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An Steady Pulsed Imaging and Labeling Scheme for Non-invasive Perfusion Imaging

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Abstract

Purpose—A Steady Pulsed Imaging and Labeling (SPIL) scheme is proposed to obtain high-resolution multi-slice perfusion images of mice brain using standard preclinical MRI equipment.

Methods—The SPIL scheme repeats a pulsed arterial spin labeling (PASL) module together with a short mixing time to extend the temporal duration of the generated PASL bolus to the total experimental time. Multi-slice image acquisition takes place during the mixing times. The mixing time is also used for magnetization recovery following image acquisition. The new scheme is able to yield multi-slice perfusion images rapidly. The perfusion kinetic curve can be measured by a multi-pulsed imaging and labeling (MPIL) scheme, i.e., acquiring single-slice ASL signals before reaching steady-state in the SPIL sequence.

Results—When applying the SPIL method to normal mice, and to mice with unilateral ischemia, high-resolution multi-slice (5 slices) CBF images could be obtained in 8 minutes. Perfusion data from ischemic mice showed clear CBF reductions in ischemic regions. The SPIL method was also applied to postmortem mice, showing that the method is free from magnetization transfer (MT) confounds.

Conclusions—The new SPIL scheme provides for robust measurement of CBF with multi-slice imaging capability in small animals.

Keywords

Pulsed Arterial Spin Labeling (PASL); Arterial Spin Labeling (ASL); UN-inverted Flow-sensitive Alternating Inversion Recovery (UNFAIR); Steady Pulsed Imaging and Labeling (SPIL); multi-pulsed imaging and labeling (MPIL); Perfusion; Cerebral Blood Flow (CBF)

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INTRODUCTION

Arterial spin labeling (ASL) perfusion MRI is a powerful technique for noninvasive measurement of cerebral blood flow (CBF) (1). The ASL technique is based on labeling water protons of feeding arterial blood using radio frequency (RF) pulses. Depending on the labeling method, the currently available ASL techniques can be divided into three major classes, pulsed ASL (PASL) (2–6), continuous ASL (CASL) (1,7,8), and velocity selective ASL (VSASL) (9). The PASL techniques have been used widely in both animal and human studies due to easy implementation, high labeling efficiency, and low specific absorption rate (SAR), but these techniques suffer from relatively low signal-to-noise ratio (SNR). For small-animal studies, CASL is a popular technique, which provides a higher SNR compared to the PASL method due to the ability to attain long bolus durations (10). However, in animals, a two-coil setup is often required to eliminate confounds due to magnetization transfer (MT) asymmetry (11–13), i.e., one transmitter coil positioned at the heart of the mouse, or neck of the rat, for spin labeling, and a second transceiver coil for imaging. This has hampered the wide application of CASL in rodents. Therefore, there is a need for new ASL techniques that use standard hardware and are capable of recording high-resolution perfusion images while suppressing the potential MT confound.

The CASL technique pseudo-continuous ASL (PCASL) has gained much attention in recent years in both human (14–17) and small-animal perfusion studies (18), due to its ability to achieve high SNR, but without requiring a special coil setup. PCASL can also suppress the MT effect efficiently. Another CASL technique, amplitude modulated CASL (8), has also been demonstrated for mouse brain perfusion studies, and showed high labeling efficiency as well as the ability to suppress MT interference. These techniques require precise positioning of the labeling plane, and the gradient must be calculated carefully to achieve adiabatic inversion of arterial blood.

In this paper, we propose an ASL scheme called Steady Pulsed Imaging and Labeling (SPIL). The new SPIL scheme borrows a principle applied in pulsed magnetization transfer (MT) (19–21) and pulsed chemical exchange saturation transfer (CEST) studies, such as VDMP-CEST (22) and FLEX (23). Both ASL and MT/CEST evaluate exchange processes. In ASL, during the exchange process, the labeled bolus enters the microvasculature and exchanges with tissue water in the imaging slice, while in CEST, water-exchangeable protons are labeled and exchange with the large water pool (24). The major difference between CEST and ASL is that the exchange process happened instantly in CEST, while an arterial transit time needed in ASL. Nevertheless, many approaches applied in ASL can be adapted for MT/CEST studies by removing the slice-selective gradient, and *vice versa*, such as conventional CASL (1,11) and CW-CEST (25), conventional pulsed ASL (26,27) and pulsed CEST (28), FAIR (29) and Selective Inversion Recovery (SIR) (30). Similarly, the ASL signal can be easily contaminated by MT effects; some techniques that have been applied in ASL studies to suppress MT effects also have been implemented in the CEST field, such as amplitude modulated CASL (8,31) and double-saturation CEST (32). In a CEST study, the labeling process is repeated many times to enhance the CEST effect and thereby achieve a discernible effect on the water signal, which allows indirect imaging of low-concentration solutes through water. This enhancement idea has been implemented in

ASL studies such as CASL, the pseudo-continuous transfer-insensitive labeling technique (pTILT) (33), and PCASL, in which a bolus is accumulated in the imaging slice by repeating the labeling process several times to extend the bolus duration before the image acquisition. Following the idea of accumulating signal by repeating the labeling scheme applied in CASL, pTILT, and PCASL, our new approach of SPIL repeats a labeling and exchanging module (LEM) consisting of an un-inverted flow-sensitive alternating inversion recovery (UNFAIR) (5) labeling unit followed by an exchange time. The multi-slice images in SPIL are acquired during the exchanging time in the LEM. In this way, the exchange time after the UNFAIR module, i.e. the bolus duration, is also used for magnetization recovery following multi-slice image acquisition. In this paper, the theory for the new SPIL scheme is developed, and its potential for CBF quantification demonstrated.

METHODS

Pulse Sequences

The SPIL consists of repeated labeling and exchange modules (LEM) of a labeling unit followed by an exchange time. The UNFAIR scheme (5) is used for labeling unit, as shown in Figure 1A. Image acquisition is performed during the exchange time between LEMs. A multi-slice 2D fast spin echo (FSE) is used for image acquisition. Perfusion images are obtained by subtraction of the labeling images from the control images. In the control image, the water spins in the whole mouse brain are inverted twice in one LEM by a pair of non-selective 180° inversion pulses. In the labeling scan, the labeling of the blood water spins is performed by non-selective inversion of the whole brain, followed by selective inversion of the imaging slice. Therefore, the water spins outside the imaging slice are inverted. Subsequently, a waiting period, t_{mix} , is applied before the next LEM to allow labeled spins to perfuse into the tissues of the imaging slice. Due to repetition of the LEM during the whole experiment, all labeled water will be accumulated in the imaging slice and reach steady-state after several LEMs. The exchange time in the LEM is used for magnetization recovery and contributes to the bolus duration time. The bolus duration τ is the time period that the labeled arterial blood flowing into the imaging slices. The major factor determining τ of one LEM is coil coverage. In this study, the excitation coil covers the majority of the mouse body. Therefore, the bolus duration for one LEM is far longer than the mixing time.

The second selective inversion slice is wider than the imaging slice, in order to ensure that the imaging slice is completely inverted. The gap between the imaging slice and the inversion slice is called a slab margin (Figure 1B). A 180° hyperbolic secant (sech) pulse (34) was applied as a selective inversion pulse, and its offset was set to the center of the imaging slab. Therefore, the offsets of each of the slice selection pulses varied significantly with respect to the offset of the inversion pulse. As a consequence, the pulse profile of the inversion pulses was critical for obtaining perfusion images without contamination, especially for the slices far away from the center of the slice package. One strategy to reduce the impact of the inversion imperfection is to apply a large slab margin. Then, only the center of the inversion pulse profile will be used, which usually demonstrates good inversion homogeneity.

The perfusion kinetic curve, i.e., the perfusion signal with respect to the total labeling duration, is needed to obtain the parameters required for absolute quantification of CBF, and was obtained here by observing the single-slice ASL signal before reaching steady-state in the SPIL sequence. The single-slice non-steady-state SPIL sequence is referred to as Multi-Pulsed Imaging and Labeling (MPIL) and is illustrated in Figures 1C and D. It follows the dynamic ASL approach introduced by others (35,36). The labeling duration in SPIL is defined by the start of the first LEM until the imaging readout, and is analogous to the labeling duration in CASL/PCASL. The variation of labeling duration is achieved by increasing exchange time in the LEMs, while keeping the number of LEMs constant.

Theory

A standard ASL single-compartment kinetic model was applied to quantify CBF, assuming instantaneous exchange of labeled spins from capillary to tissue. The observed ASL signal is described by the difference between the sum over the series of delivered magnetization units to the tissue (the arterial input function, AIF), and the clearance of the magnetization by venous flow and relaxation of the blood (the impulse residue function, IRF) (10,37,38). The description of the AIF function for SPIL is complicated because the labeled blood from the first LEM will be inverted several times by the subsequent labeling and a steady state for labeling and T_1 relaxation is generated. The AIF for the n -th mixing time ($n=1, 2, 3 \dots N$) can be calculated using a recursive method with an assumption that the effects of transition bands are negligible (see supplemental materials).

$$c_n(t) = \begin{cases} 0 & t < ATT \\ 2\alpha \frac{M_0}{\lambda} f e^{-t/T_{1a}} & ATT \leq t < ATT + t_{mix} \\ \frac{M_0}{\lambda} f \left[2\alpha e^{-\frac{t-t_{mix}}{T_{1a}}} - (2\alpha-1)e^{-\frac{t}{T_{1a}}} \right] & ATT + t_{mix} \leq t < ATT + 2t_{mix} \\ \dots & \dots \\ \frac{M_0}{\lambda} f \left[2\alpha e^{-\frac{t-(N-1)t_{mix}}{T_{1a}}} - (2\alpha-1)c_{n-1}(t) \right] & ATT + (N-1)t_{mix} \leq t < ATT + (N-1)t_{mix} + \tau \end{cases} \quad (1)$$

Where α is the labeling efficiency of one LEM, λ is the blood-brain partition coefficient for water, M_0 is the equilibrium magnetization of arterial blood, T_{1a} is the T_1 relaxation time of arterial blood, f is the CBF in units of volume of blood delivered per weight of tissue per unit time (ml/g/s), and ATT is the arterial transit time. The labeling efficiency of one LEM, α , reaches unity if the inversion pulses are perfect, while it is 0.5 if the pulses are saturation pulses, i.e., 90-degree pulses followed by dephasing, as seen from Eq.A2 in supplemental materials.

An analytical solution for the perfusion signal is difficult to obtain. However, it is still possible to obtain the solution for the steady-state situation. At the steady-state, the AIF of the SPIL sequence can be simplified to an averaged AIF. Then, the CBF values can be obtained by (see supplemental materials)

$$CBF = \frac{6000 \cdot \lambda \cdot \Delta M \cdot e^{\frac{ATT}{T_{1a}}}}{2 \cdot \alpha_{SPIL} \cdot T_{1eff} \cdot M_0} \left[\frac{\text{ml}}{100\text{g}} \right] \left[\frac{\text{min}}{\text{min}} \right] \quad (2)$$

where

$$\alpha_{SPIL} = \frac{\alpha T_{1a} (1 - e^{-\frac{t_{mix}}{T_{1a}}})}{t_{mix} (1 + (2\alpha - 1) e^{-\frac{t_{mix}}{T_{1a}}})} \quad (3)$$

is the averaged SPIL labeling efficiency, which accounts for multiple inversions by the LEMs. For the typical values used in our study, i. e. labeling efficiency for one LEM $\alpha = 0.9$ and $T_{1a} = 2.8$ s. The averaged SPIL labeling efficiency is 0.58 for a 1 s mixing time, while it will be 0.64 for a 2 s mixing time. This indicates that the train of inversion pulses saturated the arterial blood. Note that T_{1eff} is an apparent relaxation time for a monoexponential process, which accounts for the “true” T_1 relaxation of the blood, clearance of labeled blood by venous outflow, and any imperfections of inversion pulses.

The signal intensity of the control images is assumed to be equal to M_0 in the above discussion. In practice, the signal intensity of the control image is attenuated by the imperfect inversion of the 180-degree pulses, i.e., $M_{control} = \gamma M_0$, where γ is the attenuation factor. At the same time, the M images are also scaled by the attenuation factor γ , i.e., $M^* = \gamma \cdot M$, where M^* is the scaled M due to imperfect inversions. Although quantification of CBF will not be affected by the imperfect inversions if the control images are used as M_0 , by considering $M^*/M_{control} = M/M_0$, the attenuation due to imperfect pulses will reduce the overall signal-to-noise ratio of the final perfusion images. For the SPIL sequence, the attenuation factor is given by (see supplemental materials):

$$\gamma = \frac{1 - e^{-t_{mix}/T_1}}{1 - \xi e^{-t_{mix}/T_1}} \quad (4)$$

where the attenuation factor, ξ , describes the signal reduction due to the signal loss by one LEM.

Animals

The institutional animal care and use committee approved this study. Nine adult female BALB/c mice, approximately 10–18 weeks old, were used for optimizing the SPIL method. Three postnatal day-10 (P11) C57BL6 mouse pups received a right carotid artery ligation to create unilateral ischemia in the right side of the brain, and were imaged 24 hours after surgery. All animals were anesthetized using 2% isoflurane in airflow, followed by 1% to 1.5% isoflurane during the MRI scan. Mice were placed on a water-heated animal bed equipped with temperature and respiratory control. Respiration was monitored and maintained at 40–50/min. The heart rates of the adult mice were monitored by the MouseOx Plus (STARR Life Sciences Corp), and were stable at 300–350 beats/minutes with the above respiration rates.

MR Scanning

All MRI experiments were performed on a horizontal bore 11.7 T Bruker Biospec system (Bruker, Ettlingen, Germany). A 72 mm quadrature volume resonator was used as transmitter, and two sets of receiver coils were used for the current study. A 2×2 mouse

phased array coil was used for the adult mouse study, and a 10 mm planar surface coil was used for the P11 mouse study. All coils were provided by Bruker Corporation (Ettlingen, Germany). Images were acquired using a fast spin-echo (FSE) sequence with 90 degree flip angle, TE = 5 ms, RARE factor 16, slice thickness = 1 mm, a matrix size of 64×64 (with a FOV of $1.6 \times 1.6 \text{ cm}^2$) for the adult mice, and a matrix size of 64×64 (with a FOV of $1.2 \times 1.2 \text{ cm}^2$) for the ligated mice. The matrix size was reduced to 48×48 to acquire the perfusion kinetic curves. A sech pulse (13 ms pulse width) was used for the inversion pulses. The sech pulse profile was measured, and found the transition bands are about 11 % of the total bandwidth. Several sets of MRI experiments were designed to optimize and demonstrate the new sequence.

Experiment I: To evaluate MTC background and other potential contributions to ASL images—

Arterial spin labeling (ASL) techniques generally have low sensitivity, and can easily be contaminated by imaging artifacts, imperfect pulses, and magnetization transfer contrast (MTC). In order to examine whether such contamination existed, the SPIL sequences were tested on postmortem mouse brains using identical experimental parameters to those implemented on healthy mouse brain, except that two slab margins (2 mm and 6 mm) were tested.

Experiment II: To characterize the relationship between the perfusion signal and the number of LTMs using MPIL sequence—

The purpose of this experiment was to compare the performance of MPIL with a different number of LTMs, and to estimate parameters, such as effective $T_{1\text{eff}}$, and labeling efficiency, by fitting the resulting kinetic curves. The parameters obtained from the MPIL experiments were used for CBF quantification of SPIL.

Experiment III: Multi-slice perfusion imaging with SPIL—

This experiment was designed to demonstrate multi-slice perfusion image quality obtained using SPIL. Five slices with slice thickness 1 mm were acquired in each mouse brain. The slab margin was set to 6 mm, and the total inversion slab thickness was 19 mm. The total acquisition time was 8 minutes (4 minutes for each control images and labeling images) with the number of averages (NAV) 8, and the mixing time was 1 s. Two dummy scans were applied before acquiring the perfusion images.

Experiment IV: Comparison between SPIL and FAIR techniques—

The sensitivity difference between SPIL and FAIR techniques was examined by performing perfusion experiments using same coils and animals ($n=3$). The 180-degree inversion pulse and the imaging acquisition of FAIR sequence are identical to those used in SPIL scheme, except only single slice image was acquired in FAIR. The time of inversion (TI) in FAIR technique was set to 1.5 s, to maximize perfusion signal (see Figure 4A). The prescan delay for FAIR was set to 4 s to achieve the same experimental time as the SPIL scans. The M0 image of FAIR sequence was acquired by removing the inversion pulse and setting TR=10 s. With the assumption that TI is shorter than the bolus duration and ATT=0, the CBF values for each voxel can then be calculated from these data using (38)

$$CBF = \frac{6000 \cdot \lambda \cdot \Delta M \cdot e^{\frac{TI}{T_{1a}}}}{2 \cdot \alpha \cdot T_{1b} \cdot M_0 \left(1 - e^{-\frac{TI}{T_{1b}}}\right)} \left[\frac{\frac{ml}{100g}}{\min} \right] \quad (5)$$

where $\frac{1}{T_{1b}} = \frac{1}{T_{1eff}} - \frac{1}{T_{1a}}$ and TI is the time of inversion. This equation would be appropriate for human studies when $T_{1eff} \approx T_{1a}$ (17). However, the assumption $T_{1eff} \approx T_{1a}$ is invalid in mouse studies, due to the rapid clearance of the labeled blood, as seen from the dynamic perfusion study (Figure 4A). The difference between T_{1eff} and T_{1a} is more significant at high magnetic field, at which T_{1a} is much longer than clinical scanner. The SNR for the perfusion images was calculated using $\sqrt{2 - \pi/2} \Delta M / \sigma$, where σ is the noise standard derivation of the control image (18).

Experiment V: Application of the SPIL sequence to mice with unilateral ischemia—This experiment was designed to demonstrate that SPIL performs well on mice with small body size, and is able to detect unilateral reductions in brain perfusion.

Data Analysis

Images were processed using custom routines written in Matlab (MathWorks, Massachusetts). An intensity mask was applied when calculating M and CBF images. The mask was generated by taking all pixels with intensity higher than 10% of the maximum intensity of the control images ($M_{control}$). The perfusion kinetic curves were measured from the entire brain using ROI without large vessels. The CBF maps for the SPIL method were calculated using Equation 2, with the $M_{control}$ image used as M_0 . The arterial blood T_1 was set to 2.8 s (39). The value of ATT is only on the order of one hundred milliseconds for the mouse (8); therefore, it was neglected in this study. T_{1eff} was determined from the perfusion kinetic curves recorded using the MPIL sequence, and a value of 0.85 s was found for the 72 mm coil. The labeling efficiency (α) was set to 0.9, and the blood–brain partition coefficient (λ) for water was assumed to be 0.9 mL/g.

RESULTS

Simulation

Typical AIF and perfusion kinetic signals for SPIL are simulated in Figure 2A and B for a bolus duration (6 s). It can be seen that the both the AIF and the perfusion signal reach steady state after approximately five LEMs. The perfusion kinetic curve and AIF function for the MPIL scheme were simulated using parameters identical to those of the above simulations, and are illustrated in Figure 2C and D. For the MPIL sequence, the perfusion signal built up initially, with respect to the total labeling duration, and then decayed slowly due to the effective relaxation time T_{1eff} . The maximum perfusion signal recorded using one, two, and four LEMs is comparable, as demonstrated in Figure 2D.

Experiment I results

Figure 3 shows the SPIL perfusion signal from several slices over a postmortem mouse brain with two different slab margins, 2 mm and 6 mm. The perfusion signal measured at the

center slice of the brain (isoDistance = 0 mm) was less than 0.2 % (averaged over the brain) of the control images. However, the whole-brain averaged perfusion signal increased to around 0.7 % of the control for slices 3 mm from the isocenter (isoDistance = ± 3 mm) using the 2 mm slab margin. This contamination of the perfusion signal was mainly attributable to the imperfect inversion profile of the sech inversion pulses when offsets were far from the center frequency. The contamination could be suppressed significantly by increasing the slab margin to 6 mm, where a whole-brain average signal less than 0.2 % of the control value was observed for the slices 3 mm from the isocenter (see Figure 3C). Therefore, a slab margin of 6 mm was used for all the SPIL studies reported here.

Experiment II results

The perfusion kinetic data of mouse brain recorded by MPIL for one and four LTMs, together with the simulated curves, are plotted in Figure 4 for comparison. A 5 s prescan delay was used. Similar to the simulation (Figure 2D), the perfusion kinetic curve for one LEM (UNFAIR) reached a peak at 1.5 s (3.5 % control) and then decayed with $T_{1,\text{eff}}$. From the kinetic curves (Figure 4A), it can be seen that the bolus duration of one UNFAIR module is longer than 4 s, i.e., the maximum labeling time. Any bolus duration longer than 4 s will not change the curves. Therefore, a bolus duration of 6 s was used for fitting. The blood in the whole mouse body was labeled by the 72 mm coil, which led to sufficiently long bolus durations. As mentioned above, such a long bolus duration is difficult to determine accurately using kinetic curves, as any bolus duration longer than the total labeling time will give similar kinetic curves. The value of $T_{1,\text{eff}}$ was determined to be 0.85 s from the fitting. The perfusion kinetic curves acquired using four LEMs show a different pattern, compared to the UNFAIR scheme. The perfusion signal reached a peak around 2 s and then plateaued. The maximum perfusion signal was also close to 3.0% of the control images. This observation is consistent with the simulations shown in Figure 2D.

Experiment III results

Figure 5 shows typical multi-slice ASL images of the mouse brain obtained with the SPIL scheme, with a resolution of $250 \times 250 \mu\text{m}^2$. All five slices showed good brain perfusion signal. However, perfusion images between the midbrain and occipital lobes (Figure 6) show bright vascular signals, due to the posterior cerebral artery (PCA). The PCA in this region is perpendicular to the magnetic field (40). The crusher gradients of FSE in one direction was unable to suppress the PCA signal completely. Quantitative CBF analyses were performed on three brain structures: cortex, hippocampus, and thalamus. The corresponding CBF values are listed in Table 1 together with values reported by others using pCASL (18) and amplitude-modulated continuous ASL (am-CASL) (8) techniques. The thalamus showed the highest perfusion signal (264 ± 18 ml/100g/min), while the hippocampus (159 ± 13 ml/100g/min) had lower perfusion than cortex (218 ± 26 ml/100g/min) and thalamus. A similar observation has been reported by using the pCASL and FAIR techniques (18). To check for bias in estimated perfusion values in different slices, Table 2 presents average CBF values over the slices; none of the inter-slice differences reached statistical significance. The bilateral symmetry of CBF for all five mice is plotted in Figure 7. The CBF difference is less than 10% for the three brain structures. The CBF values from cortex show slightly bigger differences, which may be due to head motion. The within-ROI

standard deviations were also plotted in the same figure, and were found to be around 15% of the mean CBF values.

Experiment IV results

Typical perfusion images recorded on healthy mouse using SPIL and FAIR are presented in Figure 7. The M images on the same image slice show similar perfusion intensity except that the perfusion signal recorded using SPIL has slightly lower intensity in regions far from the coil. The perfusion intensity ratio ($M_{\text{SPIL}}/M_{\text{FAIR}}$) between SPIL and FAIR is around 0.94 for the cortical area, and is 0.84 for the hippocampal area. The results are consistent with the simulation (Figure 2) and the dynamic perfusion study on mouse (Figure 4). The CBF values obtained using SPIL are 209 ± 25 ml/100g/min and 160 ± 15 ml/100g/min for the cortical and hippocampal areas, respectively, while FAIR gave CBF values of 170 ± 13 ml/100g/min (cortex) and 121 ± 16 ml/100g/min (hippocampus). Differences in CBF estimated using the two techniques may due to slight differences in modeling, and are consistent with previous reports (18). The SNR of the perfusion images recorded using SPIL are around 22 ± 5 and 15 ± 3 for the cortex and the hippocampus, respectively, while the SNR is around 29 ± 5 (cortex) and 20 ± 3 (hippocampus) for the images obtained by FAIR.

Experiment V results

Typical perfusion images of immature (P11) mouse brain undergoing unilateral ischemia obtained using SPIL are shown in Figure 8. The T_2 -weighted images are also presented for comparison. The CBF values (Figure 8) of the unaffected hemisphere are 250 ± 30 ml/100g/min, close to the CBF values reported for adult mice (Table 1). Although the T_2 -weighted images did not depict clear ischemia on most slices, CBF showed a clear reduction in the affected right hemisphere with a mean CBF of 30 ± 20 ml/100g/min.

DISCUSSION

In this work, we introduce a new ASL scheme, dubbed SPIL, to obtain mouse brain perfusion images. The new method has several unique advantages for performing multi-slice perfusion imaging besides the typical advantages of PASL methods (i.e., no MT background interference, low SAR, and easy implementation): (i) The new scheme is robust to experimental errors considering the blood was saturated by a train of pulses, which is not sensitive to the pulse flip angle. When the bolus duration from one LEM is much longer than the exchange time, then the steady state perfusion signal simplifies the quantification of CBF, and makes it resistant to common PASL confounds, such as those related to the finite bolus duration and the presence of inversion transition bands; (ii) High acquisition efficiency for multi-slice perfusion imaging, by removing the pre-scan delays; (iii) multi-slice perfusion images recorded by SPIL do not require correction for the post-label time for each slice. In order to compare the time efficiency of SPIL scheme with other ASL methods, we define the ratio of the bolus duration time to the repetition time (TR) as the labeling time fraction. For SPIL, the labeling time fraction is 100%, while it is around 32% for PCASL with the recommended labeling parameters, i.e., 1.8 s labeling duration, 1.8 s post-label delay, and 2 s pre-scan delay (17). The final perfusion signal is a combined effect between the labeling efficiency at the imaging location α' and the labeling time fraction. In small

animal studies, the labeling efficiency at the imaging location is close to the labeling efficiency at the labeling plane α_0 , $\alpha' \approx \alpha_0$, due to the short arterial transit time. Although the labeling efficiency α_0 (around 0.5) of SPIL is lower than that of typical PCASL methods (around 0.7–0.8), the labeling time fraction (100%) has potential to enable SPIL to obtain perfusion images faster than traditional.

The SPIL scheme closely resembles a standard FSE sequence, except that one labeling unit is applied after each acquisition period. Therefore, the total experimental time is twice that of the FSE sequence. In SPIL, the bolus duration can be treated as infinite for each imaging slice. After the FSE image readout, during which spins are saturated, the labeled boluses begin to accumulate in the imaging slices similar to the post-label duration in conventional pCASL/CASL approaches. From the kinetic study using UNFAIR sequence (Figure 4A), a delay of 1.5 s between two FSE imaging on the same slice is enough to enable the labeled bolus to fully perfuse into the imaging slices. Compared to the standard PASL and CASL methods, the new scheme eliminates the prescan delay and labeling time. Therefore, SPIL theoretically has much higher acquisition efficiency (note that the pre-scan delay and labeling time are still needed in the MPIL sequence). This advantage is significant when performing multi-slice perfusion imaging, since the total experimental time does not increase with additional slices.

The time course of SPIL resembles the recently-introduced steady-pulsed ASL (spASL) approach to cardiac perfusion (41–43). However, SPIL and spASL differ in their geometry, specifically, in where labeling takes place. The labeling in spASL is identical to that in CASL and PCASL, i.e. labeling occurs as blood flow through a single labeling plane. In contradistinction, a thick slab of arterial blood is labeled in SPIL, resembling PASL. SPIL can be seen as an on-resonance version of spASL, i.e., the offset of the labeling pulses is close to zero; they are at the same frequency as the RF pulses used for imaging excitation.

The enhancement factor of SPIL is defined by the ratio of the perfusion signal recorded by SPIL to that obtained using a single LEM, i.e., UNFAIR in the current study. The enhancement factor is determined by the bolus duration of the single LEM. When the bolus duration is short, the enhancement by SPIL is high since the method can extend the bolus duration significantly; the enhancement factor is around 2.5 when bolus duration is 1 s. The signal enhancement of SPIL scheme is limited for extremely long bolus durations; the enhancement factor is 1.2 with bolus duration of 6 s, as is the case in our study. Using a long bolus duration, the perfusion signal already reaches steady state and will not gain enhancement by further increasing the bolus duration.

Multi-slice perfusion-weighted images obtained by conventional PASL and CASL methods are acquired from the first slice to the n-th slice, with no inter-image delay after the perfusion labeling period. The perfusion signal intensity will decrease from the first slice to the n-th slice due to the increased post-label delay with respect to the slice acquisition order (44). This complicates whole-brain CBF quantification, since it is challenging to model this signal decrease. This also limits the spatial resolution and the spatial coverage (i.e., number of slices). To overcome this issue requires reducing the image acquisition time by using a fast acquisition sequence, such as multi-band excitation method (45,46). The multi-slice

SPIL perfusion acquisition method is an elegant solution to this problem. Here, the slice acquisition distributes uniformly over the total labeling period. Therefore, each slice has exactly the same steady-state labeling efficiency and post-label delay; slice-wise corrections to CBF are unnecessary.

The inversion profile of the labeling pulses is critical for reducing artifacts in multi-slice SPIL. Our results show that the inversion profile is sufficiently homogeneous within the ± 3 mm region of the mouse brain, as evidenced by the cancellation of control and label images when the slab margin is 6 mm. Such a large slab margin is appropriate for most adult mouse studies. However, a smaller margin would be appropriate for much smaller animals, such as immature mice. Inversion pulses with better inversion profiles, such as frequency offset-corrected inversion (FOCI) (47–49) and bandwidth-modulated selective saturation and inversion (BASSI) (50) pulses, should enable use of smaller slab margins.

The long labeling time in SPIL not only allows time-efficient acquisition of perfusion images, but, unfortunately, may also enhance vascular artifacts. The long labeling time of SPIL ensures that blood will be labeled throughout the mouse, which can result in large signals from arterial and venous blood vessels, as shown in Figures 5 and 8. These vascular artifacts cannot be suppressed by increasing the post-labeling delay in SPIL, since the labeling time fraction is 100%. On the other hand, the long labeling time of SPIL offers opportunities for investigating slow exchange processes *in vivo*.

CONCLUSION

We present a new ASL scheme, dubbed Steady Pulsed Imaging and Labeling, or SPIL, to quantify CBF *in vivo* in the mouse brain. SPIL can improve the time efficiency and bolus duration by making use of steadily repeated image acquisition and labeling modules. SPIL offers high sensitivity and high-quality multi-slice perfusion images. Results of the present study were consistent with literature reports on mouse brain perfusion; however, full validation of the SPIL approach requires direct comparison with other PCASL/CASL methods, and with non-MRI methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Steady pulsed imaging and labeling (SPIL). **(A)** The SPIL sequence is composed of a series of label-transfer modules (LTMs), each containing two adiabatic inversion pulses, and followed by a mixing time (t_{mix}) to enable the labeled water spins to perfuse into the tissue in the image slice. During the mixing time, an image acquisition (FSE sequence) is applied. **(B)** Illustration of the inversion slab positions, with respect to the image slice. **(C)** The time diagram of the MPIL scheme. The LEM identical to the one in the SPIL sequence is repeated periodically every t_{mix} . Following the labeling duration, a single slice image is recorded. **(D)** Illustration of the inversion slab positions, with respect to the image slices, for the MPIL sequence.

Figure 2.

SPIL kinetics. **(A)** The Arterial Input Function (AIF) and **(B)** corresponding perfusion signal ($\Delta M/M_0$) for SPIL. The bolus duration of the UNFAIR LEM was assumed to be 6 s. The T_1 relaxation time of arterial blood is assumed to be 2.8 s. The labeling efficiency was set to 0.9. An effective $T_{1,\text{eff}}=1$ s and $\text{ATT}=0.1$ s were used. The mixing time was set to 1 s. **(C)** The typical Arterial Input Function (AIF) for MPIL with the parameters used in Figure A and B. The corresponding time course of the MPIL sequence with four LTMs is plotted on the top of the figure. **(D)** The simulated perfusion kinetic curves for the single slice SPIL with 1, 2, and 4 LTMs, respectively. When the number of LTMs is set to one, the MPIL sequence is the UNFAIR sequence.

Figure 3. Post-mortem SPIL results. **(A)** Control images (M_{control}). Only three slices located at -3 mm, 0 mm, and 3 mm from the isocenter of the magnet are shown. **(B, C)** Difference images ($\Delta M/M_{\text{control}}$) recorded using the SPIL scheme, with slab margins of 2 mm (B) and 6 mm (C). The mixing time is 1 s, NAV= 8 , with five slices with a slice thickness of 1 mm, and a slice gap of 0.5 mm (total slice package 7 mm).

Figure 4.

Experimental perfusion kinetic curves, with respect to total labeling time, for MPIL with one LTM, i.e., the UNFAIR sequence **(A)**, and four LTMs **(B)**. Data from healthy adult mouse brains. Solid lines are theoretical fitting curves. The $T_{1,\text{eff}} = 0.85$ s, $\text{ATT} = 0.12$ s, and a labeling efficiency $\alpha = 0.8$ was obtained for the fitting. The labeling duration was assumed to be 6 s.

Figure 5.

Representative multi-slice perfusion maps (middle row) of a normal mouse brain acquired using the SPIL scheme. Five slices with a slice thickness of 1 mm were acquired. The mixing time was 1 s. The M_{control} images are shown above the perfusion images for comparison (top row). The CBF maps were calculated by assuming a labeling efficiency of 0.9, $T_{1a}=2.8$ s, and $T_{1,\text{eff}}=0.85$ s.

Figure 6. Bilateral symmetry of CBF for three brain regions in five mice: cortex (green triangle), hippocampus (red circle), and thalamus (blue diamond). Error bars represent the in-ROI standard deviations. Typical ROIs for the three brain regions are illustrated in the figure inset.

Figure 7.
Typical M maps from a healthy mouse brain recorded with SPIL (A) and FAIR (B) techniques. The bottom row shows quantitative CBF maps acquired with SPIL (C) and FAIR (D) techniques.

Figure 8.

Representative multi-slice perfusion maps (bottom row) on postnatal mouse brain with a stroke model, acquired using SPIL. The T_2 -weighted images ($TE=60$ ms) are shown above the perfusion images for comparison (top row). Five slices with a slice thickness of 1 mm and a slice gap of 0.1 mm were applied.

Table 1

Regional perfusion values (n=5) estimated using SPIL. Regional CBF values reported in the literature are provided for comparison.

Brain region	CBF (ml/100g/min)	CBF (ml/100g/min) Ref. (18)	CBF (ml/100g/min) Ref. (8)
Cortex	218±26	160–175	209–240
Hippocampus	159±13	153	277
Thalamus	264±18	227	199

Table 2
Regional perfusion values (n=3, ml/100g/min) for different slices estimated using SPIL.

Brain region	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5
Cortex	193±19	205±18	215±19	212±23	204±19
Hippocampus	161±19	150±20	162±19	\	\
Thalamus	251±18	241±18	\	\	\