duration are two other considerations in this study. We determined that a voxel size of 50×50 μm² represents a good tradeoff. At this resolution, an ICA typically covers 36±5 voxels (N=8) and a VA covers 17±6 voxels (N=8). This level of voxel count is comparable to that of human PC MRI protocols (26). To further reduce the scan duration, we applied two additional schemes, lowering TR and reducing FOV along the phase-encoding direction. We found that a TR of 15 ms is sufficient to preserve signal intensities in the vessels. This is physiologically sensible given our slice-thickness of 0.5 mm. Specifically, based on the fact that mean flow velocity in VA is 3.8 cm/s (Figure 3b), simple calculation suggests that it would take 13.0 ms for blood spins in the imaging slice to be completely replaced by fresh, unsaturated spins. Therefore, TR can be shortened to this range without losing sensitivity. We further found that reducing the phase-encoding FOV to 2/3 of the full-FOV value can save scan time (by 1/3) without having a negative impact on data quality. This is because ICAs and VAs are fortuitously located in the middle of the

brain, thus slight foldover does not result in overlapping of tissue signals with the vessel voxels.

The limited total blood volume in mice places an intrinsic barrier for CBF measurements using PET. As a potential solution, Temma et al. proposed to define a volume of interest (VOI) in the heart to estimate the arterial input function (28). With this method, the CBF of mice was reported to be 60~80 ml blood/100 g tissue/min. In contrast, CBF measured by PC MRI in the present study was 274.1 ± 10.9 ml blood/100 g tissue/min. As an additional comparison, CBF measured by arterial spin labeling (ASL) technique was ~210 ml blood/100 g tissue/min (29,30). Thus, PC MRI revealed more consistency with ASL than with VOI-PET. The remaining CBF discrepancy between PC and ASL may be attributed to technical factors such as labeling efficiency in ASL.

Based on our results, the intra-session CoV of whole-brain BF values (without repositioning) is estimated to be $2.0 \pm 0.4\%$, according to Study 3. For inter-session CoV, our data suggested that it is approximately 10.7%, based on mice in Study 4. For inter-strain CoV, we recently measured blood flow in another cohort of BALB/c mice (N=3) and, compared to the C57BL/6 mice in Study 4, the group-level difference is 5.3%. Therefore, it appears that inter-session differences, potentially due to variations in anesthesia level and animal physiological state, represent a larger source of variability compared to mouse strains or measurement noise.

In this study, the blood flow evaluations have been restricted to major feeding arteries of the brain, i.e., internal carotid artery and vertebral artery. We expect that PC MRI can also be applied to measure blood flow in smaller arteries. However, some considerations in imaging parameters are necessary. For example, the spatial resolution will need to be increased such that one can identify at least 15–20 voxels in the vessel. With a smaller voxel size, the number of averages should increase to preserve sufficient SNR. Additionally, because flow velocity in smaller arteries tends to be lower, TR of the sequence will likely need to be increased to allow refreshing of saturated spins.

Although the optimization studies conducted in this work were performed at 11.7T, some of the findings are expected to be also applicable for experiments at lower magnetic field strengths (e.g. 7T, 9.4T). VENC and in-plane resolution primarily depend on the vessel diameter and flow velocity, thus is not field-dependent. Additionally, the efficacy of reduced FOV is related to tissue geometry near the vessels, and is also independent of the magnetic field. However, the SNR of the image will be field-dependent. At a lower field, the intrinsic SNR of the data is expected to be lower. On the other hand, transverse signal decay at lower fields will be less due to a longer T_2 and T_2^* . Thus, the exact SNR loss at a lower field will depend on the imaging parameters such as TE.

A limitation of the present work is that we did not perform physiological challenge experiments, such as CO₂ alteration or varying anesthesia depth, to modulate blood flow and use the proposed technique to characterize its sensitivity. These further testings should be the goal of future studies.

CONCLUSIONS

We have optimized PC MRI for non-invasive quantification of CBF in mice at 11.7T. With the optimized protocol, it takes 96 sec to evaluate CBF with a test-retest variability of approximately 5%. PC MRI may be a valuable tool in studies of disease mouse models and contrast-agent or drug effects.

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Figure 1:

Flowchart and representative images in the positioning of PC MRI scan. (a) Flowchart of major steps. (b) Slice positioning of coronal TOF scan overlaid on a localizer image. (c) Maximum-intensity-projection (MIP) image of coronal TOF scan. (d) Single-slice view of coronal TOF in the slice that contains ICAs. (e) Single-slice view of coronal TOF in the slice that contains VAs. (f-i) Sagittal TOF images that allow visualization of LICA, RICA, LVA, and RVA, respectively. The arteries of interest are pointed out by arrows. PC MRI slice positioning is illustrated for LICA.

Figure 2:

Representative PC MRI images for (a) LICA, (b) RICA, (c) LVA, and (d) RVA. For each PC MRI scan, one obtains two magnitude images (corresponding to flow-encoding gradients of opposite polarities), one complex-difference image, and one velocity map. A zoomed-in version of the target artery is shown in the inset.

Figure 3:
Summary of (a) peak and (b) mean blood flow velocities in the four arteries of interest. Error bars indicate standard deviations across mice.

Figure 4:

Resolution dependence of PC MRI results. (a) Representative complex-difference images (zoomed-in version only) of an LICA at different resolutions. The yellow contours illustrate manually drawn ROIs. (b,d) ICA and VA BF values obtained at different resolutions. Statistically significant differences based on paired t-test are shown. (c,e) Coefficient-of-variation (CoV) from repeated scans for ICA and VA at different resolutions. Error bars indicate standard deviations across mice.

Figure 5:
TR dependence of PC MRI results. (a,b) Complex-difference images and velocity maps at different TR. (c,d) ICA and VA BF values obtained at different TR. (e,f) Coefficient-of-variation (CoV) from repeated scans for ICA and VA at different TR. Error bars indicate standard deviations across mice.

Figure 6:

Results of PC MRI with reduced FOV. (a) A representative ICA velocity map from a full FOV scan and a reduced FOV scan. Regions that are folded are also indicated. Arrows indicate the vessel of interest. (b) Summary of BF values obtained by the full FOV scan and the reduced FOV scan. Error bars indicate standard deviations across mice.